THE EFFECTS OF SEA LICE ON JUVENILE PINK SALMON PREDATION SUSCEPTIBILITY

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Paul A. Mages
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Name: Paul Mages

Degree: Master of Science

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Examining Committee:

Chair: Dr. L. Quarmby, Professor

Dr. L. Dill, Professor Emeritus, Senior Supervisor
Department of Biological Sciences, S.F.U.

Dr. R. Ydenberg, Professor
Department of Biological Sciences, S.F.U.

Dr. I. Côté, Professor
Department of Biological Sciences, S.F.U.

Dr. B. Roitberg, Professor
Department of Biological Sciences, S.F.U.
Public Examiner

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ABSTRACT

Juvenile pink salmon (*Oncorhynchus gorbuscha*) infected by sea lice (*Lepeophtheirus salmonis*) are known to be more susceptible to predation, but the mechanisms by which this occurs are unknown. This thesis used a predation risk framework to understand how *L. salmonis* may increase juvenile pink salmon predation susceptibility. Infected juvenile pink salmon increased their exposure to predators by returning sooner after a simulated heron attack when infected with a single adult louse. However, when attacked by a model heron, they appear to be equally likely to escape as non-infected fish. When infected with adult female lice, juvenile pink salmon were not able to swim as far in a swim tunnel against a gradually increasing current; suggesting a reduced condition. The effect of infection on condition is a potential driver of the increased risky behaviour (exposure) and has broader implications for predation susceptibility and for juvenile pink salmon early marine survival.

Keywords: parasite-host interaction; predation susceptibility; pink salmon; sea lice; prey behaviour; escape response; prolonged swimming

IN LOVING MEMORY OF MY MOTHER AND A BOUNDLESS FUTURE FOR CLAIRE

The journey to obtain my masters began during the time my mother, Velda, was losing her battle with breast cancer. It closes with the recent birth of my daughter, Claire. My mother always supported my dreams, encouraged my curiosity and taught me much about life. My journey finishes nearly 5 years after my mother's death and yet her encouragement lives on the spirit of Claire, who has given my life a new challenge, direction and purpose. Thank you Mom, for all you taught me, and Claire, I hope that I instil a love and curiosity in the natural world as well as the determination and will to follow what dreams may come.

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CHAPTER 1:

GENERAL INTRODUCTION

The direct act of predation is an important factor in shaping the ecology of organisms. However, the *risk* of predation is itself a great enough force to alter prey behaviour and ecology. Animals must make behavioural decisions in a manner that considers the risks and benefits associated with their actions in order to maximize their fitness. These decisions are often heavily influenced by their current state of health (or "condition") and the environmental context in which they occur and may mean the difference between life and death.

Prey behaviours which increase or decrease the likelihood of predation have been of great interest to ecologists (Lima and Dill, 1990; Angradi, 1992; Mesa *et al.*, 1994; Mesa *et al.*, 1998; Barber *et al.*, 2000; Lank and Ydenberg, 2003). Many behaviours, for example feeding, rearing of young, and mating, cannot be performed simultaneously with anti-predation behaviours or vigilance (Lima and Dill, 1990). Thus, animals must make trade-offs to maximize the net benefits of these behaviours.

The risk of predation can be defined as the product of the probabilities of encountering a predator, being attacked by that predator, and being able to escape that predator if attacked (Lima and Dill, 1990). Intrinsic habitat properties, the prey's physiological condition and its behaviour all influence the risk of mortality through their effects on one or more of these probabilities. All habitats have inherent features that influence predation risk, such as light level, the availability of cover, and the density of predators. These factors must be assessed by prey while determining how to allocate time to potential activities in the habitat (Lima and Dill, 1990; Lank and Ydenberg, 2003), and influence

foraging behaviours, such as when and where to feed, what to eat, and how to consume or handle the food (reviewed by Lima and Dill 1990). For example, prey often reduce feeding activity when they sense the presence of predators (Milinski, 1985; Angradi, 1992; Levri, 1998; Soto *et al.*, 2005).

While in a given habitat, a prey's physiological and morphological characteristics limit its ability to detect, avoid, and escape a predator. For example, reduced condition has been shown to decrease the probability of escape by prey (Mesa *et al.*, 1994; Murray, 2002). This could be the result of not detecting or reacting slowly to an attack, or to a reduced physical ability to escape. It is the prey's assessment of the habitat's inherent risk, its own escape abilities, and physiological condition that determine its behaviour, and thus influence overall susceptibility to predation.

Any factor, such as age, injury, starvation, disease, or parasitism that reduces the prey's condition can influence the risk of predation. Parasites are unique in the fact that their fitness is dependent on how they can exploit their host. The nature of a parasite is to remove resources from its host, either by direct absorption of the food (e.g., tapeworm) or by consumption of body fluids and tissue (e.g., ticks and lice). In the latter case, the host must expend energy to replace or repair what was lost to the parasite in order to avoid a reduction in condition. Infection costs are often subtle as hosts can often mitigate the increased energetic demand up to some point, for example by foraging more frequently, however this may increase the probability of a predator encounter (Magnhagen, 1988; Barber *et al.*, 2000; Lank and Ydenberg, 2003).

While parasites with complex life cycles sometimes increase the probability of host mortality due to predation, in order to facilitate transmission among hosts, many parasites (generally those with simple life cycles) have evolved low virulence when host survival is vital to their transmission (Poulin, 2007). Less virulent parasites can still have negative impacts if they occur in unnaturally high densities or infect juveniles. In these cases, parasites can reduce the host's condition sufficiently to affect behaviours and may impact the host's susceptibility to predation in ways previously described (i.e., increased exposure and reduced escape ability; Barber *et al.*, 2000).

Because many simple life cycle parasite/host systems have evolved to some evolutionary equilibrium, it is difficult to assess the effects of these parasites in the wild. However, with increased anthropomorphic changes in the marine environment come opportunities to investigate novel ecological interactions between host and parasite. One such example is the anthropogenically altered sea louse (*Lepeophtheirus salmonis*) - Pacific salmon (*Oncorhynchus* spp) host-parasite system. *Lepeophtheirus* salmonis is a directly transmitted ectoparasite commonly found on marine salmonids. A caligid copepod, it has a ten-stage life cycle (Figure 1.1) consisting of two free-living planktonic naupliar larval stages, a free-living infective copepodid stage, four parasitic attached chalimus stages, two motile pre-adult stages and one motile reproductive adult stage (Johnson and Albright, 1991). Once in the motile stage sea lice can transfer to other hosts, likely to find a mate (Tully and Nolan, 2002).

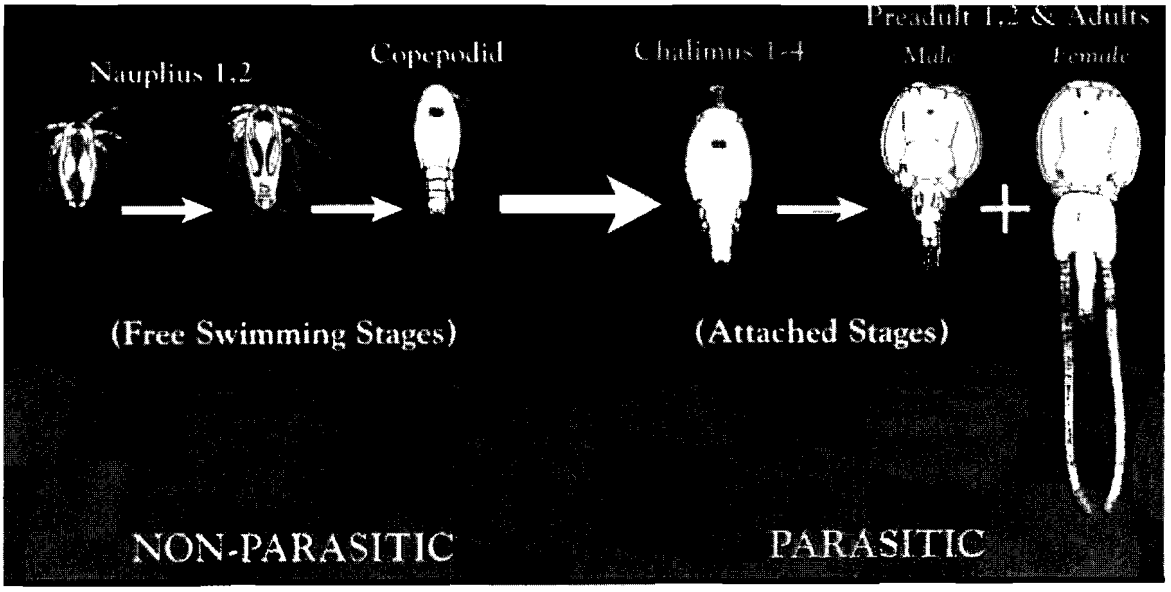

Figure 1.1 Diagram of sea lice stages
http://www.spaquaculture.com/default.aspx?pageid=42

Female lice produce hundreds of eggs, which develop and hatch from strings attached to the female. Once hatched, the nauplii are released into the water and, upon moulting to the copepodid stage, search out a suitable host. Once attached to a host, *L. salmonis* grazes on the skin and mucus or punctures the skin to obtain a blood meal.

The pink salmon, *O. gorbuscha*, is one of the smallest of all the Pacific salmonid species, returning to their natal streams after only a year in the open ocean. Upon emerging from the gravel, the fry migrate directly to the ocean where they grow from approximately 35 to 90 mm within a few months in the nearshore environment (Groot and Margolis, 1991). In July and August, these larger juveniles mix with the returning wild adult salmon. Sea lice transmission from adults to juvenile smolts occurs at this time (Krkosek *et al.*, 2007b).

The dynamics of this naturally evolved host/parasite system have been changed with the advent of fish farms (Krkosek *et al.*, 2007a, b). With the introduction of fish farms, large numbers of infective sea lice are now present on the salmon's migration pathways in the early spring (Orr, 2007) and small, scale-less juvenile salmon are infected shortly after they enter salt water (Morton *et al.*, 2004; Morton and Williams, 2004; Morton *et al.*, 2005). Krkosek *et al.* (2005) found lice infestations near a fish farm in the Broughton Archipelago, BC (Figure 1.2) to be three to four orders of magnitude greater than in areas away from farms. Pink salmon returns to the Broughton Archipelago have been

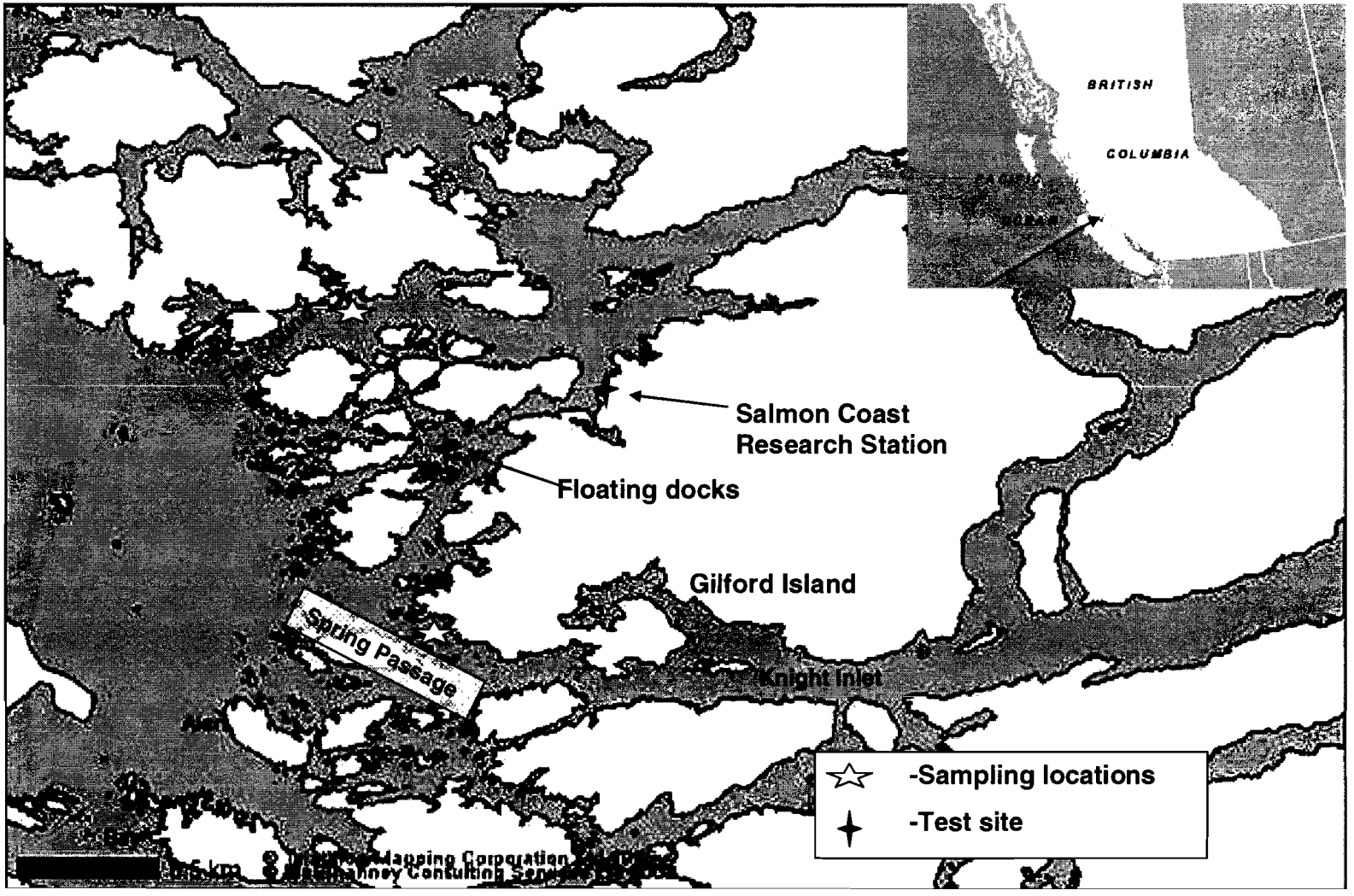

Figure 1.2 Map of the study area located in the Broughton Archipelago of British Columbia.

declining and this has been linked to decreased early marine survival resulting from an increased level of louse parasitism on juveniles (Krkosek *et al.*, 2007a). Recent evidence has shown that lice infected juvenile pink salmon (JPS) are more susceptible to predation than non-infected conspecifics (Marty Krkosek, University of Alberta; Brendan Connors, Simon Fraser University, pers. comm.). In these studies they subjected groups and individual JPS to coho (*O. kisutch*) salmon smolts and anadromous cut-throat trout (*O. clarki*) predation, respectively (see Connors *et al.*,2008). In both sets of experiments, predators consumed more sea lice infected JPS than non-infected ones, however, the mechanism driving this selective predation is unknown. This thesis looks at three possible mechanisms by which infection with sea lice can increase JPS susceptibility to predation. Chapter 2 tests the hypothesis that infected JPS increase their exposure to predators. Specifically, I tested the prediction that when frightened by a simulated heron attack, infected JPS would emerge from protective cover and resume feeding sooner than uninfected fish. Lice infection may also increase JPS predation risk by impairing their escape ability. This hypothesis was tested by subjecting naturally infected JPS to a simulated attack by a heron model. I predicted that as lice loads increase, JPS would be less likely to react, have greater reaction latency, and/or exhibit slower escape swimming (Chapter 3). These two hypothesized mechanisms are based on the premise that lice infection degrades JPS condition. If so, when subjected to increasing flow velocities in a prolonged swimming endurance test, naturally and experimentally infected JPS should be able to swim less distance than non-infected JPS; I

tested this in Chapter 4. Not only is swimming endurance an indicator of condition, but parasite impacts on it would have further ecological consequences, for example on escaping predation, competitiveness and reduced migratory ability, ultimately impacting JPS fitness.

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CHAPTER 2:

RISK-TAKING BEHAVIOUR OF JUVENILE PINK SALMON (*ONCORHYNCHUS GORBUSCHA*) PARASITIZED BY SEA LICE (*LEPEOPHTHEIRUS SALMONIS*)

2.1 Abstract

Parasitic infections can cause reduced health or condition in their hosts, which can make them more vulnerable to predation. A parasitized host with reduced condition may attempt to offset energetic costs associated with infection by increasing its foraging activity, thereby increasing its risk of predation. I investigated the possible effects of sea lice (*Lepeophtheirus salmonis)* infection on the risk taking behaviour of juvenile pink salmon (JPS; *Oncorhynchus gorbuscha)*. After a simulated predator attack, fish infected with one adult female sea louse returned to a high risk feeding area sooner than did non-infected JPS. These results suggest that sea lice cause JPS to accept greater predation risks to offset the costs of infection.

2.2 Introduction

Predators often consume those prey that are most vulnerable and vulnerability may arise for several reasons (Lima and Dill, 1990). For example, a reduction of health or condition (e.g. energy stores), or any increased energetic demand on prey, can lead to higher predation risk (Hudson *et al.*, 1992; Mesa *et al.*, 1994; Steen *et al.*, 2002; Soto *et al.*, 2005). A change in prey behaviour that increases its susceptibility to predators may be caused by either reduced condition or the prey's efforts to restore or maintain its condition (Lima and Dill, 1990). Parasites are a factor that can affect vulnerability by decreasing a host's condition, resulting in changes in host/prey behaviour (Holmes and Zohar, 1990). However, it is often difficult to determine if parasites actively manipulate host behaviour to facilitate transmission to their definitive host or if behavioural changes, resulting in predation, are a side-effect of infection costs to the host which do not necessarily increase the parasite's fitness (Poulin, 2007).

A parasitic infection can increase energetic demand of a host and thereby reduce the latter's physiological health or condition, especially in fish (Barber *et al.*, 2000; Barber and Wright, 2006). For example, parasitized fish can have increased physical costs associated with locomotion (e.g., increased drag; Ostlund-Nilsson *et al.* 2005) and/or physiological costs (for example, costs of osmoregulation, immune response to remove parasites and/or repair tissue damage). The main impact of parasites may be the removal of nutrients crucial to a host's energy budget (Barber and Wright, 2006). Therefore, hosts must

mitigate these increased energetic costs in order to avoid loss of condition and thus reduced fitness (current or future) (Holmes and Zohar, 1990).

A host can alter its behaviour to offset the costs associated with parasite infection. An infected fish could reduce its activity level to conserve energy (Brassard *et al.*, 1982), move to a less costly environment (Webster *et al.*, 2007), or offset costs by increasing its foraging effort (Barber *et al.*, 2000). However, these behaviours result in a trade-off. Host foraging decisions (e.g., where, how long and what to feed on) can increase food intake, but any increases in foraging time, for example, will result in less time being available for other activities such as predator vigilance (Dill, 1983; Lima and Dill, 1990; Barber *et al.*, 2000). Predator density often increases with foraging patch quality, therefore, in high quality patches, prey must balance increased foraging benefits with the cost of increased exposure to predation (Milinski, 1985; Giles, 1987; Godin and Sproul, 1988; Hugie and Dill, 1994; Damsgard and Dill, 1998). Prey with increased energetic demands caused by parasites may be willing to trade-off increased predation risk with increased foraging in high quality patches if such behavioural alteration offsets the metabolic cost of the parasites sufficiently.

Parasites can influence multiple components of predation risk (encountering a predator, being attacked by and escaping that predator; Lima and Dill, 1990). This study investigates whether a parasitized host will trade-off safety for increased foraging opportunities in order to offset metabolic costs of infection. If so, a parasitized host is expected to resume foraging sooner after a predator attack relative to a non-parasitized host, increasing the likelihood that

the former will be seen and attacked by a predator. However, many parasites with simple life cycles are relatively benign so as to not kill their host (or cause the host to be killed) before they are able to be transmitted or reproduce. Therefore, condition of these hosts (and the predation risk factors it influences) may only be affected when parasites are highly abundant or the host is especially weak to start with. Juvenile fish, for example may suffer reduced condition as a result of infections typically occurring in adults or larger juveniles.

Studies show increasing numbers of ectoparasitic sea lice, *Lepeophtheirus salmonis*, on out-migrating juvenile salmon in areas near fish farms in the Broughton Archipelago, British Columbia, causing up to 95% mortality (Morton *et al.*, 2004, 2005; Krkosek *et al.*, 2006). This study system provides a unique opportunity to utilize a new host/parasite interaction (Krkosek *et al.*, 2007) to look at the effects of a "novel" parasite on host behaviour.

During their seaward migration juvenile pink salmon (JPS) experience heavy mortality from bird and fish predators (Parker, 1968, 1971). Krkosek and Connors (pers. comm.) found *L. salmonis*-infected JPS to be more susceptible to predation from salmonid predators than non-infested conspecifics. Increased risk of predation due to sea lice infestation could therefore decrease the probability of salmon surviving their early marine life.

While it is now known that sea lice dramatically increase JPS mortality, the mechanism by which this happens remains unclear. One possibility is that the increased risk of predation of parasitized JPS could be related to a shift in behaviour of infected fish. Jones *et al.* (2007) found JPS to have an immune

response to sea lice infection, which increases energetic demands on the fish. Moreover, infected JPS prefer freshwater and incur higher energetic costs when in saltwater, which may be due to osmotic challenges associated with sea lice (Webster *et al.*, 2007). Increased energetic costs due to sea lice means there is less energy available for growth. JPS have evolved a fast growth strategy to escape size-selective predation (Lebrasseur and Parker, 1964; Parker, 1968, , 1971) such that growth is crucial to their early marine survival (Mortensen *et al.*, 2000; Beamish *et al.*, 2004). Therefore, parasitized JPS should attempt to mitigate infection costs and reduced growth rates by increasing foraging behaviour, thus accepting a short-term increase in predation risk, which may impact JPS early marine survival.

This study investigates the possible effects of sea lice infection on risk-taking behaviour of juvenile pink salmon. If sea lice infection does increase the energetic demand of JPS, they should take greater risks to obtain more food by returning to forage sooner after a predator disturbance than unparasitized fish. Unparasitized fish should not resume foraging as quickly because the benefit of the increased feeding is outweighed by the increased risk of predation.

2.3 Methods

2.3.1 Collection and maintenance of fish

From 20 to 28 June 2007, wild JPS were collected from Spring Passage SW Gilford Island, Broughton Archipelago, BC (Figure 1.2), using a beach seine (30m x 2m net, 3mm mesh). Fish were dip-netted into 20 L buckets and visually sorted into non-infected and infected groups (i.e., those with adult female *L. salmonis* visible on the skin and those without). Non-infected and infected groups were further examined as in Krkosek *et al.* (2005) and classified according to the following criteria: fish were deemed to be non-infected if they had no lice (attached or motile *L. salmonis* or *Caligus clemensi*), no history of infection (i.e., attached or motile louse scars) and no evidence of non-lice damage (e.g., predation scars). Fish were deemed infected if they had one adult female *L. salmonis,* evidence of an infection history (i.e., motile louse scars), no other lice species present, and no evidence of non-lice damage. To be classified as infected, fish had to meet all of these criteria.

Fish were taken to a floating dock (Figure 1.2; (Krkosek *et al.*, 2006)), where three non-infected and three infected groups (the number of fish, 33-41 per group, matched in opposing groups) were haphazardly selected and placed randomly in six separate identical flow-through floating tubs (1.5 m x 1.5 m x 0.5 m deep). Artificial kelp, made from floating strips of black plastic bags and fastened to the floor of the tub in the corners, provided cover. A 1 m diameter circle of white corrugated plastic was fastened to the bottom, in the centre of each tub, to provide high contrast of the fish so they would be more visible on

video. A floating feeding ring made of closed cell foam was held in the centre on the surface by fishing line. The darker edges of the tub, where fake kelp was fastened, comprised a low-risk zone whereas the open centre with the white bottom and feeding ring was designated as a high-risk zone (Figure 2.1).

Fish were fed commercial fish food (EWOS micro #0-1) in the tubs by sprinkling food in the ring on the water surface each hour of daylight (between 0500-2200). A total of ~3% body weight per day per fish was fed after the fish were put into tubs (fish went into tubs between 1600-1800) and the following day, but not on the test day except in the test itself. This feeding schedule was adopted to acclimate the wild fish to the commercial fish food and for the fish to associate the open area and ring with a foraging opportunity. A camera model was suspended approximately 1.75 m above each tub to habituate the fish to the overhead presence of the real camera used during testing. On each of 23 and 26 June, 2007, six groups (three non-infested control and three infested) of fish were tested. An additional two groups were tested on 28 June, resulting in a sample size of seven groups each of infected and non-infected fish (Table 2.1). All trials took place between 1300 -1730 and were performed in random order within each day.

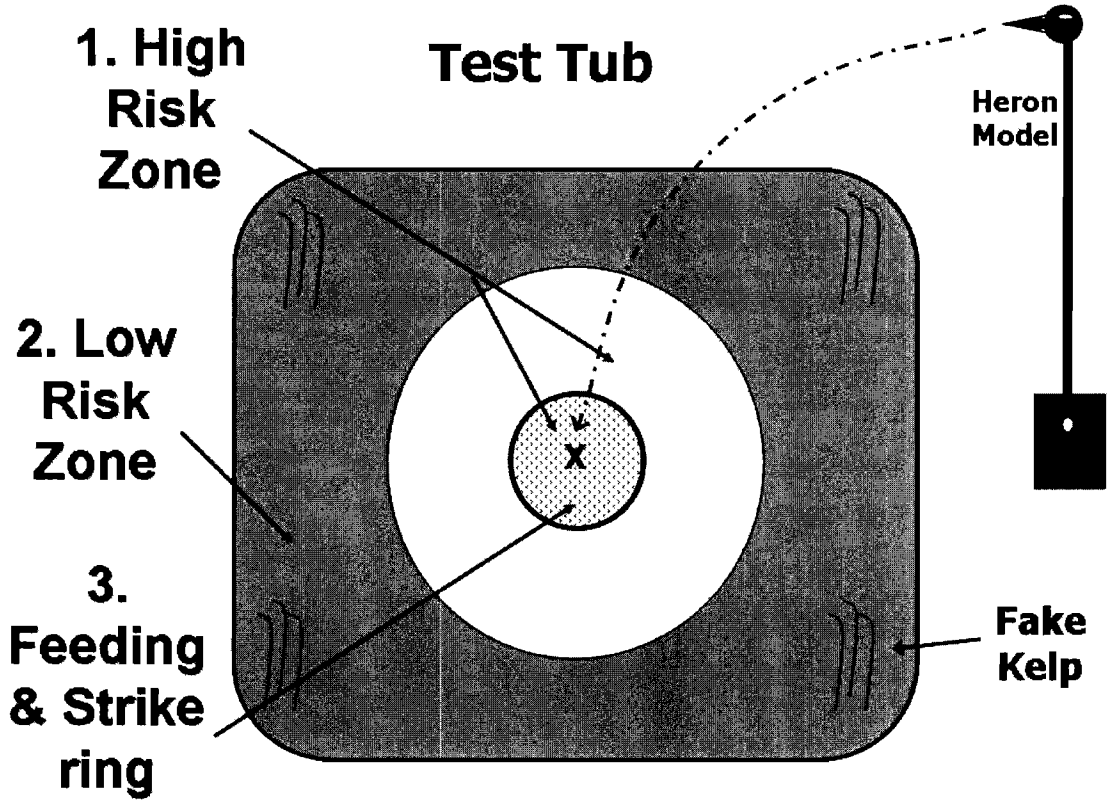

Figure 2.1 Schematic drawing of test arena.
Strike was initiated when >50% of the fish were in the central hatched area.
View is from above as recorded by camera.

Table 2.1 **Trial data for juvenile pink salmon (JPS).**
Number of fish per group, return times, group fish sizes (mean ± SD), lice prevalence (percentage of JPS with lice) and motile scars (mean ± SD) at the end of the trials. Trial 1 was performed on June 23; Trial 2, June 26; Trial 3, June 28. Return times with + denotes censored data (true value is unknown but greater than shown).

Trial	Lice	Group size	Return Time (s)	Length (mm)	Adult Female (%)	Adult Male (%)	Pre-Adult (%)	Adult Caligus (%)	Motile Scars
1	Yes	40	70	74.8±6.4	100	32.5	0	5.0	8.3±4.5
1	Yes	41	210	73.6±5.7	100	12.2	2.4	7.3	9.6±5.8
2	Yes	33	65	77.8±5.3	60.6	9.01	12.2	0	14.4±14.2
2	Yes	35	150	77.0±5.6	45.7	28.6	0	2.9	9.9±8.2
2	Yes	35	65	77.2±5.5	74.3	14.3	2.9	2.9	13.0±11.0
3	Yes	34	115	75.7±6.5	76.5	29.4	2.9	8.8	9.7±7.4
1	Yes	39	660+	73.0±4.8	74.4	7.7	2.6	0	10.4±7.1
1	No	37	90	72.3±3.8					
1	No	40	130	73.1±4.8					
2	No	39	615	73.7±4.9					
2	No	39	155	75.0±4.2					
1	No	38	295+	73.3±3.7					
2	No	33	805+	75.2±5.9					
3	No	36	315+	76.7±7.8					

2.3.2 Test procedure

Prior to each trial, the camera model above the test tub was replaced with a Sony™ Hi8 Handicam to record the trial. At the same time, a wooden model heron head and beak attached to the top of a pivoting pole was placed at the edge of the dock so it would strike the water inside the floating ring upon release (Figure 2.1). The fish were acclimated to the placement of these items and the presence of the observers for 15 min before food was sprinkled in the floating ring. Video recording commenced when the food was added and continued for the 15 min trial duration. The model heron "attacked" (i.e., the head was released so that the beak hit the water) when a majority of the fish (≥ 60% by visual estimation) was feeding at the surface, inside the ring. The model heron rebounded but remained above the water to represent a continued perceived threat until the end of the trial. Each group was tested only once, following which the forklength of each fish was measured, motile scars counted and lice recounted (Table 2.1) as in Krkosek *et al.* (2005). The fish were then released seawards from their point of capture.

2.3.3 Analysis

The videos were converted to digital format by Dazzle^{TM}150 interface, Pinnacle Studio 8^{TM} software, burned to CD and played back using LG Cyberlink™ PowerDVD 6 on a PC connected to a flatscreen monitor. The videos were used to create a "presence" timeline by counting the proportion of fish in the low- and high-risk areas of the tub every 5 seconds for the duration of each test. The trial began (t = 0) when the food was placed in the centre of the tub and the

time for 50% of the fish present in the high risk area at the time of the simulated heron attack to return to the risky area was obtained from this timeline (these were not necessarily the same individuals returning). However, due to variation in the time at which different groups began feeding, the attack did not always occur at the same point in the timeline. This resulted in some trials having less time for fish to return following the "attack", and thus in any censoring occurring at different times after "attack", although always 15 min. after food addition.

Time to event (survival) analysis was used to quantify the effect of lice infection on the time to return (to the high-risk area) of JPS because the dataset included censored data (5 of 14 groups did not return to the high-risk area before the trial ended). Kaplan-Meier (KM) estimates of the probability of not returning were obtained using observed return times. A parametric survival model with lice infection as a fixed factor were fitted to the KM survival estimates by log-likelihood using exponential, Weibull and lognormal error distributions. Akaike Information Criterion corrected for small sample sizes (AIC_c) was used to select the distribution which best fit the data (Burnham and Anderson, 2002). AIC_c model weights (*w;* the probability that the given model is truly "best" among the candidate models) were calculated. There were no differences between infected and non-infected group sizes (non-infected mean number of JPS (± SD) 37.29 ± 2.28 JPS, infected 36.71 ± 3.20 JPS; $t\,(12) = 0.38$, $p > 0.05$) or mean (± SD) fork length (non-infected 74.18 ± 1.52 mm, infected 75.6 ± 1.83 mm; $t\,(12) = -1.57$, $p > 0.05$), so these were not included as covariates in the analysis. T-tests were performed in SPSS release 16.0 (SPSS Inc.) and survival analysis performed

using R version 2.6.1 (R Development Core Team 2007). Analysis was also conducted using the alternative time of return criteria of (a) 50% of the entire group, and (b) a fixed number of 14 fish returning, both of which gave results similar to those described below.

2.4 Results

Groups of JPS infected with sea lice returned to the risky area more quickly than non-infected fish (Figure 2.2). The lognormal and exponential distributions were nearly equally likely to provide the best fit to the data ($w = 0.46$ and 0.45, respectively; Table 2.2). However, only the model with the lognormal error distribution had a significant fit to the data when compared to the null model (likelihood ratio $X^2_{(1,11)} = 3.95$, $p < 0.05$). This model indicated a significant effect of lice (Table 2.3). The predicted median times (based on the lognormal model) for 50% return to the risky area to feed for infected and non-infected JPS were 146.70 and 460.28 seconds, respectively (Figure 2.2). Fish in these trials lost adult female lice and gained adult males and *C. clemensi* adults (presumably from the surrounding water) between the time they were put into groups and the end of the trial; however, this is not likely to influence the outcome because all infected fish had lice-induced damage when tested (i.e. motile lice scars; Table 2.1).

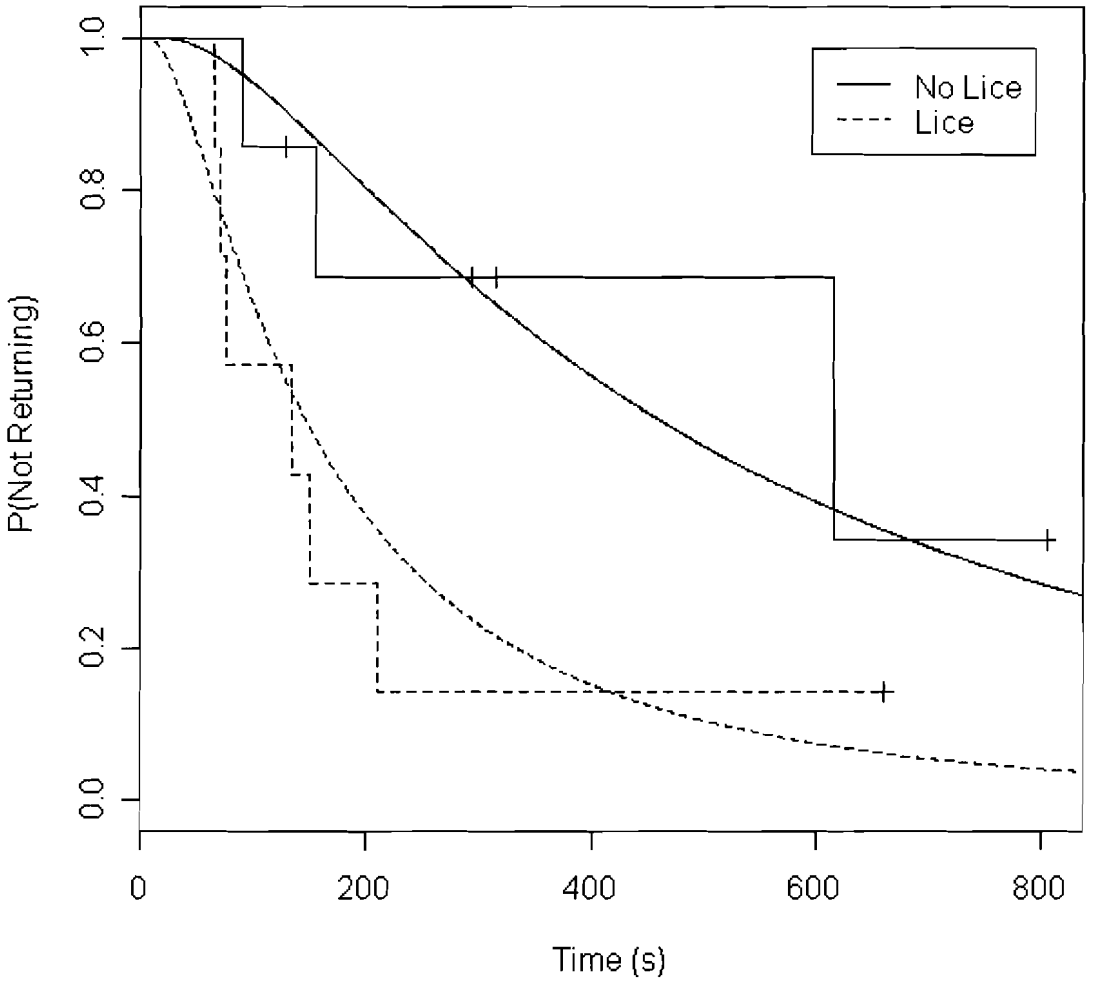

Figure 2.2 Time until juvenile pink salmon returned to a high-risk foraging area after a simulated heron attack.
Data are represented as a Kaplan Meier step plot overlaid by predicted return times based on a best-fit survival regression model with a lognormal error distribution (Table 2.3). The y-axis is the proportion of groups in which 50% have not returned by time (x). Tic marks denote censored data points which are minimum return times because 15 min trials ended before fish had returned to the high-risk area (see Table 2.1).

Table 2.2 **Summary of models for time to return data.**
Model = log (T) = β0 + β1 * Lice infection + σ*e, where T is return time, σ is a scale parameter and e is an error term based on an Exponential, Lognormal, or Weibull error distribution. For each model: N = 14, K = number of model parameters, -2LL = -2*maximum log-likelihood estimate, AICc = Akaike's Information Criterion corrected for small sample size, Δ = the change in AICc between the model and the model with the lowest AICc (highlighted in bold), and w = Akaike weight (the likelihood of superiority over other models in the set).

	Distribution	K	-2LL	AICc	Δ	W
1	**Lognormal**	**3**	**119.65**	**128.05**	**0.00**	**0.46**
2	Exponential	2	122.98	128.07	0.02	0.45
3	Weibull	3	122.96	131.60	3.31	0.09

Table 2.3 **Parameter estimates of time to return models.**
Parameter estimates from the survival regression model (shown in Table 2.2 with the lognormal error distribution) of time to return to a risky area after a simulated predator attack. Lice infection in the model is 0 or 1 based on absence or presence of sea lice.

	Estimate	Standard error	*P* value
Intercept	6.14	0.461	< 0.0001
Lice infection	-1.19	0.591	< 0.05
Scale	-0.008	0.248	> 0.05

2.5 Discussion

Fish infected with one adult female sea louse returned to a high-risk foraging area more than 5 min sooner than non-infected JPS after a simulated predator attack. Fish returning after the attack appeared to be searching for or consuming remaining food. These data support the hypothesis that sea lice infection causes JPS to accept greater predation risk to obtain a feeding opportunity, likely because they need to offset the costs of sea lice infection to prevent a loss of condition and growth. However, it should be understood that these results are correlational. The direction of causation was not tested here, thus it is also possible that risk taking JPS may be more likely to become infected by sea lice.

The costs associated with sea lice infection of JPS may be considerable. Webster *et al.* (2007) found that sea lice infected-JPS will leave a food-rich patch in saltwater for an area of lowered salinity where osmotic costs are lower, suggesting that osmotic costs increase with infection. The immune response of JPS when infected by sea lice found by Jones *et al.* (2007) would further increase costs for JPS. Other studies have shown increased physiological costs associated with sea lice infections on larger Atlantic salmon (*Salmo salar*): osmoregulation failure (Grimnes and Jakobsen, 1996), increased immune response (Fast *et al.*, 2006), reduced swimming performance, and blood loss capable of causing anemia (Wagner and McKinley, 2004). Dawson *et al.* (1999), showed compromised osmoregulatory function and skin damage, in *S. salar* smolts associated with infections of pre-adult sea lice. As sea lice feed on blood

and skin (Tully and Nolan, 2002) salmon need not only to recover and repair these tissues, but also to fight off bacterial and viral infections (Johnson *et al.*, 1996), further increasing energetic costs.

If JPS alter their foraging behaviours and are able to offset sea lice-induced costs, the true effects of lice infections to JPS health and condition observed in the wild and in laboratory studies may be underestimated. Studies thus far have reported no reduction in condition (i.e., Fulton's condition factor) due to sea lice infection (except for near-moribund infected JPS; Morton and Routledge, 2006). The results presented here suggest JPS may be attempting to offset the costs of low infection levels by altering their foraging behaviour. However, in doing so, they are likely increasing their exposure to predators, a key component of predation risk, which may play a role in the increased predation mortality observed by Krkosek and Connors (pers. comm.). Future field studies are needed to determine the true costs associated with various infection levels, durations of infection and if JPS can actually offset them by increased foraging effort.

Increased predation risk-prone behaviours induced by parasites as seen here are similar to studies where prey are under stress and need to increase food intake (Barber *et al.*, 2000). For example, increased energetic demands caused by rapid growth of trout (*O. mykiss*) injected with growth hormone, induced increased foraging in the presence of a predator as compared to non-injected fish (Jonsson *et al.*, 1996). Food deprivation in coho (*O. kisutch*) smolts (Damsgard and Dill, 1998) and juvenile pink and chum salmon (Magnhagen,

1988) caused fish to increase their foraging in dangerous patches (predator present) relative to satiated fish. These observations are not limited to salmonid fishes. Grorud-Colvert & Sponraugle (2006) experimentally created high- and low-condition bluehead wrasse (*Thalassoma bifasciatum*) juveniles and found that low-condition larvae sought cover less and ate more after a simulated predator attack. Numerous studies show increased foraging behaviours of parasitized threespined sticklebacks (*Gasterosteus aculeatus*) resulting in increased predation risk for the hosts (Milinski and Heller, 1978; Giles, 1983, , 1987; Godin and Sproul, 1988). In contrast to these studies, Dawson *et al.* (1999) and Webster *et al.* (2007), showed reduced food intake by *S. salar* post smolts and JPS fry, respectively, when infected with pre-adult stage lice although food consumption returned to pre-infection levels once lice grew to the adult stage in the former study. However, this reduction of feeding may be a sign of increased stress or sickness; Morton and Routledge (2005) showed infected JPS stopped feeding at the onset of being "loners" or moribund. This behaviour was not observed in any of my groups, so it is unlikely that fish used in the current study had reached this point.

Behavioural changes, as shown here, that increase predation susceptibility are not commonly seen in the hosts of parasites with simple lifecycles (Vaughan and Coble, 1975; Smith Trail, 1980; Barber *et al.*, 2000) and are likely to only be seen in novel interactions between sea lice and juvenile hosts. Many of the studies showing that parasites increase a host's predation risk by altering host behaviour (Giles, 1983; Milinski, 1985; Barber *et al.*, 2004)

involve parasites with a complex lifecycle which requires predation by its definitive host to complete its lifecycle (Milinski, 1990). In the sea lice – juvenile pink salmon system, sea lice do not rely on predation to complete their life cycle. Therefore, the observed increase in predation risk is more likely a side-effect of infection costs to the fish. There is evidence of motile sea lice transferring to salmonid predators during handling of infected JPS (Connors *et al.*, 2008), but transfer is likely a result of motile lice's ability to switch hosts when in danger and not an evolved strategy. The main difference between these two systems is that sea lice must transfer to the predator to avoid death not to reproduce.

This study provides evidence that costs associated with sea lice infection potentially increase the risk of predation of JPS due to increased exposure to predators. Juvenile pink salmon infected with sea lice emerged from cover to feed more rapidly than did uninfected fish, a behavioural mechanism that may contribute to the documented high mortality of lice-infested fish. However, a prey's ability to escape a predator attack also influences its predation risk. Determining if sea lice affect JPS escape swimming performance is therefore also important to understand why infected JPS are preyed upon more than non-infected ones. This is the subject of the next two chapters.

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CHAPTER 3:

EFFECTS OF SEA LICE (*LEPEOPHTHEIRUS SALMONIS*) ON THE ESCAPE RESPONSE OF JUVENILE PINK SALMON (*ONCORHYNCHUS GORBUSCHA*) TO A SIMULATED AVIAN ATTACK

3.1 Abstract

A prey's ability to escape predation is paramount to its survival. A factor such as parasitism can affect survival if it reduces the likelihood of escape. In the Broughton Archipelago of British Columbia, juvenile pink salmon (JPS) (*Oncorhynchus gorbuscha)* infected with ectoparasitic sea lice (*Lepeophtheirus salmonis*) are more likely to be preyed upon than non-infected conspecifics. This study investigates four escape performance measures of wild-caught, naturally infected JPS as possible mechanisms of increased predation risk. After a simulated heron attack, the probability of JPS reaction, reaction time, swimming velocity and acceleration were measured. To test the prediction that *L. salmonis* negatively affects one or more of these responses, predictive models were created and then compared using Akaike information criterion. *L. salmonis* had no effect on escape performance; however, *Caligus clemensi*, a generalist parasite also found on JPS, negatively affected escape acceleration. It is likely that *L. salmonis* infection levels were not sufficient to impair the escape parameters measured here, although it is expected that escape ability should be highly conserved under virtually all but the highest intensities of infestation, as it is critical to JPS early marine survival.

3.2 Introduction

A prey's risk of predation is the product of the probabilities that a prey encounters a predator, is attacked by that predator, and given attack, is able to escape that predator (Lima and Dill, 1990). Predator abundance, the "inherent risk" of the environment (e.g. light level and cover), the prey's physiological and morphological constraints, and its behavioural decisions all influence the risk of mortality through their effects on one or more of these probabilities. Therefore a prey's ability to detect, avoid, and escape a predator is essential to its survival. Reduced physiological condition (i.e., general health or energy stores) has been shown to decrease the probability of escape by prey (Murray, 2002). For example, predators often attack the weakest or slowest in a group (Fitzgibbon and Fanshawe, 1989). This increase in predation risk could be caused by a reduction in escape ability, which may take the form of slow reaction to an attack, failure to react, or a reduced ability to flee from the predator (Mesa *et al.*, 1994). Factors that can reduce prey condition include injury, starvation, disease, and parasitism. A reduction in condition from any of these causes can therefore increase predation risk if it reduces escape ability.

A parasite that reduces the host's energy stores may directly impact the host's ability to react to and escape from a predator (Murray, 2002; Alzaga *et al.*, 2008). Locomotor function may be negatively affected if parasite infection results in less energy being available for high-cost movements such as the predator escape swimming modes used by fish. These swimming modes are powered by fast-twitch white muscles which depend on anaerobic energy sources (Webb,

1994). These sources are quickly depleted, thus limiting the duration of high-speed swimming, especially in smaller fish; however, they are usually sufficient for prey to reach cover (McDonald *et al.*, 1998; Kieffer, 2000). Atlantic cod (*Gadus morhua*) in poor condition caused by starvation had reduced sprint and endurance swimming abilities due to a reduction of anaerobic metabolite fuel capacity (Martinez *et al.*, 2004). If host condition is reduced due to parasitic infection in a similar manner as from starvation, then heavy parasite burdens on small fish may decrease fast-start swimming abilities (see Blake *et al.* 2006).

Unusually high numbers of ectoparasitic sea lice, *Lepeophtheirus salmonis,* have been reported on out-migrating juvenile pink salmon (JPS) (*Oncorhynchus gorbuscha)* in areas near fish farms in the Broughton Archipelago, British Columbia, Canada (Morton and Williams, 2004; Krkosek *et al.*, 2005). *Lepeophtheirus salmonis* is a caligid copepod with a direct lifestyle that is specific to marine salmonids. These infestations can cause up to 95% mortality in migrating juvenile pink and chum salmon (*O. keta)* (Morton *et al.*, 2004, 2005; Krkosek *et al.*, 2006). Krkosek and Connors (pers. comm.) found that *L. salmonis*-infected JPS were more susceptible to salmonid predators than non-infected conspecifics. However, the mechanisms by which lice may influence vulnerability are unknown.

Lepeophtheirus salmonis has a range of deleterious effects on their salmonid hosts. They feed on the skin, mucus, and blood of their hosts and have been known to cause severe skin lesions (Pike and Wadsworth, 2000). They can impair host osmoregulation ability and cause immunosupression (Tully and

Nolan, 2002). Grimnes and Jakobsen (1996) experimentally infected Atlantic salmon (*Salmo salar*) post-smolts with lice, which caused severe physiological damage and death to their host once lice reached adulthood. The damage caused by *L. salmonis* increases stress hormone levels which have widespread effects on the physiology of the fish (Tully and Nolan, 2002). Adult Atlantic salmon were found to have reduced swimming abilities due to a decreased cardiac output after exercise when infected with *L. salmonis* at levels of 0.13 lice·g^{-1} (Wagner *et al.*, 2003). Jones *et al.* (2007) found experimentally infected JPS to have an increased immune response compared to uninfected controls, and Webster *et al.* (2007) found that infected JPS prefer fresh-water and incur higher energetic costs when in saltwater, which suggested osmotic challenges associated with *L. salmonis*. Infections levels that are relatively benign on adults and larger fish could have a greater impact on small juveniles (Johnson *et al.*, 1996). These increased costs associated with *L. salmonis* infection may affect the survival of JPS by reducing the fish's condition and increasing its probability of experiencing mortality by predation.

This study system provides a unique opportunity to investigate the impact of an ectoparasite on the predation risk of a novel host (Krkosek *et al.*, 2007). The aim of this study is to determine if the costs, in terms of reduced condition, imposed by sea lice on juvenile salmon translate into a reduced ability of young salmon to react to, and escape from, a predator. I predict that as *L. salmonis* on the host increase in number and in age/size, JPS escape ability (probability of

reaction, reaction time, and escape swimming performance) will be negatively affected.

3.3 Methods

3.3.1 Collection of wild fish

Each day from May to June 2005, schools of wild juvenile pink salmon were caught using a beach seine (20m × 1.5m, 4mm mesh) at various locations throughout the Broughton Archipelago (Figure 1.2). From these schools, 30-50 fish were selected haphazardly to ensure that a range of *L. salmonis* intensities and lice stages were used in the experiment. Captured fish were removed from the seine using a "zipnet" made from a Ziploc™ baggie fastened to a net frame, with small holes cut in the bag to allow water to drain out slowly. Thus the fish were never taken out of the water, and lice were not lost due to abrasion by the net. The fish were transported by boat in 20 L buckets to the floating dock at the Salmon Coast Research Station (Gilford Island) where the experiment took place (Figure 1.2). Fish were held overnight (12-16 h) in 200 L plastic barrels with 4 mm mesh across both ends to allow water to flow through; these were suspended off the dock. Fish were not fed prior to testing, although it is possible that small amounts of JPS prey entered the barrels, but not likely in sufficient quantity to impact results.

3.3.2 Testing procedure

A 1-m diameter plexiglass octagon (Figure 3.1) was used as the arena in which test fish responded to artificial predatory attacks by a model heron. In the

centre of the octagon, a holding area for the test fish, made by a net (20 cm diameter) glued to the bottom and held up above the water by fishing line, ensured that test fish would consistently be in the same general area when "attacked". A 15 cm reference line was marked on the bottom so measurements taken from video could be calibrated. Black plastic surrounded the arena to reduce disturbance.

Individual fish were removed from their holding barrels, quickly transported in a "zipnet" to the test arena, and placed in the net barrier for a 15 min acclimation period. A Sony^{TM} Hi8 camcorder (30 frames·s^{-1}) mounted ~2 m above the arena began recording just before the observer (looking though a slit in the black plastic) dropped the net barrier. The "attack" was initiated only when the fish was positioned over the edge of the collapsed net, which ensured that the fish would not try to use the net as cover. Recording was stopped ~ 5 s after the simulated attack, and the fish captured and placed in a Whirlpak^{TM} bag. The fish were then euthanized by a sharp blow to the head, placed on ice, and frozen within an hour. Fork length (FL, nearest 1 mm), body depth (BD, 1 mm), wet weight (0.1 mg), and parasite load were later recorded. Fish were thawed and viewed under a dissecting microscope to check for lice. The lice were carefully removed and identified by species, sex, and life stage (Galbraith, 2004). Some JPS were infected with *Caligus clemensi* (a generalist parasitic caligid copepod)

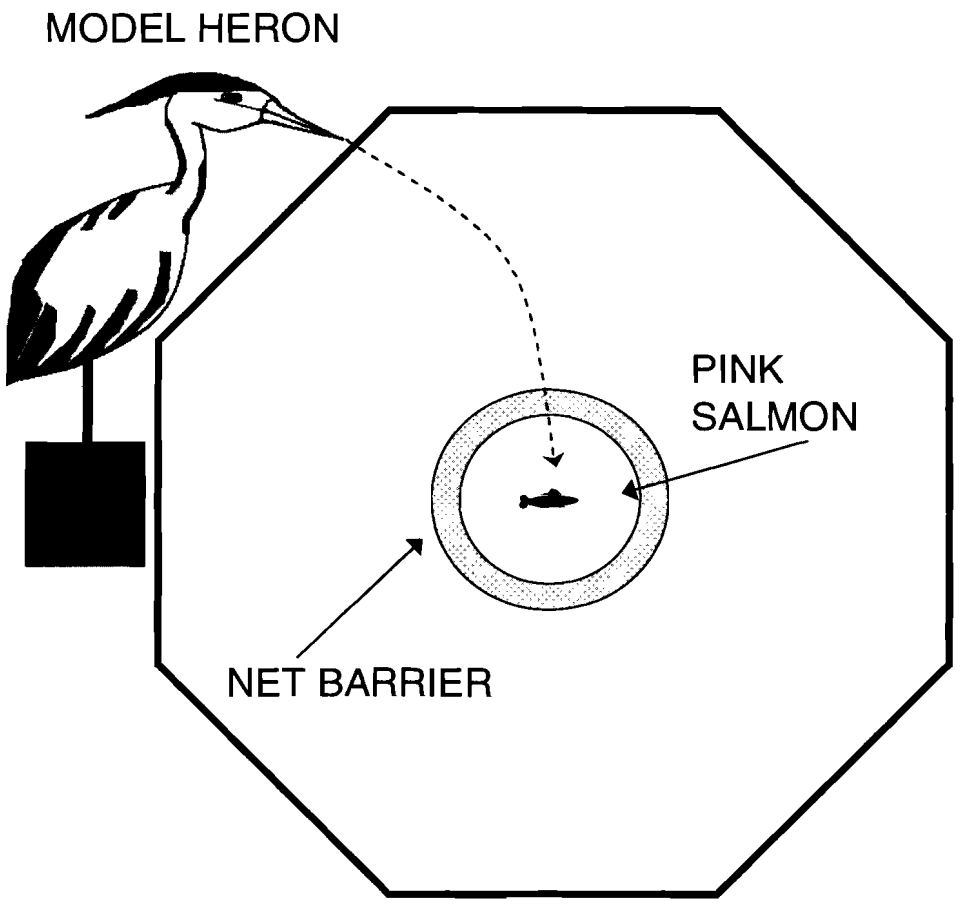

Figure 3.1 **Drawing of test arena.**
A 1-m plexiglass octagon was used as the test arena. In the centre, a holding area for the test fish made by a net (20 cm dia.) glued to the bottom, and held up above the water by fishing line ensured that test fish would consistently be in the same general area when "attacked". A hidden observer released the fishing line to drop the barrier. A plastic model heron, placed at the edge of the arena, was pivoted by the observer releasing a string, causing it to fall and strike the surface of the water in the middle of the arena and net barrier.

and were included in the analysis. Due to the differences in size between lice stages and species, the numbers of lice were scaled by lice metabolic load (LML) using an allometric scaling constant $L^{2.25}$ (calculated from length^3 = mass, and metabolic rate = mass$^{0.75}$; Peters 1983) where L is the average total length of each stage and species of louse (Table 3.1). This was an attempt to estimate lice load in metabolic units, which is most relevant to presumed effects on JPS energetic state.

3.3.3 Escape response analysis

The videotape of each trial was converted to a digital version by Dazzle^{TM}150 interface and Pinnacle Studio 8^{TM} software, and the resulting MPG files played back in Apple Quicktime Pro^{TM}. Each trial video was played back frame-by-frame to measure: (1) if there was a reaction, denoted by a C-start (Weihs, 1973), (2) the reaction time (number of frames from the heron model's first movement until the fish reacted / 30 frames·s^{-1}), and (3) the distances travelled in 5 and 20 frames. The positions of the fish's head (this was used as it was the most distinguishable point on the fish due to video quality) at each frame (starting one frame before the reaction until 20 frames after) and the reference line were marked on a transparency overlaid on the monitor screen. The transparencies were then placed on a Compaq^{TM} Tablet PC T1000 and the length of the escape path measured using Image Tool^{TM} version 3.0 (an image analysis program available free online from UTHSCSA at

Table 3.1 **Size and lice metabolic load (LML) by species and stage.**
LML is calculated as ($L^{2.25}$) where L is the total length (mm) of the louse at that stage. Lice stages are: copepodid (CP), chalimus 1 (CH1), chalimus 2 (CH2), chalimus 3 (CH3), chalimus 4 (CH4), pre-adult 1 male (PA1M) and female (PA1F), pre-adult 2 male (PA2M) and female (PA2F), adult male (AM) and female (AF). *C. clemensi* has only 1 pre-adult stage. Lengths are averages obtained from Parker and Margolis 1964, Kabata 1972, Johnson and Albright 1991, and Galbraith 2005.

		CP	CH1	CH2	CH3	CH4	PA1M	PA1F	PA2M	PA2F	AM	AF
L. salmonis	**L**	0.70	1.21	1.52	2.20	2.77	2.90	3.70	5.40	4.27	5.40	9.96
	LML	0.45	1.54	2.57	5.89	9.90	10.97	18.99	44.45	26.21	44.45	176.23
C. clemensi	**L**	0.66	0.91	1.31	1.35	2.94	4.50	4.10	na	na	4.83	4.34
	LML	0.39	0.81	1.84	1.96	11.32	29.49	23.92	na	na	34.58	27.19

http://ddsdx.uthscsa.edu/dig/itdesc.htrnl). Velocity was calculated by dividing the distance travelled by the fish in 20 frames by time (20 frames / 30 frames·s^{-1}). As a measure of initial burst performance, acceleration (not instantaneous acceleration), calculated by dividing the velocity reached in the first 5 frames by time (5 frames / 30 frames·s^{-1}), was also determined.

3.3.4 Statistical analysis

Binomial (i.e., reaction versus no reaction) logistic regression models were fit to the data using maximum-likelihood to test if *L. salmonis* and *C. clemensi* affected the likelihood of reaction of JPS to the heron. JPS that reacted to the heron were used in separate multiple linear regression analyses to determine the effects of *L. salmonis* and *C. clemensi* on reaction time, velocity and acceleration. Analysis of velocity and acceleration included only those JPS that were not swimming at the time of the reaction.

Eight to 10 candidate models were selected for each response measure to represent competing hypotheses. Models for likelihood of reaction and reaction time included water temperature to statistically control for its possible effects. Models also included the main effect terms of total LML values of *L. salmonis* and *C. clemensi,* and the interactions 'temperature * lice' and 'salinity * lice'. Analyses of velocity and acceleration used the same models but included a body size variable (and body size interactions with both louse species) to control for body size influences on swimming performance (Webb, 1976). Because FL, BD, and mass were highly correlated (Pearson's r = 0.8-0.9, $p > 0.001$) FL was used as the measure of body size. Within each model set the null model (i.e.,

response = mean response of all fish) and the model without lice terms represented no effect of lice.

Akaike information criterion for small sample sizes (AIC_c) was used to rank the candidate models based on the relative differences (ΔAIC_c) between the model with the lowest AIC_c value and all the other models within the set for that analysis. AIC_c model weights (*w*) (the probability that a particular model is truly best among the candidate models) were calculated. Because there is uncertainty about which model is actually "best", all model *w*'s were used to calculate the estimated parameter likelihoods (i.e., importance of variables) and the estimated parameter values along with their unconditional SE. Only parameters with values with confidence intervals (± 2SE) that did not encompass zero were considered to have an effect (Burnham and Anderson, 2002). Analyses were performed using SPSS version 16.0 and R version 2.6.1 (R Development Core Team, 2007).

3.4 Results

Juvenile pink salmon (N=306) with a mean (± SD) FL of 6.27 ± 0.04 cm were infected by an average of 2.02 ± 3.55 *L. salmonis* and 0.41 ± 1.18 *C. clemensi* (max 31 and 16, respectively). Mean water temperature and salinity were 11.6 ± 1.1°C and 23.5 ± 5.0 ppt. (Table 3.2).

Table 3.2 **Description of escape responses, environmental variables, JPS fork length, and sea lice loads.**
Lice prevalence is the number of JPS infected with lice divided by the total number of JPS. LML is lice metabolic load.

Prob of reaction	Temp (°C)	Salinity (ppt)	Number reacting	FL (cm)	*L. salmonis*		*C. clemensi*	
					# Lice	LML	# Lice	LML
N = 306			269					
Lice prevalence					62.4%		26.1%	
Mean	11.61	23.52		6.27	2.02	95.89	0.41	3.62
SD	1.11	4.99		0.70	3.55	151.58	1.18	10.75
Min	10.00	14.00		4.40	0.00	0.00	0.00	0.00
Max	13.50	32.40		8.10	31.00	1018.48	16.00	77.30
Total					619.00	29343.20	126.00	1107.13

Reaction time	Temp (°C)	Salinity (ppt)	Reaction time (s)	FL (cm)	*L. salmonis*		*C. clemensi*	
					# Lice	LML	# Lice	LML
N = 258								
Lice prevalence					40.0%		26.0%	
Mean	11.60	23.80	0.57	6.25	1.75	88.95	0.34	3.62
SD	1.14	4.99	0.12	0.71	3.08	144.86	0.66	10.42
Min	10.00	14.00	0.07	4.41	0.00	0.00	0.00	0.00
Max	13.50	32.40	1.27	8.10	31.00	1018.48	4.00	61.77
Total					452.00	22949.58	88.00	935.00

Velocity	Temp (°C)	Salinity (ppt)	Velocity (cm/s)	FL (cm)	*L. salmonis*		*C. clemensi*	
					# Lice	LML	# Lice	LML
N = 108								
Lice prevalence					58.3%		28.7%	
Mean	11.48	24.40	37.11	6.21	1.73	73.93	0.45	4.48
SD	1.21	5.52	15.30	0.68	2.77	117.10	0.99	12.54
Min	10.00	14.00	7.95	4.60	0.00	0.00	0.00	0.00
Max	13.50	32.40	73.02	7.70	14.00	579.94	7.00	77.30
Total					187.00	7984.80	49.00	484.03

Acceleration	Temp (°C)	Salinity (ppt)	Acceleration (cm/s^2)	FL (cm)	*L. salmonis*		*C. clemensi*	
					# Lice	LML	# Lice	LML
N = 80								
Lice prevalence					55.0%		37.5%	
Mean	11.46	25.24	355.16	6.29	1.86	71.45	0.59	5.26
SD	1.26	5.61	131.51	0.73	3.06	120.42	1.11	12.72
Min	10.00	14.00	36.72	4.60	0.00	0.00	0.00	0.00
Max	13.50	32.40	637.92	8.10	14.00	579.94	7.00	77.30
Total					149.00	5715.68	47.00	421.04

3.4.1 Reaction to attack

The heron model elicited reactions in 88% of JPS (Table 3.2). The top four models were 2-11 times as likely as models representing no lice effect (null and temperature-only models) to be the best and the uncertainty surrounding the averaged parameter estimates was low (low *w* of null model relative to the other models; Table 3.3). The highest ranking models included *L. salmonis* and *C. clemensi* and their interaction terms (Table 3.3); however, the parameter estimates based on these models suggest that only *L. salmonis* and its interactions with temperature and salinity significantly increased the probability of a reaction (Table 3.4). The lice effect models are more informative than the null model, but they fail to explain much of the variation in the likelihood of reacting to the simulated heron strike (rightmost column in Table 3.3).

3.4.2 Reaction time

The mean JPS reaction time was 0.57 ± 0.12 s (Table 3.2). The top five models were 9-33 times more informative than the null model and thus the uncertainty surrounding the averaged parameter estimates was very low (Table 3.5). Temperature had a negative but very small effect on JPS reaction time (Table 3.6). Despite their inclusion in the top models neither *L. salmonis, C. clemensi*, nor their interaction terms influenced JPS reaction time. The temperature-only model (no lice effect) was the top ranked model but was only slightly more informative than models including a lice effect ($w = 0.33$ vs. 0.25; Table 3.5). The uncertainty over which model is best supported and the fact that

Table 3.3 **Summary of models for likelihood of JPS reaction to simulated heron attack. N=306. Model = Log (reaction) = Intercept + model variables + error (σ). Model variables include the main effects of temperature (Temp), *L. salmonis, C. clemensi,* and interaction terms of *L. salmonis and C. clemensi* with Temp and salinity (Salt). For each model: K = number of model parameters (variables + intercept + error (σ)), maximum log-likelihood estimate (MLE), Akaike's Information Criterion corrected for small sample size (AICc). Δ is the change in AICc between the model and the model with the lowest AICc (highlighted in bold), *w* is the Akaike weight (the likelihood of superiority over other models in the set), and R^2_{LS} is the Homer-Lemeshow goodness of fit statistic.**

	Model	K	MLE	AICc	Δ	*w*	R^2_{LS}
1	**Temp + *L. salmonis* + *C. clemensi* + *C. clemensi* * Temp + *C. clemensi* * Salt + *L. salmonis* * Temp + *L. salmonis* * Salt**	**8**	**211.94**	**-95.90**	**0.00**	**0.46**	**0.14**
2	Temp + *L. salmonis* + *C. clemensi*	4	219.34	-93.75	2.15	0.16	0.06
3	Temp + *L. salmonis* + *L. salmonis* * Temp + * Salt	5	217.98	-93.59	2.31	0.14	0.07
4	Temp + *C. clemensi*	3	221.72	-92.50	3.40	0.08	0.02
5	Temp + *C. clemensi* + *C. clemensi* * Temp + *C. clemensi* * Salt	5	219.20	-91.88	4.02	0.06	0.06
6	Null	1	225.67	-91.17	7.74	0.04	n/a
7	Temp + *L. salmonis*	3	223.02	-90.72	5.19	0.03	0.02
8	Temp	2	225.37	-89.55	6.35	0.02	0.00

Table 3.4 **Parameter estimates for reaction likelihood models. Parameter likelihood (importance of variable), estimates and unconditional SE calculated using *w* of all models of likelihood of reaction to heron attack (values are rounded to nearest 0.01). Parameter estimates ± 2 unconditional SE not overlapping 0 are highlighted in bold.**

Parameter	Parameter Likelihood	Parameter estimates	Unconditional SE
Intercept	1.00	1.14	2.42
Temp	0.96	0.08	0.21
L. salmonis	**0.79**	**0.25**	**0.02**
C. clemensi	0.76	-0.02	0.55
***L. salmonis* * Temp**	**0.60**	**0.005**	**0.001**
***L. salmonis* * Salt**	**0.60**	**0.02**	**0.0002**
C. clemensi * Temp	0.52	0.0018	0.04
C. clemensi * Salt	0.52	-5.4 E-06	0.01

Table 3.5 **Summary of models fit (by least sum of squares) to JPS reaction times to simulated heron attack.**
N=258. See Table 3.3 caption for model terms and symbol definitions. RSS is the residual sum of squares of the models.

	Model	K	RSS	AICc	Δ	*w*	R^2
1	**Temp**	**3**	**3.339**	**-1115.54**	**0.00**	**0.33**	**0.04**
2	Temp + *L. salmonis*	4	3.319	-1114.99	0.54	0.25	0.04
3	Temp + *L. salmonis* + *L. salmonis* * Temp + *L. salmonis* * Salt	6	3.274	-1114.34	1.20	0.18	0.05
4	Temp + *C. clemensi*	4	3.338	-1113.49	2.05	0.12	0.04
5	Temp + *L. salmonis* + *C. clemensi*	5	3.319	-1112.93	2.61	0.09	0.04
6	Temp + *C. clemensi* + *C. clemensi* * Temp + *C. clemensi* * Salt	6	3.335	-1109.58	5.95	0.02	0.04
7	Temp + *L. salmonis* + *C. clemensi* + *C. clemensi* * Temp + *C. clemensi* * Salt + *L. salmonis* * Temp + *L. salmonis* * Salt	9	3.268	-1108.42	7.12	0.01	0.06
8	Null	2	3.463	-1108.16	7.38	0.01	n/a

Table 3.6 **Parameter estimates of reaction time models.**
Parameter likelihood (importance of variable), estimates and unconditional SE calculated using *w* of all models of reaction time (values are rounded to nearest 0.01). Parameter estimates ± 2 unconditional SE not overlapping 0 are highlighted in bold.

Parameter	Parameter likelihood	Parameter Estimates	Unconditional SE
Intercept	1.00	0.53	0.33
Temp	**0.99**	**-0.02**	**0.01**
L. salmonis	0.53	0.00	0.00
C. clemensi	0.23	0.00	0.00
C. clemensi * Temp	0.03	-2.53E-06	1.64E-05
C. clemensi * Salt	0.03	-2.42E-06	4.97E-06
L. salmonis * Temp	0.19	-1.87E-05	1.97E-05
L. salmonis * Salt	0.19	1.29E-06	2.33E-06

the models explain very little of the variation suggest that they do not provide a reliable prediction of reaction time.

3.4.3 Velocity

Mean escape velocity attained by JPS was 37.11 ± 15.30 cm/s (Table 3.2). The top five models were 3-10 times better supported than the null model; however, the top two models represented both a lice effect and no lice effect and were virtually equally supported (models 1 and 2 respectively; $w = 0.31$ vs. 0.23, Table 3.7). Given the lack of clear support for a single model, the fact the models explain little of the variation, and that the parameter values ± 2 SE span zero (Table 3.8), there is no evidence that *L. salmonis* reduces velocity of the escape response.

3.4.4 Acceleration

JPS had a mean acceleration 355.16 ± 131.51 cm/s^2 in the first 5 frames of the escape response (Table 3.2). The top model, which included *C. clemensi* and its interaction terms, was 8 times as likely as the next ranked model (including all terms) and 770 times more likely than the null model to be the superior model and the uncertainty surrounding the averaged parameter estimates was low (Table 3.9). This model is more informative than the null model, and suggests that *C. clemensi* negatively affects acceleration (Table 3.10), although the interaction between *C. clemensi* and FL was positive. The top model explained 28% of JPS acceleration variance (Table 3.9) providing some evidence that infestation by *C. clemensi* reduces acceleration, but there is

Table 3.7 Summary of models fit (by least sum of squares) to JPS escape velocity data. N=108. See Table 3.3 caption for model terms (note addition of fork length) and symbol definitions. RSS is the residual sum of squares of the models.

	Model	K	RSS	AICc	Δ	*W*	R^2
1	**Temp + Fork length + *C. clemensi* + *C. clemensi* * Temp + *C. clemensi* * Salt + *C. clemensi* * Fork length**	**8**	**21241.76**	**587.87**	**0.00**	**0.31**	**0.15**
2	Temp + Fork length	4	23228.71	588.46	0.59	0.23	0.07
3	Temp + Fork length + *L. salmonis*	5	22808.14	588.68	0.82	0.20	0.09
4	Temp + Fork length + *C. clemensi*	5	23053.87	589.84	1.98	0.11	0.08
5	Temp + Fork length + *L. salmonis* + *C. clemensi*	6	22641.69	590.14	2.27	0.10	0.10
6	Null	2	25052.96	592.35	4.48	0.03	n/a
7	Temp + Fork length + *L. salmonis* + *C. clemensi* + *C. clemensi* * Temp + *C. clemensi* * Salt + *L. salmonis* * Temp + *L. salmonis* * Salt + *C. clemensi* * Fork length + *L. salmonis* * Fork length	12	20430.79	593.49	5.63	0.02	0.18
8	Temp + Fork length + *L. salmonis* + *L. salmonis* * Temp + *L. salmonis* * Salt + *L. salmonis* * Fork length	8	22415.80	593.68	5.81	0.00	0.11

Table 3.8 Parameter estimates of escape velocity models.
Parameter likelihood (importance of variable), estimates and unconditional SE calculated using *w* of all models of escape velocity (values are rounded to nearest 0.01).

Parameter	Parameter likelihood	Parameter Estimates	Unconditional SE
Intercept	1.00	2.49	13.78
Temp	0.97	1.90	2.39
Fork length	0.97	-0.66	2.16
C. clemensi	0.54	-1.60	1.65
C. clemensi * Temp	0.32	0.01	0.04
C. clemensi * Salt	0.32	0.03	0.02
C. clemensi * Fork length	0.32	0.12	0.11
L. salmonis	0.32	0.01	0.01
L. salmonis * Temp	0.02	4.76E-05	7.43E-05
L. salmonis * Salt	0.02	6.51E-06	4.68E-05
L. salmonis * Fork length	0.02	-3.21E-04	6.15E-04

Table 3.9 Summary of models fit (by least sum of squares) to JPS escape acceleration data.
N=80. See Table 3.3 caption for model terms (note addition of fork length, FL) and symbol definitions. RSS is the residual sum of squares of the models.

	Model	N	K	RSS	AICc	Δ	*W*	R^2
1	**Temp + Fork length+ *C. clemensi* + *C. clemensi* * Temp + *C. clemensi* * Salt + *C. clemensi* * Fork length**	**80**	**8**	**977581**	**770.89**	**0.00**	**0.77**	**0.28**
2	Temp + Fork length + *L. salmonis* + *C. clemensi* + *C. clemensi* * Temp + *C. clemensi* * Salt + *L. salmonis* * Temp + *L. salmonis* * Salt + *C. clemensi* * Fork length + *L. salmonis* * Fork length	80	12	904194	775.28	4.39	0.09	0.34
3	Temp + Fork length + *C. clemensi*	80	5	1135515	775.66	4.76	0.07	0.17
4	Temp + Fork length	80	4	1193585	777.37	6.48	0.03	0.13
5	Temp + Fork length + *L. salmonis* + *C. clemensi*	80	6	1130286	777.63	6.73	0.03	0.17
6	Temp + Fork length + *L. salmonis*	80	5	1189476	779.37	8.48	0.01	0.13
7	Temp + Fork length + *L. salmonis* + *L. salmonis* * Temp + *L. salmonis* * Salt + *L. salmonis* * Fork length	80	8	1130589	782.53	11.63	0.00	0.17
8	Null	80	2	1366297	783.80	12.91	0.00	n/a

Table 3.10 Parameter estimates of acceleration models.
Parameter likelihood (importance of variable), estimates and unconditional SE calculated using *w* of all models of acceleration (values are rounded to nearest 0.01). Parameter estimates ± 2 unconditional SE not overlapping 0 are highlighted in bold.

Parameter	Parameter likelihood	Parameter Estimates	Unconditional SE
Intercept	1.00	5.55	183.72
Temp	1.00	17.96	11.78
Fork length	1.00	24.39	21.58
C. clemensi	**0.96**	**-49.92**	**20.43**
C. clemensi * Temp	0.86	0.92	0.98
C. clemensi * Salt	0.86	0.42	0.22
***C. clemensi* * Fork length**	**0.86**	**4.17**	**1.80**
L. salmonis	0.13	-0.37	0.43
L. salmonis * Temp	0.09	0.01	0.02
L. salmonis * Salt	0.09	0.003	0.003
L. salmonis * Fork length	0.09	0.03	0.04

no support for the hypothesis that *L. salmonis* reduces escape acceleration of JPS.

3.5 Discussion

Lepeophtheirus salmonis, at the levels tested in this study, did not reduce the likelihood of a reaction, increase the reaction time, or reduce the escape velocity and acceleration of juvenile pink salmon in response to a simulated heron attack. However, *C. clemensi* had a negative effect on JPS acceleration.

Reflex responses (such as the ones measured in this study) are crucial to surviving a predation attack (Howland, 1974; Walker *et al.*, 2005), and thus should be highly conserved and resistant to fluctuating health or condition. A reduced escape response may only occur when fish condition is highly compromised. However, infected JPS do not exhibit a reduced condition (measured as Fulton's condition factor; Jones and Nemec, 2004) until they become "loners" ~1-2 d prior to death (Morton and Routledge, 2006). "Loners" are infected JPS that are moribund and unresponsive to overhead stimuli (Morton and Routledge, 2005). The fish tested in this study had not reached this debilitated state and thus did not experience sufficient reduction in condition to affect their fast-start escape abilities.

From a parasite's perspective, whether or not it should cause its host to be eaten or die will depend on whether host mortality will increase parasite fitness. The majority of studies on parasites affecting host predation mortality have

focused on parasites with complex multi-host life cycles where parasite fitness depends on transmission (Barber *et al.*, 2000). In such species, the effects of infection on fish escape behaviour depend on whether the fish host is an intermediate or definitive host. Thus, Blake *et al.* (2006) found that infections by the trematode *Bunodera* spp. did not affect fast-start (stages one and two) performance of its definitive host, the three-spined stickleback (*Gasterosteus aculeatus*). By contrast, host performance was reduced by the tapeworm *Schistocephalus solidus*, which uses the stickleback as its intermediate host. *Lepeophtheirus salmonis* and *C. clemensi* have direct life-cycles and their fitness does not rely on transmission to another host. If a predator kills their host before they can reproduce or move to another host, their fitness would be directly, negatively affected. Consequently, their effects on hosts may be expected to be similar to those of parasites with complex life cycles infecting their definitive host, i.e., relatively little effect on the host's predator escape ability. Any reduction in louse fitness as a result of increased host predation could be an indirect effect of unnaturally high infection levels on JPS.

The effect of *C. clemensi* I found is surprising given that this ectoparasite species is smaller and less physically damaging than *L. salmonis* and relatively few were found on JPS in this study (Figure 3.2 and Table 3.2). Several of the slowest fish were the most heavily infected with motile *C. clemensi.* Since this species readily transfers between hosts when in close proximity (Connors, pers. comm.), and we do not know the infection history of the wild-caught test fish, I

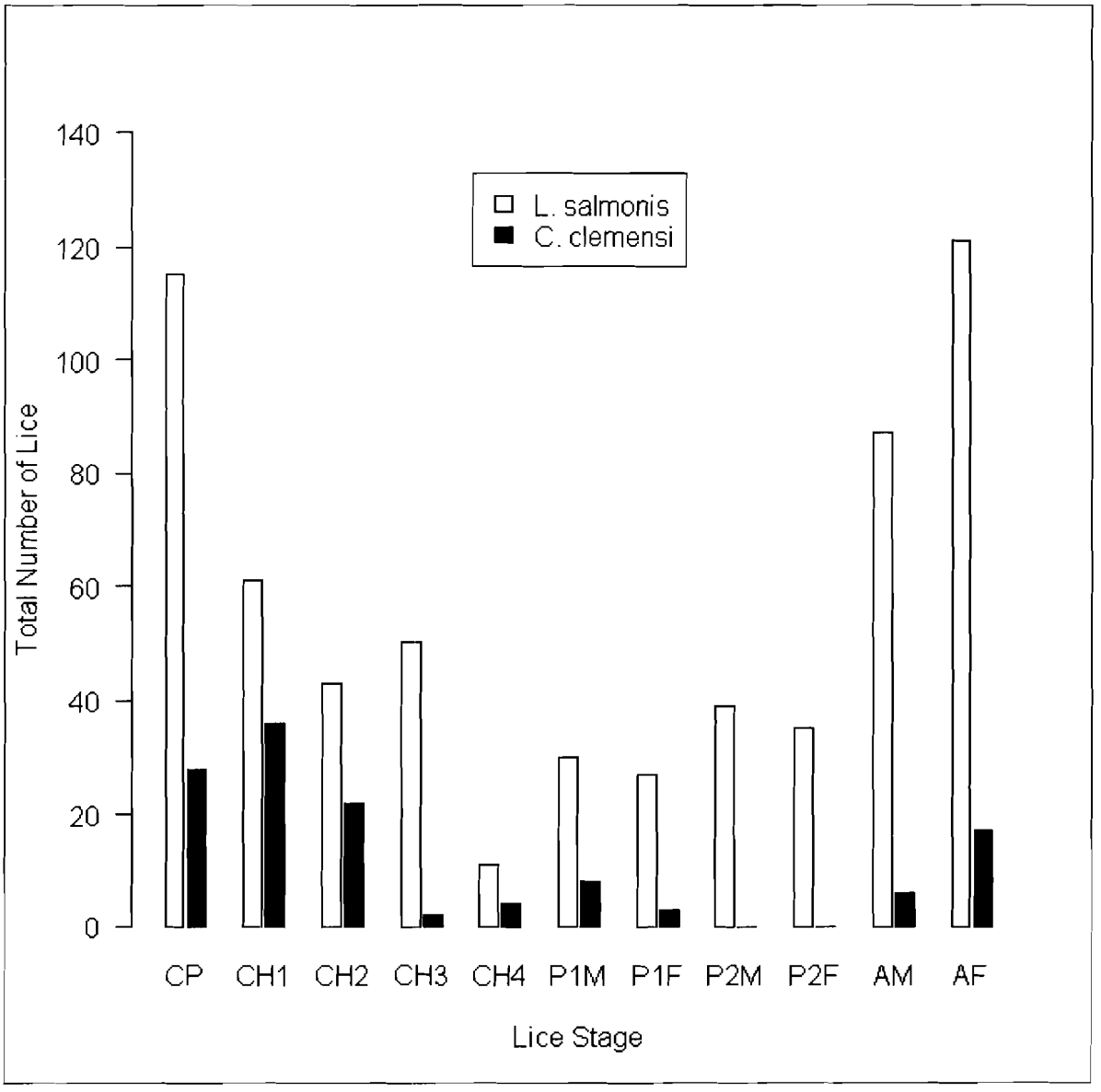

Figure 3.2 **Total number of *L. salmonis* and *C. clemensi* stages infecting 215 of 306 JPS used in this study.**
Lice stages are: copepodid (CP), chalimus 1 (CH1), chalimus 2 (CH2), chalimus 3 (CH3), chalimus 4 (CH4), pre-adult 1 male (P1M) and female (P1F), pre-adult 2 male (P2M) and female (P2F), adult male (AM) and female (AF). *C. clemensi* has only 1 pre-adult stage.

cannot discern if motile *C. clemensi* originated on, or jumped to the poorer performing fish.

It is possible that I could not detect an effect of *L. salmonis,* even if it were there, due to limitations of my study. This study captured a "snapshot" of the current infection state on naturally infected JPS without knowledge about the history of the fish or its infection (i.e., duration and intensity). JPS have been shown to lose lice (Jones *et al.* 2007; Krkosek, pers. comm.), or gain motile stages from nearby fish. Therefore, the observed lice load may not correlate with current fish condition. Without experimentally infecting JPS or tracking individual fish and their lice over time, we cannot be certain of the infection history of the host.

My ability to detect a lice effect was reduced because of the large amount of variation observed in JPS behaviour. This may be due in part to differences in JPS position, or angle of orientation to the heron model prior to the attack introducing variation in the likelihood of reaction and reaction times. Velocity and acceleration varied as much among non-infected as among infected JPS (Figures 3.3 and 3.4) which may be partly explained by the fact that these measurements were derived from all three stages of the fast start, compared to other studies which used only stages one and two. These first two stages are essentially a reflex response but the fish has control of its swimming mode in the third stage (ranging from braking to continued swimming, depending on motivation; Weihs, 1973). Inclusion of this final stage likely introduced variation

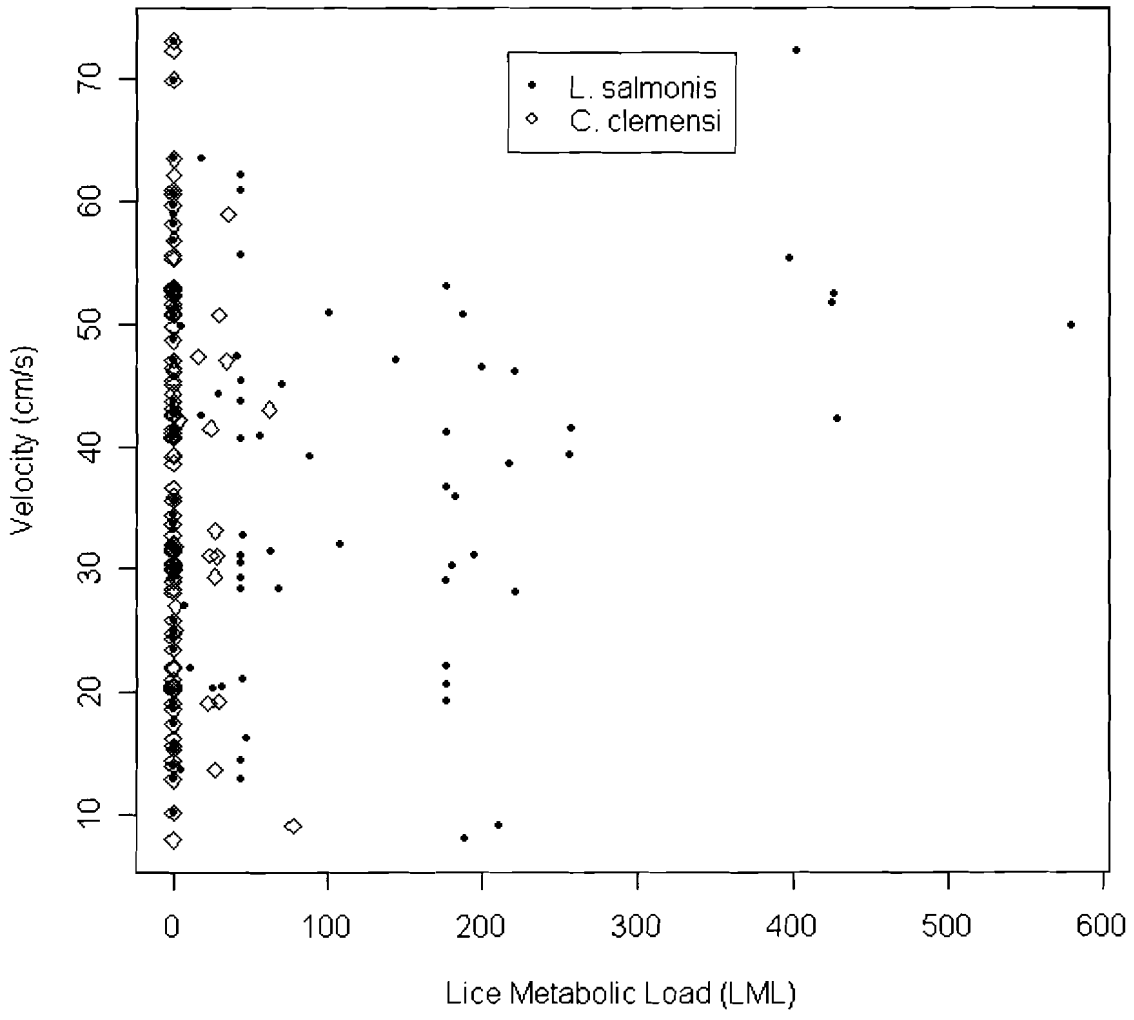

Figure 3.3 Plot of JPS escape velocity vs. Lice Metabolic Load (LML).

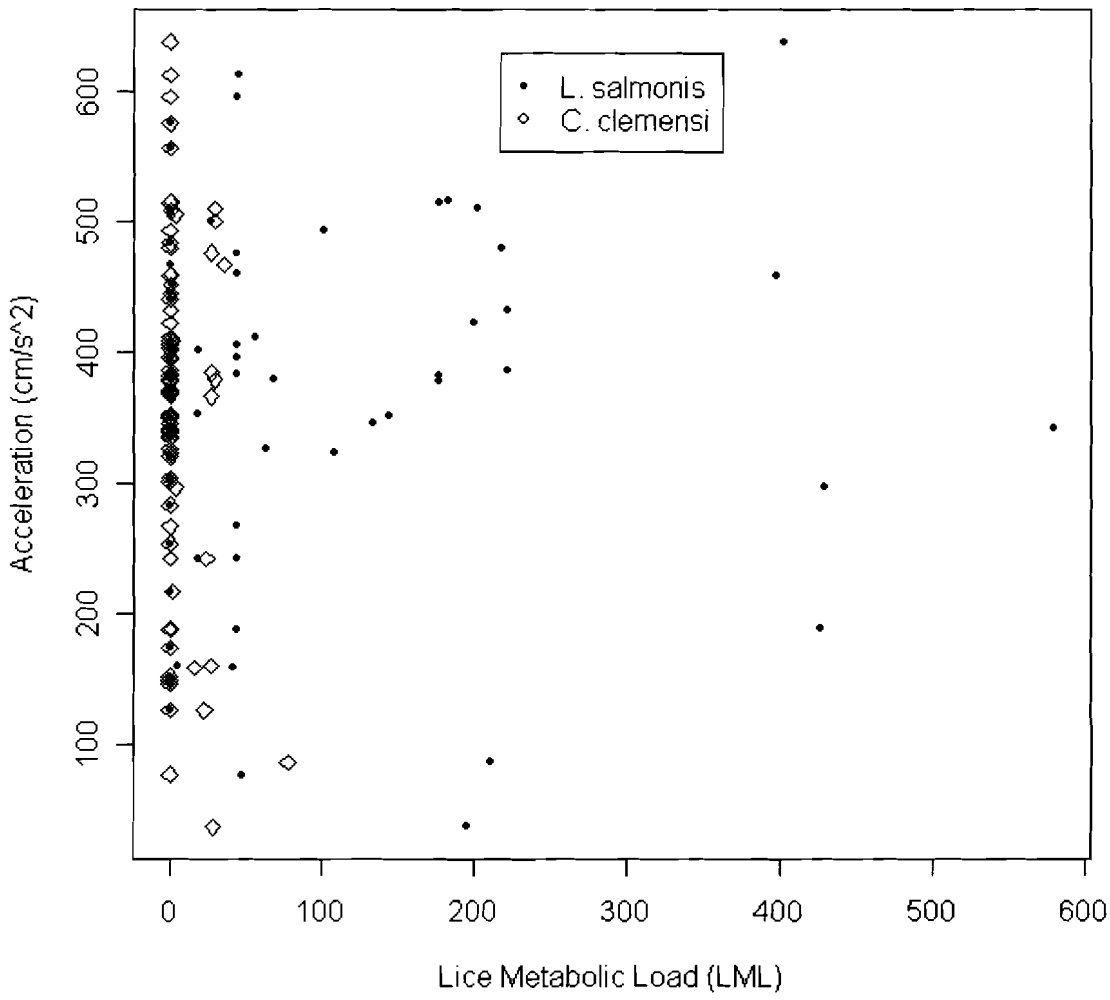

Figure 3.4 Plot of JPS escape acceleration vs. Lice Metabolic Load (LML).

due to individual differences in fish swimming behaviour, which could not be measured or controlled. However, my index of acceleration captured less of stage three than my measure of velocity, which may be why models explained more variance in the former than in the latter response (Tables 3.9 and 3.7, respectively).

Lice-infected JPS are more susceptible to salmonid predators (Krkosek and Connors, pers. comm.). The data from my study suggest that since *L. salmonis* does not affect the escape response or fast start abilities, infected JPS are equally likely to escape an attack as non-infected fish. However, these results should be viewed with caution, as they do not necessarily apply to escapes from the coursing salmonid predators used by Krkosek and Connors (pers. comm.). Escape responses and fast start capabilities are important, but successful evasion of coursing predators also requires more energy than darting away from an ambush predator (e.g. heron) and thus would likely be more sensitive to JPS condition. This remains to be examined.

Even if sea lice do not increase predation risk through reduced escape abilities, they can still influence predation risk through other components such as reducing JPS swimming endurance, which might reduce their ability to escape coursing predators (Chapter 4), or by increasing JPS exposure and conspicuousness to predators (Chapter 2).

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CHAPTER 4:

THE EFFECT OF SEA LICE ON JUVENILE PINK SALMON SWIMMING ENDURANCE

4.1 Abstract

A parasitic infection, which reduces host condition, is likely to influence how the latter interacts with its environment. A large proportion of juvenile pink salmon (JPS; *Oncorhynchus gorbuscha)* in the Broughton Archipelago of British Columbia have been found to be infected with the ectoparasitic sea louse, *Lepeophtheirus salmonis*. Previous work has shown that infected JPS are more likely to be preyed upon by coho salmon (*O. kisutch*). If *L. salmonis* reduce JPS condition this could play a role in the increased predation susceptibility. The present study measured the swimming endurance of naturally and experimentally infected JPS to determine the effects of *L. salmonis* on pink salmon condition. Juvenile pink salmon naturally infected with adult male and pre-adult stage lice did not appear to have a reduced condition. However, JPS experimentally infected with adult female lice showed a reduced ability to swim as far as control fish (i.e., uninfected fish), and this effect increased with lice load. A reduced swimming endurance is not only likely to influence predation risk for JPS, but may have other ecological implications, such as slower seaward migration. This research demonstrates the use of experimental infections in the field and provides a baseline measure of JPS swimming abilities, setting the stage for further investigation into the effects of sea lice on the health and swimming capabilities of juvenile salmon.

4.2 Introduction

Parasites play a significant role in the ecology and survival of their hosts and can affect the behaviours and abilities of their hosts to carry out their day-to-day routines. For example, if the parasites create an energetic drain on the host, reducing its condition or health, less energy is available for locomotion, especially costly movements (e.g. foraging forays, migration, escaping predators; Barber *et al.*, 2000; Munderle *et al.*, 2004; Alzaga *et al.*, 2008). However, hosts may attempt to mitigate this energy deficit by altering their behaviours to increase energy intake, which can increase their susceptibility to predation (Lima and Dill, 1990).

Whereas a parasite should be relatively benign unless it gains a fitness advantage by increasing the probability of transmission to its next host, situations may occur where parasites reduce host condition to the point of death, e.g. if abnormally abundant or infecting juvenile hosts. An example of the latter has arisen as studies show high numbers of ectoparasitic sea lice, *Lepeophtheirus salmonis* on out-migrating juvenile salmon in areas near fish farms in the Broughton Archipelago, British Columbia, causing up to 95% mortality (Morton *et al.*, 2004, 2005; Krkosek *et al.*, 2006). This study system provides a unique opportunity to utilize a new host/parasite interaction (Krkosek *et al.*, 2007a) to look at the effects of a "novel" parasite on juvenile host condition.

Many studies have examined the costs of *L. salmonis* infection to salmonid hosts. For example, increased physiological damage and death in Atlantic salmon (*Salmo salar*) post-smolts was associated with infections by

motile stage sea lice (Grimnes and Jakobsen, 1996). Sustained swimming tests on adult *S. salar* found reduced swimming ability due to a decreased cardiac output after exercise when infected with *L. salmonis* at 0.13 lice·g^{-1} (Wagner *et al.*, 2003). Webster *et al.* (2007) found that infected juvenile pink salmon, (JPS, *Oncorhynchus gorbuscha*) prefer freshwater and incur higher energetic costs when in saltwater which suggested there were osmotic challenges associated with *L. salmonis* infection. Some studies have found no difference in Fulton's condition factor of infected and non-infected JPS (Jones and Nemec, 2004; Butterworth *et al.*, 2008). However, Morton and Routledge (2006, 2008) argue that condition factor is not a valid assessment of the impact of sea lice on JPS as their data suggest that condition factor is only reduced when the fish are moribund. Aside from the use (or misuse) of condition factor, there has not been any direct test of the effects of *L. salmonis* on JPS condition.

I used a prolonged swimming test to assess the impact of sea lice on the condition of naturally and experimentally infected JPS. Prolonged swimming tests, defined as high intensity swimming which lasts between 20 s and 120 min and ends in fatigue, are widely used to assess swimming capacity, performance, and condition (Beamish, 1978; Mesa and Olson, 1993; Hammer, 1995; Farrell *et al.*, 1998; Martinez *et al.*, 2003; Munderle *et al.*, 2004; Blake *et al.*, 2005; Grorud-Colvert and Sponaugle, 2006). Measuring the point at which failure occurred, I tested the hypothesis that *L. salmonis* infection reduces JPS swimming endurance, thus indicating a reduced condition.

4.3 Methods

4.3.1 Fish collection and maintenance

4.3.1.1 Natural infections

Wild JPS naturally infected with sea lice were collected with a beach seine (30.5 x 2.5 m with 4 mm mesh) near Twin Lagoon in Fife Sound, Broughton Archipelago, BC (Figure 1.2). In order to be used in the experiment, JPS had to have a fork length (FL) of 55 ± 2 mm and meet the following criteria: non-infected fish had to have zero lice (of any species) and no visual scarring, either from previous lice infections or predators; infected fish had to be infected with at least 1 motile *L. salmonis*, have a chalimus louse scar (evidence of infection history), no other species of louse, and no predator scars. Fish were visually assessed as in Krkosek *et al.* (2005a). They were sorted and placed in individual, floating, 1 L flow-through containers, which minimized handling of the fish while transporting them to the swim chamber, and eliminated the possibility of lice transferring between fish. The containers were transported in 80 L coolers to the Salmon Coast Research Station where they were placed in a 250 L flow-through tank until the swim trials were conducted the following day.

4.3.1.2 Experimental infections

Fish used in experimental infection trials were collected from Spring Passage Channel, Broughton Archipelago, BC (Figure 1.2). Test JPS were seined and selected with the same criteria as the non-infected fish above. Adult female *L. salmonis* (AF) were collected by sorting AF infected JPS and holding them separately from test fish. All fish were taken back to a floating dock system

and placed in separate flow-through floating tubs (described in Krkosek *et al.,* 2006). Infection of previously non-infected JPS with adult female *L. salmonis* occurred the following day (see below). Following infections, fish were returned to the same tub and held for 20-24 hrs., after which they were placed in individually marked containers and taken to the research station for swim trials the following day, approximately 38-46 hr after infection. From this point forward naturally infected JPS will be referred to as NI and experimentally infected JPS as EI.

Non-infected JPS were selected haphazardly from holding tubs and placed into two 20 L buckets, which were randomly assigned as treatment or control groups. Treatment groups were manually infected with one, two, three, or four AF and the control group (no lice) was "sham infected" (i.e., all handling was the same except the placement of lice) at the same time as each of the other treatment groups. JPS infected with AF were netted and placed into a clear plastic Zip-lock® bag and the louse was gently removed by dislodging it and placing it on its back on the experimenter's finger. After louse removal the host fish was returned to the water and later released. The fish to be infected was held in a 250 ml container filled with water. To facilitate attachment of the louse to the fish (and not the container), the water was drained until the skin of the fish was exposed to the air and the louse physically placed on the fish. Following AF attachment, the water was refilled. This process was repeated until the appropriate number(s) of lice were attached. JPS were held in these containers for 5 min to assure attachment of the lice and then placed together in a 20 L

bucket for 30 min before being returned to the holding tub. Because fish movement stimulated AF attachment and to control for the possible effects of anaesthetic on adult lice, JPS were not anesthetized during infections. Infections generally took less than 5 min and JPS were not out of water for more than 30 sec. Handling times were kept consistent across treatment groups to ensure any difference between treatment levels were due to lice and not to increased emersion due to repeating the procedure up to four times.

4.3.2 Swim tunnel design

The prolonged swimming tests were carried out in a swim tunnel comprising four separate fish swimming chambers running in parallel (Figure 4.1). A 760 L plastic water reservoir was connected to a 10.2 cm diameter PVC pipe fitted with a butterfly valve that was then reduced to 7.6 cm and split by Y-fittings into four 7.6 x 76.2 cm clear PVC swim chambers. A bundle of drinking straws was placed upstream of each chamber to straighten the flow and minimize turbulence. A 4 mm plastic mesh screen, held in place by 0.5 mm dia. stainless steel fishing wire, was placed at the front of each swim chamber approx 10 cm from the straws to prevent the fish from utilizing micro-eddies behind the straws. Fish were placed in the chamber through a lid that was sealed with a foam gasket and secured by hose clamps. A grid made of stainless steel fishing wire was mounted in a gate valve housing in the rear of

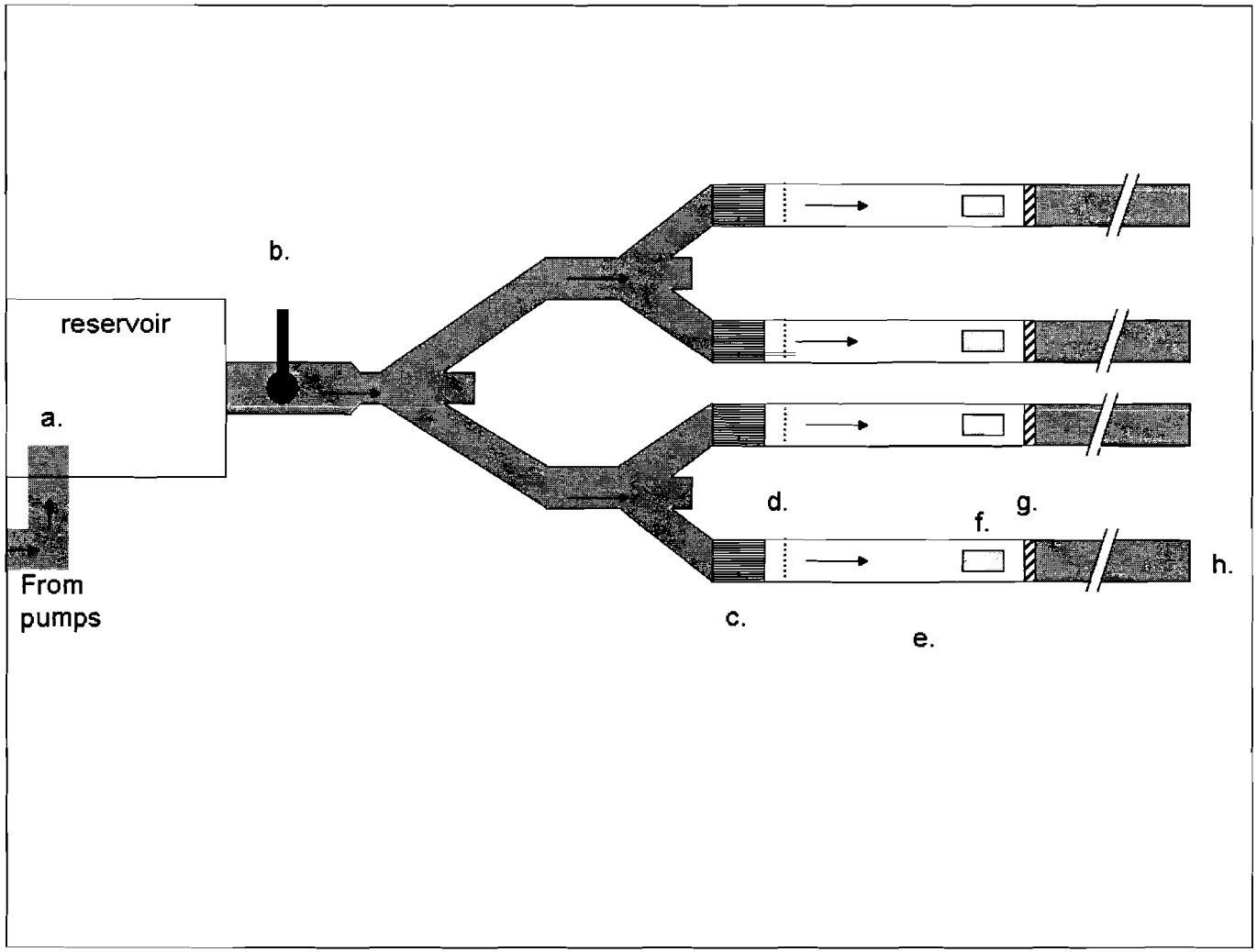

Figure 4.1 Schematic drawing of swim tunnel as seen from above.
Water from pumps discharges into reservoir (a). Components are: valve (b), flow straightening straws (c), mesh screen (d), swim chamber (e), door (f), electrified grid (g), and outflow (h).

each swim chamber (which allowed the grid to be raised to allow fish removal). Each grid was controlled by an on/off switch connected to a variable transformer set to 2V AC. The grids remained electrified during trials and were switched off when the fish reached the endpoint (fatigue). The slight electrical current motivated the JPS to continue swimming until complete exhaustion rather than resting against the grid.

A submersible pump (Barnes Pump Canada; model 3SE 514L) supplied water to the reservoir. To ensure a stable temperature and salinity, the pump was placed 12.2 m. below mean low water level in front of the research station. A second pump (American Products, model 382204) with an intake 6.1 m below the surface provided additional water as needed to maintain sufficient water supply to the header tank. Pump intakes were screened (~ 0.5 cm mesh), and a 200 µm filter bag was placed around the pump hoses discharging into the reservoir to prevent fouling of the swim chambers. The water velocity in the chambers was regulated by adjusting the height differential between the water level in the reservoir and the outflow pipe. Because the pump output was not adjustable, an adjustable overflow valve in the side of the reservoir allowed excess water to spill over once the desired water level height was achieved. These water levels were calibrated to specific flow velocities (see below) by recording the time to fill a 20 L bucket from the swim tunnel outflows.

4.3.3 Test protocol

Prior to each trial the reservoir and swim tunnel were flushed and filled with seawater. Four fish were chosen for each trial (when possible, two control and two treatment fish were swum simultaneously), placed randomly in a chamber and allowed to acclimate with no flow for five minutes. The swimming test was a constant acceleration test modified from Tierney *et al.* (2007) whereby water velocity was gradually increased to the initial velocity of 8.25 cm/s over 1 minute and then increased by 2.75 cm/s every 5 min thereafter. Because maximum velocity attainable (37.75 cm/s due to pump limitations) was reached before some fish failed, the final stage was extended for an additional 30 min in those cases. The trial was ended at that point regardless of JPS failure. The endpoint of the test was when the fish failed to continue swimming against the flow and remained against the grid for longer than 2 sec. The electric grid remained on until the endpoint was reached, at which point it was switched off for that chamber. The stage and time of failure were recorded for each fish. Due to flow dynamics of the system, an exhausted fish could not be removed until the trial was ended because the remaining fish would experience an interruption of flow. At the end of the trial, flow was stopped by closing the main butterfly valve, the header tank was filled, and the gate valve grids in each chamber were raised. When the main valve was re-opened, the fish were flushed out the end of the chambers where they were caught in a net. NI fish were returned to the containers for later release seawards from their collection point. EI fish were sedated with clove oil and killed with a sharp blow to the head so that motile louse scars could be counted accurately as an indicator of lice damage.

4.3.4 Analysis

Critical swimming speed calculations could not always be performed because the final swimming stage sometimes had a longer duration and some fish did not fail. Therefore, total or maximum distance swum (d_{max}) was calculated for each fish as a measure of fish performance: the time (s) swum at each stage multiplied by the velocity at that stage, and summed for all stages swum. As this was the cumulative distance swum at various velocities it is assumed to be an ecologically relevant measure of fish condition.

Time to event (survival) analysis was used to quantify the effect of lice infection on JPS swimming endurance because both datasets (NI and EI fish) included censored data (31% and 11% respectively). Those fish who did not fail before the trial was ended were still able to contribute to the data as they were assigned an "at least distance swum" without failure. Kaplan-Meier (KM) estimates of the probability of JPS swimming a given distance were obtained using observed maximum swimming distance (d_{max}). Parametric survival models (Table 4.1) representing competing hypotheses were fit to the KM survival estimates by log-likelihood with exponential, Weibull and lognormal error distributions for a total of 12 models. All models (excluding the null model) included the main effects of fork length and chamber to account for the effects of fish size and any consistent variation in water velocity between swim chambers. Models used to test for an effect of lice included a lice variable: presence/ absence of lice for the naturally infected fish, and AF group (number of adult

female lice) for the experimentally infected fish. The number of motile scars was not included in the models as it was highly correlated with AF.

Akaike information criterion corrected for small sample sizes (AICc) was used to rank the candidate models based on the relative differences (ΔAICc) between the model with the lowest AICc value and all the other models within each set. AICc model weights (*w;* the probability that the given model is truly "best" among the candidate models) were calculated. Because all models contribute some information and there is uncertainty about which single model is actually "best", all model *w*'s were used to calculate the estimated parameter likelihoods (importance of variable) and the estimated parameter values, along with their unconditional SE. Only parameter values ± 2 SE that did not encompass zero were considered to have an effect (Burnham and Anderson, 2002). All analyses were performed in R version 2.6.1 (R Development Core Team, 2007).

Table 4.1 **Models representing competing hypotheses.**
For experimentally infected JPS, lice = the number of adult females 0-4; naturally infected fish, lice = infected or non-infected group.

	Model	Hypothesis
1	Lice + Forklength + Chamber	Lice Effect
2	Lice + Forklength + Chamber + Lice*Forklength	Lice Effect
3	Forklength + Chamber	No Lice Effect
4	Null	No Lice Effect

4.4 Results

4.4.1 Natural infections

A total of 84 JPS with a mean (± SD) FL of 55.1 ± 1.3 mm were swum between 15-27 May 2007. Water temperature averaged 8.67 ± 0.24°C and salinity ranged between 28-32 ppt. JPS in the naturally infected group (n_{NI} = 42) had a mean (± SD) of 1.31± 0.60 motile lice, however a majority were pre-adult stages and adult males (Figure 4.2). Some JPS were also infected with other juvenile stages of lice. Median distance swum (i.e., the distance at which 50% failed) was 676.7 and 719.6 m for infected and non-infected JPS, respectively (Table 4.2 and Figure 4.3). Despite this 6% difference, the null model with a lognormal distribution was eight times more likely than the models incorporating an effect of lice to be the "best" model. Because of the superiority of the null model, no parameter estimates were calculated. These results suggest JPS swimming endurance is not reduced by natural infections of *L. salmonis* at the levels tested here.

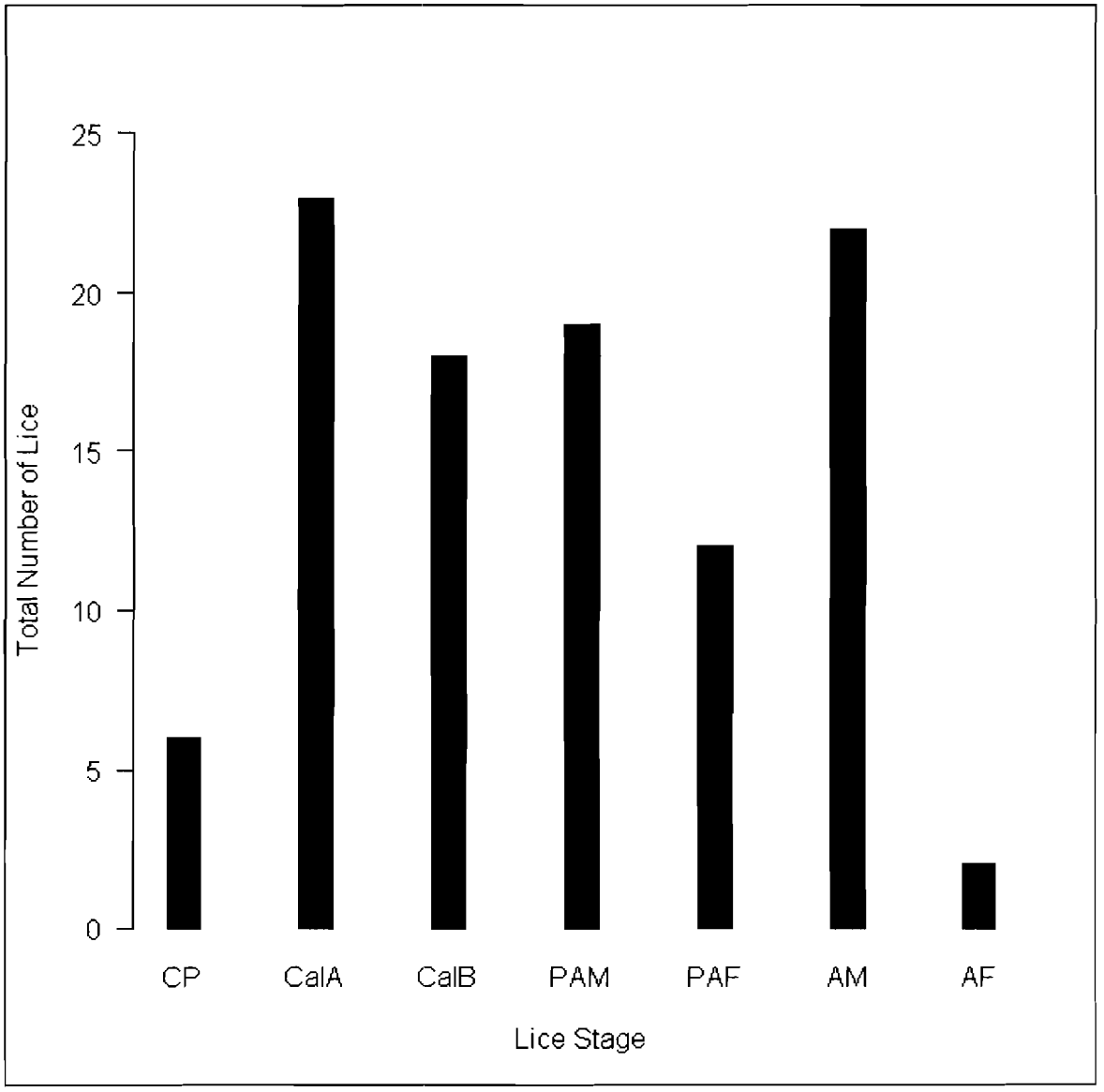

Figure 4.2 **Numbers of *L. salmonis* stages on the naturally infected JPS (N=42) used in this study.**
Lice stages are: copepodid (CP), chalimus 1 and 2 (CalA), chalimus 3 and 4 (CalB), pre-adult 1 and 2 male (PAM), pre-adult 1 and 2 female (PAF), adult male (AM) and adult female (AF).

Table 4.2 **Swimming data for naturally and experimentally infected juvenile pink salmon. Naturally infected fish (N=84) are grouped by lice infection: no motile lice (NL), motile lice (L). Experimentally infected fish (N=54) are grouped by number of adult females 0-4). JPS were experimentally infected for 40 hrs before testing. Censored data are the number of fish that did not fail. Median dmax is the distance at which 50% of fish failed and motscr is the number of motile scars on JPS, counted after the trial.**

Infection Trial	Group	FL (mm)	SD	N				Median d_{max} (m)	Median motscr
				Infected	Survived to testing	Failed	Censored		
Natural	NL	55.3	1.3	n/a	42	27	15	719.56	n/a
	L	54.9	1.3	n/a	42	32	10	676.66	n/a
Experimental	0	54.9	1.3	17	17	13	4	753.17	0
	1	54.9	0.9	14	13	12	1	590.70	2
	2	54.3	1.2	14	12	12	0	573.02	10
	3	55.4	1.6	16	9	8	1	439.89	12
	4	53.7	0.6	16	3	3	0	169.29	19

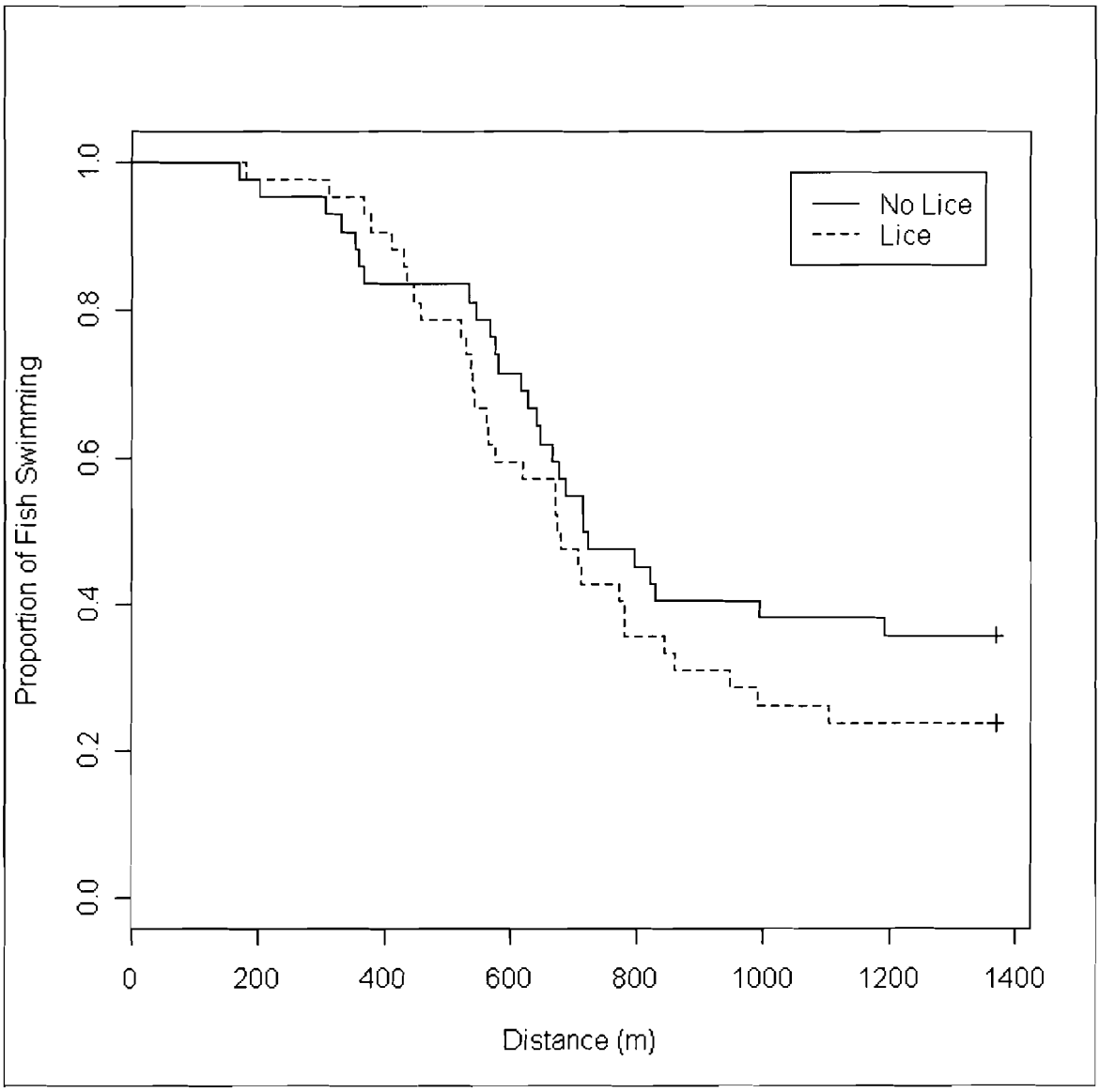

Figure 4.3 Kaplan-Meier plot of the proportion of non-infected and naturally infected juvenile pink salmon still swimming after having swum a given distance. Each step indicates the distance where a swim failure event occurred and + indicates censored data (fish did not fail).

4.4.2 Experimental infections

A total of 37 JPS infected with 1, 2, 3 or 4 AF and 18 control JPS were swum in this experiment (Table 4.2). Trials were performed between 6-21 June 2007; water temperature was 8.6 ± 0.2°C and salinity varied between 28-32 ppt. during this period. The median distances infected JPS could swim before they failed were less than for control fish (Table 4.2), and as the number of AF lice increased, the distances JPS could swim declined in a continuous fashion (Table 4.2; Figure 4.4). The top ranking models included lice and FL-AF interactions with a lognormal or Weibull error distribution and were 2-3 orders of magnitude more informative than the competing models (Table 4.3). The number of adult females had a large and significant negative effect on JPS swimming endurance (Table 4.4). The interaction of FL and AF had a significant positive effect on endurance, which can be interpreted as female lice having less of an effect on larger fish. Lice damage, as indicated by the number of motile scars, was highly correlated with the number of AF lice (Table 4.2; Spearman's rho = 0.815, $p < 0.01$).

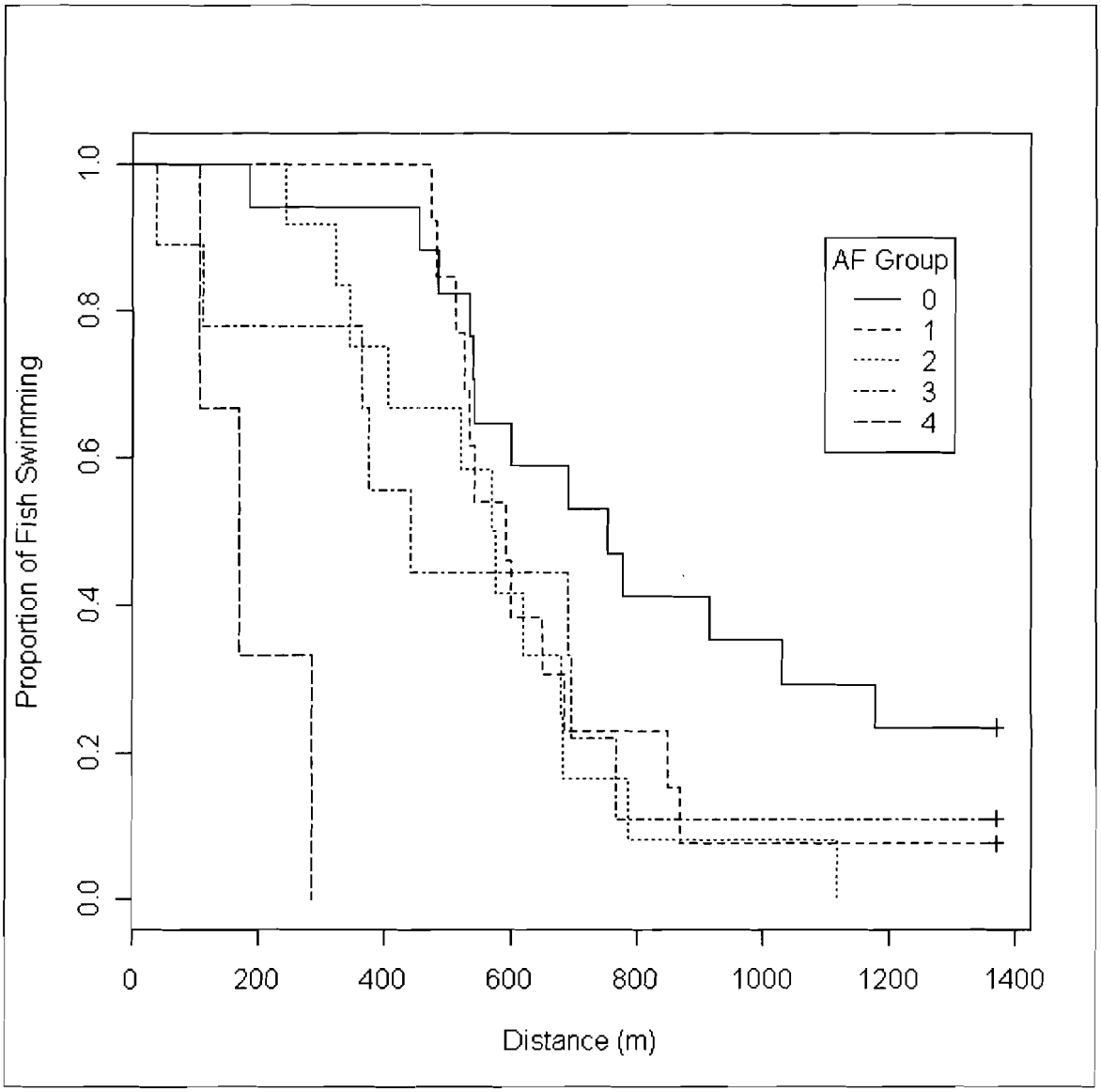

Figure 4.4 Kaplan-Meier plot of the proportion of experimentally infected juvenile pink salmon still swimming after having swum a given distance. Each step indicates the distance where a swim failure event occurred and + indicates censored data (fish did not fail).

Table 4.3 **Summary of models for experimentally infected JPS. Model is Log (dmax) = Intercept + model variables + σ*e, where σ is a scale parameter and e is an error term based on Weibull or Lognormal distributions (exponential error distributions did not provide an adequate fit). For each model: N = 54, K = number of model parameters, LL = maximum log-likelihood estimate, AICc = Akaike's Information Criterion corrected for small sample size, Δ = the change in AICc between the model and the model with the lowest AICc (highlighted in bold), and w = Akaike weight (the likelihood of superiority over other models in the set). Only models with w > 0.001 are shown.**

	Model variables	Distribution	K	LL	AICc	Δ	*W*
1	**adult female + forklength + chamber + adult female*forklength**	**Weibull**	**7**	**-556.7**	**1127.40**	**0.00**	**0.80**
2	Adult female + forklength + chamber + adult female*forklength	Lognormal	7	-558.1	1132.63	2.80	0.20

Table 4.4 **Model parameters for experimentally infected JPS. Parameter likelihoods (importance of variables) and estimates with unconditional SE, calculated using w of all experimental swimming models (Table 4.3; other models, not shown, have w = 0, and thus do not influence parameter estimates). Values are rounded to the nearest 0.01. Parameters estimates ± 2 unconditional SE not overlapping 0, and therefore considered significant, are highlighted in bold.**

Parameter	Parameter likelihood	Parameter estimates	Unconditional SE
Intercept	**1.00**	**20.31**	**5.26**
Adult female	**1.00**	**-11.17**	**2.43**
Forklength	1.00	-0.16	0.09
Chamber	1.00	0.08	0.32
Adult female*forklength	**1.00**	**0.20**	**0.04**
Σ	1.00	-0.75	0.76
E	1.00	0.42	NA

4.5 Discussion

When experimentally infected with adult female lice JPS had reduced endurance, suggesting that infection with adult female *L. salmonis* had a negative impact on JPS condition. This seems biologically reasonable given the large size and energetic needs of the female parasites, who are acquiring resources to produce eggs, and was supported by evidence of females having fed on the test fish (motile scars; Table 4.2). While there was a large overall effect, it was most evident for higher lice loads, particularly in view of the fact that the majority of these fish died before they could be tested (Table 4.2). Given that I swam only the fish capable of withstanding the challenge of three and four lice, the estimate of impact of adult female lice on swimming endurance must be a conservative one. My findings are similar to those from the swimming tests performed by Wagner *et al.* (2003) on large Atlantic salmon, and to those reported for other parasite-host systems: parasites have been shown to reduce prolonged swimming ability in the European eel (*Anguilla anguilla*) and the cardinal fish (*Cheilodipterus quinquelineatus*) (Munderle *et al.*, 2004; Ostlund-Nilsson *et al.*, 2005, respectively). The effect is similar to that seen in studies using prolonged swimming tests to investigate the magnitude of reduced heath and condition in unfed Atlantic cod (*Gadus morhua*) (Martinez *et al.*, 2003, 2004).

A parasite-induced decline in swimming endurance indicates a reduced condition, suggesting that lice infection is energetically costly to JPS. There are several possible reasons for this. For example, adult females feed on blood, and its removal can induce anaemia (Wagner and McKinley, 2004). To obtain the

blood meal, a louse punctures the skin (causing the motile scars) which ruptures the osmotic barrier, allowing Na ions to enter the body, and increasing the energy required to maintain osmotic homeostasis (i.e., excretion of Na ions). Repairing this tissue damage and fighting off secondary infections from these wounds is an additional cost. As lice infections cause an increase in energy requirements they may lead to reduced condition if JPS cannot compensate for lost resources.

In contrast to the results for experimentally infected salmon, naturally infected JPS showed no diminution of endurance and, by inference, condition. There are at least two possible explanations for this inconsistency.

First, males (pre-adult and adult) comprised 83% of the louse stages naturally infecting JPS. *L. salmonis* males are much smaller than females and likely have lower energy requirements; since they are seeking out mating opportunities they may not feed as much as females and thus may be less detrimental. Studies of the effects of sea lice on Atlantic (Dawson *et al.*, 1999; Pike and Wadsworth, 2000; Wagner *et al.*, 2003; Wells *et al.*, 2006) and Pacific salmonids (Morton and Routledge, 2005; Krkosek *et al.*, 2006) found that the onset of pathogenicity began with the pre-adult stages, but did not look at gender-specific effects.

A second possibility is that the current level of motile lice on a host JPS is not a good indicator of past lice intensity on that fish, or the duration of that infection, and thus is only weakly correlated with the host's current condition. I attempted to account for this by testing fish which had a chalimus scar, as evidence of prior infection, but given the transient nature of motile lice and the

fact that JPS are able to rid themselves of lice (Jones *et al.*, 2007); M. Krkosek, University of Alberta, pers. comm.), this may have been insufficient to fully account for their infection history. Due to this uncertainty, it would be premature to conclude that there is no effect of the lice stages represented on the NI salmon, but rather that snapshot tests such as these are insufficient to determine effects on condition. Future studies should use experimental infections or track infections over time.

Reduced swimming endurance could have ecological consequences for JPS. Because JPS migrate in large shoals, a fish with reduced swimming endurance may be less able to compete for food, maintain its position within the shoal, or remain with the shoal at all, thereby losing the benefits which the shoal provides, such as protection from predators. Reduced swimming endurance may also increase predation susceptibility if it makes infected individuals less likely to escape from a coursing predator, such as a coho salmon (*O. kisutch*) or cutthroat trout (*O. clarkii*). The costs incurred from infections may also increase infected JPS susceptibility to predation if, in an attempt to mitigate these costs, they increase their foraging effort and thus their exposure to predators (see Chapter 2).

This study is the first to use field based swimming tests on wild juvenile pink salmon and demonstrates that the experimental infection methods used here are essential when testing for the effects of lice on performance or condition. These methods are ecologically relevant, and practical for use in field experiments. As suggested by the NI and EI control groups having very similar

median swimming distances (Table 4.2), fish were not placed under additional stress due to the experimental infection methodology. These observations suggest that the infection protocol used in this study is a valid method with which to assess the effects of motile lice on JPS condition.

My overall findings illustrate the need for further work using swimming tests and experimental infections to investigate the effects of other lice stages and infection durations; these tests should be run concurrently with physiological tests to understand the mechanisms underlying any effects. Information gained from such studies will shed light on the impact *L. salmonis* infections have on wild juvenile pink salmon. Pink salmon populations are depressed and continuing to decline, likely as a result of their exposure to sea lice (Krkosek *et al.*, 2007b). This is the first study to show an effect of sea lice on JPS condition, and while direct mortality may be an important driver of population declines, reductions in condition leading to reduced swimming endurance may contribute to the impact of sea lice on pink salmon populations.

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CHAPTER 5:

GENERAL DISCUSSION AND CONCLUSIONS

Pink salmon (*Oncorhynchus gorbuscha*) populations in the Broughton Archipelago of British Columbia are depressed, and continuing to decline, likely as a result of their exposure to sea lice from salmon aquaculture(Krkosek *et al.*, 2007a). Direct sea louse induced mortality may be an important driver of these declines. However, reductions in condition, and increases in risky behaviour (both of which may lead to increases in predation), likely also contribute to the impact of sea lice on pink salmon population dynamics as predation by other salmonids is a major source of the early marine mortality of juvenile pink salmon (JPS). This thesis has examined the effect of sea lice on JPS condition and anti-predator behaviour. It should be noted that the sampling of naturally infected JPS in these studies may have "censored" the true population in that the fish most vulnerable to infections may have already died and thus were not available to be tested. This could possibly underestimate the true effects of lice on JPS at the population level.

The negative effect of sea lice on JPS condition, for which evidence was provided in Chapter 4, is likely to influence multiple components of predation risk, from increased risk-prone behaviours to reduced escape abilities. To avoid a loss of condition, infected JPS alter their behaviours, increasing their predation risk by returning from cover sooner than non-infected fish (Chapter 2). JPS thus appear to trade-off the increased risk of exposure to a predator for the benefit of increased feeding. If mitigating the cost of infection by increased foraging is successful, then JPS may be able to avoid the loss of higher order capabilities such as those used in fast-start escapes (Chapter 3). It is possible that the costs

of increased exposure were low because JPS escape abilities had not been reduced by infection. However, JPS did experience a reduced condition when infected with adult female lice (Chapter 4), which was likely due to a failure to offset infection costs completely.

The loss of condition and subsequent reduction in swimming endurance probably also influences other components of predation risk, for example, a decreased ability to escape coursing predators, or to maintain position within the shoal, as shown by Krkosek and Connors (pers. comm.). Predation becomes extremely likely once infection has reduced JPS condition so severely that they become "loners" (Morton and Routledge, 2005, 2006), alone at the surface and unresponsive to stimuli.

While JPS condition is a major driver of increased susceptibility to predators through the above mechanisms, other conspicuous behaviours associated with lice infection, such as leaping from and rolling on the water surface (Webster *et al.*, 2007) are also noteworthy. These behaviours are energetically costly and thus unlikely to be associated with a reduced condition, yet they will make the fish more conspicuous and thereby increase susceptibility to predators. It is these effects on all components of predation risk (Lima and Dill, 1990) that eventually leads to the increased consumption of lice-infected JPS by salmonid predators.

Future research should continue to advance the work presented here to understand the mechanisms behind JPS mortality. While there are knowledge gaps in our understanding of the effects of sea lice, my work adds to a growing

body of evidence on mechanisms behind sea lice-related mortality (both direct and indirect), supporting the conclusion of other researchers (Krkosek *et al.*, 2007a) that sea lice are at least partly responsible for the declines of wild salmon populations in the Broughton Archipelago, BC.

Sea lice are found on juvenile salmon at such low levels in areas without salmon aquaculture that the effects of lice on JPS predation risk are likely to arise only in the presence of fish farms. Sea lice and salmon interactions have evolved to a state such that when infected as large juveniles or adults, the fish can fight off or withstand even relatively heavy infections, but smaller or weaker hosts cannot. The anadromous nature of Pacific salmon offers young fry a time and place where infection pressure is very low, and transmission occurs when returning infected adults and large juveniles are in sympatry. These newly infected hosts are probably large enough to not succumb to the lice, although parasite mortality is often underestimated in natural systems (Rohde, 1984). This natural cycle has been altered by the introduction of net cage salmon aquaculture into nearshore waters where much smaller juveniles, just entering the ocean, are being infected by lice which have overwintered on farmed salmon (Krkosek *et al.*, 2007b). Thus, infections occurring on smaller JPS are more likely to have effects not seen with larger fish, or in naturally evolved parasite-host systems.

This novel interaction between JPS and sea lice allows investigation of the mechanisms by which parasites affect predation susceptibility of their hosts, without concern for co-evolutionary dynamics. The findings from this research

have contributed to the fields of parasitology and behavioural ecology, providing insight into how parasites influence host decisions and abilities. This has broader potential implications for the host's use of its habitat and interactions with both competitors and predators, ultimately impacting their survival.

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