

**BEHAVIOURAL INTERACTIONS OF SEA LICE,
THREESPINE STICKLEBACKS, AND JUVENILE PACIFIC
SALMON**

by

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ABSTRACT

There is scientific controversy over the role played by sticklebacks (*Gasterosteus aculeatus*) in transmitting sea lice (*Lepeophtheirus salmonis*) to juvenile pink salmon (*Oncorhynchus gorbuscha*). Sticklebacks could be either ‘sources’ or ‘sinks’ for sea lice infecting salmon. I investigated host choice of adult male lice and a novel cleaning behaviour by sticklebacks towards juvenile salmon. Behavioural assays showed that sea lice preferred water that had contained salmon over controls, with no preference between salmon and stickleback cues. Infection trials showed that lice had lower survival on sticklebacks than on salmon. Experiments showed that cleaning behaviour by sticklebacks reduced louse loads on salmon. Sticklebacks preyed more strongly on adult female lice than males, and they also cropped their egg-strings. These results suggest that sticklebacks are unsuitable hosts for sea lice and cleaning could potentially reduce sea louse populations. Thus, sticklebacks are more likely to be ‘sinks’ for sea lice than ‘sources’.

Keywords: host-parasite transmission, sea lice; cleaning; host choice; sex-selective; semiochemical; source; sink;

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1 GENERAL INTRODUCTION

In their simplest form, source-sink dynamic models are characterized by different habitat types resulting in recruitment exceeding mortality (source) or mortality exceeding recruitment (sink), which can ultimately contribute to the growth or decline of entire populations (Pulliam 1988). However, Many variants of theoretical source-sink dynamic models have been proposed over several decades. One area of disagreement pertains to the persistence of sink populations over time; despite very elevated mortality, sink populations can become evolutionarily stable if active dispersal occurs (Pulliam 1988). Thus, habitat selection resulting from active individual choice becomes critical in determining the dynamics of source-sink systems (Delibes *et al.* 2001). If density-dependent competition for high quality habitat exists, then one can expect poorer competitors to occupy sink habitats and fail to survive there, which can create a stable maladaptation whereby individuals continually select sink habitat but these populations are sustained by immigration and no selection takes place within the sink (Dias 1996). The theoretical work explaining the intricacies of source-sink dynamics, however, is more impressive than the empirical evidence provided to support it (Pulliam 2000). When applied to ectoparasites, habitat selection of a host that offers decreased survival and/or depressed reproductive success creates an opportunity for application of source-sink dynamics.

Sea lice are a group of ectoparasitic caligid copepod species that commonly infect bony fish in the marine environment. The term ‘sea louse’, however, has become most closely associated with *Lepeophtheirus salmonis* (Krøyer) – a louse that is usually found on salmonids and is ubiquitous throughout the northern hemisphere. Sea lice (*Lepeophtheirus* as well as *Caligus* spp.) are the most widespread pathogenic parasites of farmed salmon and cost the marine aquaculture industry an estimated US\$100 million every year (Johnson *et al.* 2004). Sea lice have long been problematic for European salmon farms where, in large numbers, *L. salmonis* can cause skin erosion and hemorrhaging as a result of their feeding on mucous, skin, and blood of their hosts, which can lead to direct death or death from secondary infections (Pike & Wadsworth 2000). Sea lice epizootics related to salmon aquaculture and the transmission of parasites from cultured to wild fish have drawn a great deal of attention from environmentalists and others in some regions of the world (Johnson *et al.* 2004).

In British Columbia (BC), Canada, sea lice epizootics related to salmon aquaculture were reported relatively recently (Morton *et al.* 2004) when compared to similar European outbreaks that date back four decades (Pike & Wadsworth 2000). Considerable scientific and public attention has focused on the transmission of sea lice, particularly *L. salmonis*, to wild juvenile pink (*Oncorhynchus gorbuscha*) and chum (*O. keta*) salmon. Contrasting opinions of the population effects of these parasites have generated a polarized debate in the scientific literature with published evidence for both negligible (Beamish *et al.* 2006) and considerable (Ford & Myers 2008; Krkosek *et al.* 2005, 2006, 2007) negative impacts on BC’s wild salmon populations, although evidence for the latter appears to be increasing. The Broughton Archipelago in the southern

coastal region of BC (Figure 1.1) has been the primary area of concern, as it is home to the province's highest concentration of fish farms, capable of producing as many as 1.6 billion *L. salmonis* eggs in a period of only two weeks (Orr 2007).

L. salmonis emerge from the eggstrings of an adult female louse as planktonic nauplii. They molt from this into the parasitic copepodid stage, which seeks out and attaches to the skin or gill of a host and then molts into the first of four attached chalimus stages. These chalimi are tethered to one location on the host and are thus immobile. It is not until the preadult and adult stages that lice are motile and can move on and between hosts. The rate of development for *L. salmonis* is mediated by temperature and the egg-adult cycle is complete in approximately 400 degree-days (Johnson & Albright 1991). Although sea lice have direct life cycles (Johnson *et al.* 2004), and *L. salmonis* is considered to be a salmonid-specialist parasite, this species has been documented on non-salmonid fishes sympatric with salmon aquaculture, including saithe (*Pollachius virens*) in Europe (Bruno & Stone 1990) and the threespine sticklebacks (*Gasterosteus aculeatus*) in BC (Jones *et al.* 2006). The stickleback, in particular, has attracted a lot of attention due to its high louse load with an unusual characteristic – virtually no adult lice are found infecting sticklebacks. It has been “concluded” that lice switch to salmonid hosts upon reaching motile adult stages on sticklebacks (Jones *et al.* 2006), and they are thought of as a source of lice in the Broughton Archipelago (Beamish *et al.* 2006) despite any evidence that this actually occurs. On the basis of current evidence, it is unclear whether sticklebacks really do amplify sea louse infections on wild juvenile salmonids, or if sticklebacks are merely scapegoats, diverting attention from the potential role of fish farms as sources of sea lice.

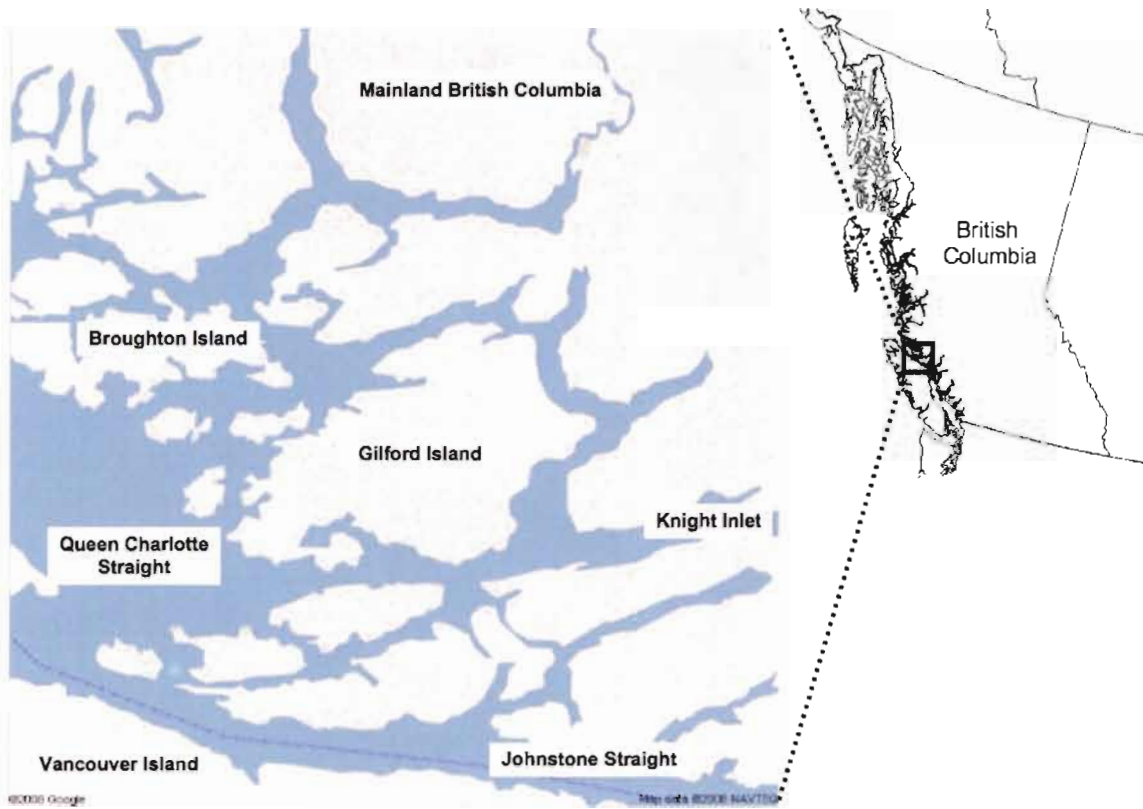


Figure 1.1 Map of the Broughton Archipelago in coastal British Columbia. Salmon Coast Field Station, where this research was conducted, is located on the north shore of Gilford Island (maps courtesy of Google®).

From this paucity of evidence has arisen a debate which I will refer to as ‘source vs. sink’ concerning the role of sticklebacks in transmission of sea lice between farmed and wild salmon in the Broughton Archipelago. Lice may mature on sticklebacks to the preadult stage at which point they switch hosts to a salmon (wild or farmed) and are thus a ‘source’ of lice for wild juvenile salmon. Alternatively, it can be hypothesized that sticklebacks offer zero fitness for lice and that any copepodid louse that infects this species does not survive to adulthood (consistent with the lack of adults on sticklebacks). In this case sticklebacks would act as a ‘sink’ for the louse population in the Broughton Archipelago as they remove lice that could otherwise infect wild salmonids.

With ‘source vs. sink’ as the overarching question motivating my thesis research, stickleback-salmon transmission of sea lice was investigated along two avenues by using wild-caught fish and lice in the Broughton Archipelago in field-based laboratory experiments. First, the host choice behaviour of adult *L. salmonis* was investigated in response to host-derived cues. Sea lice have been found to exhibit directional responses to chemical cues (Hull 1997), thus behavioural assays were used to determine active choice in adult male *L. salmonis* to water conditioned with the semiochemicals of the two potential hosts. This was complemented by host-specific infection and survivorship studies of adult male lice, which incorporate all potential host cues, not only chemosensory input (Chapter 1). Second, the discovery of a novel cleaning behaviour whereby sticklebacks remove and consume lice from the skin of infected juvenile salmon was investigated. Evidence for biased consumption of female lice was quantified and the general characteristics of this novel interaction were described (Chapter 2). This behaviour was captured on video (Appendix B). An investigation into the host-specific

survivorship of chalimus-stage lice was also conducted; these results are presented in Appendix A.

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2 HOST CHOICE BEHAVIOUR OF SEA LICE, *LEPEOPHTHEIRUS SALMONIS*, AND THE ROLE PLAYED BY STICKLEBACKS, *GASTEROSTEUS ACULEATUS*, IN SALMON PARASITE TRANSMISSION DYNAMICS

2.1 Abstract

Sea lice, *Lepeophtheirus salmonis*, are generally considered to be a salmonid-specific parasite. However, their immature lifestages have been found infecting threespine sticklebacks, *Gasterosteus aculeatus*, at very high levels in the Broughton Archipelago, BC. To investigate whether sticklebacks ultimately serve as a source of infection of juvenile salmon by sea lice, or as a sink, behavioural assays were performed to establish host preference using semiochemical cues. Single species infection trials were also performed. Both salmon- and stickleback-derived cues caused a statistically significant increase in the proportion of lice that became active in a Y-tube apparatus compared to control seawater. Adult male lice chose salmon-conditioned water significantly more often than control water, although there was no preference for salmon when offered simultaneously with stickleback chemical cues. Adult lice infected salmon hosts significantly faster than they did sticklebacks, and survived significantly longer on salmon hosts than on stickleback hosts. These results suggest that sticklebacks are an inferior, dead-end host for *L. salmonis* and may not be a source for infection of wild juvenile salmon.

2.2 Introduction

Ectoparasitic infestations are a recurrent problem for the aquaculture industry (Costello 2006). One particularly widespread parasite is the sea louse, *Lepeophtheirus salmonis*, which is ubiquitous in the northern hemisphere and completes its entire parasitic life cycle on salmonid fishes (Pike and Wadsworth 2000). On the coast of British Columbia, Canada, and in particular in the Broughton Archipelago, where Atlantic salmon (*Salmo salar*) farms are numerous, sea lice affect not only farmed fish but also wild salmon. Sea lice occur at very high prevalence on wild juvenile pink (*Oncorhynchus gorbuscha*) and chum (*O. keta*) salmon (Morton *et al.* 2004). Curiously, lice have also been found at higher intensity and prevalence on threespine sticklebacks (*Gasterosteus aculeatus*) than on juvenile pink and chum (*O. keta*) in the Broughton Archipelago, although only in the immature, attached chalimus lifestages (Jones *et al.* 2006b). This absence of preadult and adult lifestages on sticklebacks suggests two alternative hypotheses: (1) lice cannot complete their life cycle on sticklebacks and, thus, sticklebacks are a dead-end host (the ‘sink’ hypothesis), or (2) lice mature to the motile stages, at which point they switch to a salmonid host to complete their life cycle (the ‘source’ hypothesis). Jones *et al.* (2006a) concluded the latter to be true based on the virtually complete absence of adult sea lice on sticklebacks in the wild. To truly evaluate these hypotheses, however, host choice behaviour of sea lice must first be understood.

Ectoparasitic copepods, such as sea lice, have an ultimate requirement to locate a suitable host in a relatively expansive environment (Luntz 2003). In the marine

environment, turbulent odour plumes form a complex chemical landscape of cues through which these arthropods must navigate (Zimmer and Butman 2000). Sea lice have been shown to exhibit a directional response to host-derived cues detected by two chemoreceptors on the first antennae (Hull 1997). This directional response is characterized by a positive, upstream rheotaxis as part of their host location behaviour (Ingvarsdottir *et al.* 2002a). This upstream swimming is then changed to side-to-side casting movements when the odour plume can no longer be detected (Devine *et al.* 2000). The semiochemical compounds responsible for eliciting these behaviours in *L. salmonis* have been isolated from salmon-conditioned water and identified as isophorone and 6-methyl-5-hepten-2-one, which are structurally similar to analogous compounds detected by terrestrial arthropods (Bailey *et al.* 2006).

Host location in *L. salmonis* can occur at two stages in their lifecycle. Free-swimming copepodids attach to a host for the first time following a planktonic nauplius stage (Johnson and Albright 1991) and motile preadult and adult stages exhibit host-switching behaviour (Ritchie 1997), both within and between species of salmonid. Both copepodid (Bailey *et al.* 2006) and adult male (Devine *et al.* 2000, Ingvarsdottir *et al.* 2002a) lice have been shown to exhibit behavioural attraction to water conditioned with Atlantic salmon. Knowing this behavioural property of sea lice has prompted the proposal of pest management plans via attraction/repulsion tactics using host- and non-host-derived cues (Ingvarsdottir *et al.* 2002a,b, Luntz 2003).

Host preference can help to inform the ‘source’ and ‘sink’ debate, as a dead-end host should not be chosen over a more suitable host when offered simultaneously (Pulliam, 1988). We therefore ran behavioural assays using wild lice and host-derived

chemical cues to determine any host preference in these lice. These were coupled with species-specific infection trials to include non-chemical cues in the host choice decision of sea lice. A subset of these infected fish were then used to evaluate differential survivorship of lice on the two hosts.

2.3 Methods

2.3.1 Sea Lice

All lice were obtained from naturally infected juvenile pink salmon captured in the Broughton Archipelago, in spring 2007. In order to assess what decisions are being made by lice at the host-switching lifestage, only adult male lice were used in the experiments, as *L. salmonis* is known to exhibit a male-biased inter-host transfer ability (Hull *et al.* 1998) and adult male lice have a similar tendency to switch hosts as both male and female preadults (B. Connors, unpublished data). It was not possible to obtain adult male lice from sticklebacks, as they do not occur on this species in the wild (Jones *et al.* 2006b) and lice have not been successfully reared in laboratory conditions (Jones *et al.* 2006a). Lice were removed from the skin of juvenile salmon using gentle tactile manipulation – an adaptation of the methodology used by Krkosek *et al.* (2005). No anaesthetics were used to avoid any potential effects on louse behaviour. Salmon were returned to a recovery area, and lice transferred directly to a holding container where they were held *en masse* until needed in the experiment (0-2 hours).

2.3.2 Conditioned Water

Threespine sticklebacks were caught using minnow traps and seine nets, and juvenile pink salmon were caught using seine nets. Ambient seawater (28-33‰; 9-13°C) was conditioned using 10 g of live fish•L⁻¹ housed in a well-aerated container for 24 hours. This method has proven to be effective in creating standardized conditioned water for salmonid and non-salmonid species of fish that successfully elicits behavioural responses from *L. salmonis* in other studies (Devine *et al.* 2000, Ingvarsdottir *et al.* 2002a). Although I did not measure the concentration of the conditioned water, the goal was to present lice with an ecologically relevant choice between equal numbers of two potential host species. The conditioning tanks were kept in a flow-through bath of ambient seawater to buffer temperature variation. Control water was kept in identical containers for 24 hours without any fish. All water was then filtered (100 µm mesh) into tanks forming part of the Y-tube apparatus described below, and used immediately in behavioural assays.

2.3.3 Y-Tube Behavioural Assays

Water was siphoned into the vertical Y-tube apparatus from tanks containing equal amounts of each of the conditioned and/or control water. The apparatus was adapted from that used by Devine *et al.* (2000) and Ingvarsdottir *et al.* (2002a), and is illustrated in Figure 2.1. Flow was regulated at the downstream end using a veterinary-

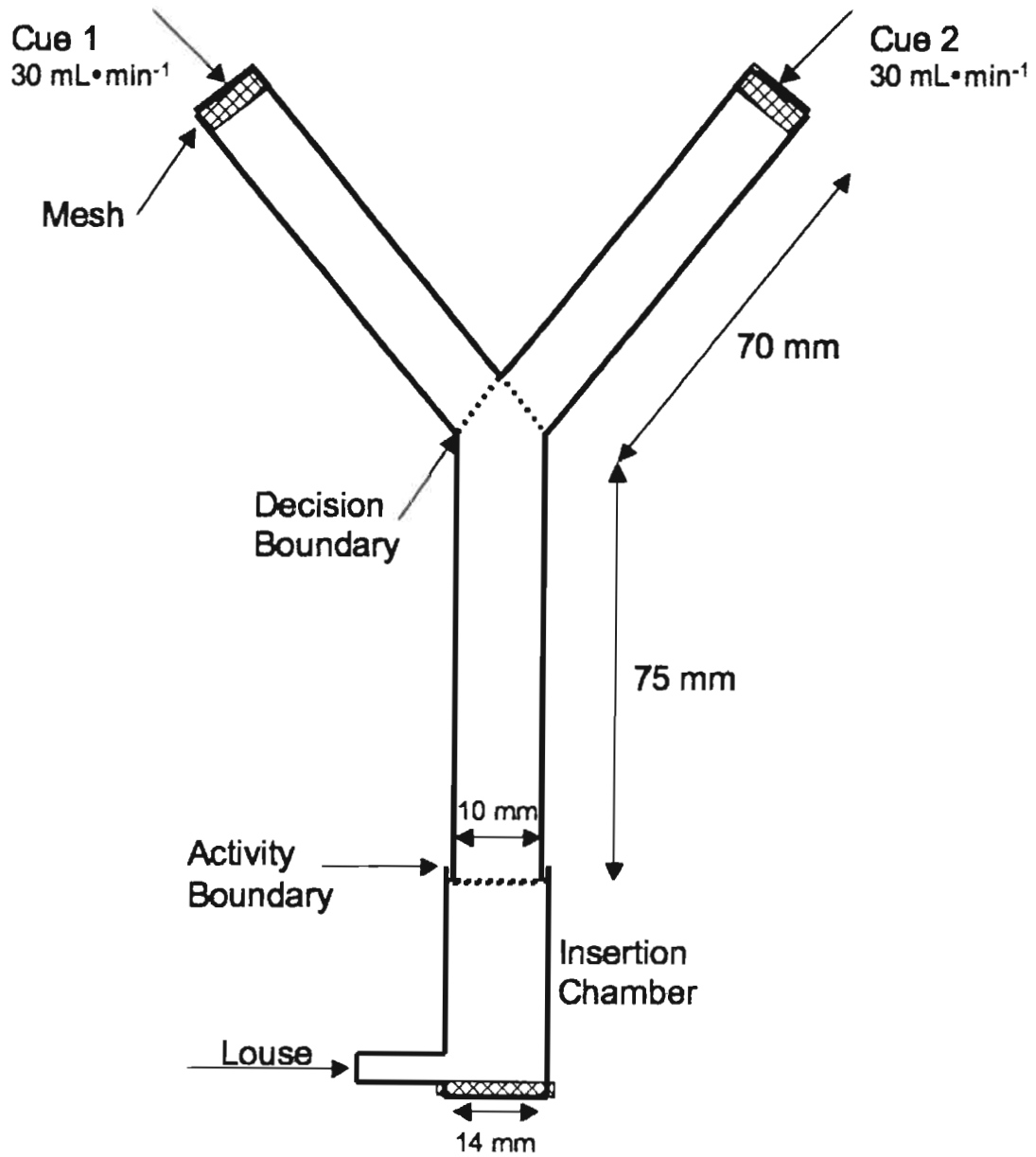


Figure 2.1 Diagram of the Y tube apparatus used in behavioural assays. Dashed lines indicate decision lines used for evaluation of louse behaviour during observation, not physical boundaries

grade intravenous drip valve ($15 \text{ drops} \cdot \text{mL}^{-1}$), which allowed for fine-tuning of flow rates. Water was siphoned into each of the upper arms at a rate of 30 ml/min through 1cm ID Nalgene® tubing. Food colouring was used in mock trials to confirm complete segregation of the two cues. Inlets into the upper arms and the outlet of the lower arm were screened with 1 mm mesh to ensure that lice remained in the apparatus.

After a short time (2-3 min) to allow establishment of the cues in the apparatus, the test louse was inserted into the chamber attached immediately below the Y-tube, where it was exposed to both chemical cues simultaneously. Each louse was then given 10 minutes to respond. Behaviours were categorized as: (1) distinct choice, (2) increased activity, or (3) no activity. Distinct choice involved the louse swimming up the bottom arm of the Y-tube and fully entering either one of the upper arms. At this point, the choice and time were recorded and the trial ended. Increased activity was recorded if the louse left the insertion chamber but made no choice (did not enter either arm) in the 10-minute time limit. No activity meant the louse did not leave the insertion chamber. These latter lice were deemed behaviourally deficient and did not contribute to the target sample size ($N=50$) for each cue combination (salmon-control, stickleback-control, and salmon-stickleback). The cues were alternated through the two arms of the Y-tube between trials to control for any arm-bias.

2.3.4 Host-Specific Time to Infection

These trials were used to identify any difference in time to infection between host species. This allows for chemosensory cues as well as mechanosensory and visual ones, all of which are important in host location (Genna *et al.* 2005), to be used by the lice.

Ten adult male lice were placed in a small container containing 500 mL of ambient seawater to increase likelihood of infection. One uninfected stickleback or one uninfected juvenile pink salmon was then placed into each container and observed. All fish in these trials were of similar length (mean \pm 1 SE; salmon: 58.17 \pm 1.08 mm, stickleback: 60.11 \pm 1.31 mm; independent sample t_{33} =1.14, p =0.26). All trials occurred under artificial fluorescent lighting. Any attachment of lice was recorded and time-to-attachment noted. A subset of those fish that were successfully infected was then transferred to larger aquaria where they were held individually and monitored at regular time intervals (1, 3, 6, 12, 18, and 24 h) for the presence of the lice. This allowed the assessment of stickleback's and salmon's viability as hosts for adult lice, which was evaluated using a Kaplan Meier Log-Rank survivorship analysis.

2.4 Results

2.4.1 Y-Tube Behavioural Assays

Sea lice were approximately four times more active when exposed to either type of fish-conditioned water than when exposed only to control seawater (χ^2 =41.59, df =3, p <0.001). There was no difference in the proportion of lice active between the three cue combinations tested (χ^2 =0.63, df =2, p =0.73; Fig. 2.2).

Sea lice preferred salmon-conditioned water when paired with control seawater (χ^2 =6.72, df =1, p <0.001), but showed no preference for stickleback-conditioned water over control water (χ^2 =0.44, df =1, p =0.51; Fig. 2.3). When both types of conditioned

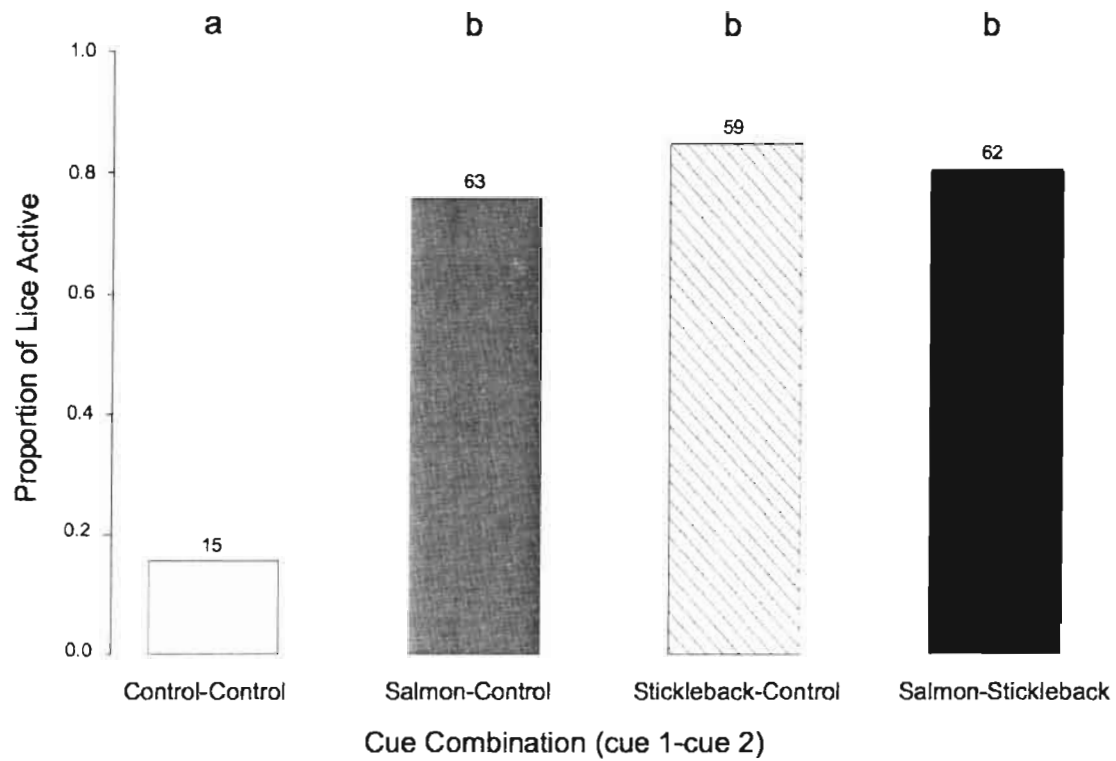


Figure 2.2 Proportion of lice that were active under each combination of chemical cues in Y-tube bioassays. Numbers above bars indicate sample size for that treatment. Only the control-control differs significantly from the other 3 pairs (as indicated by letters above the bars).

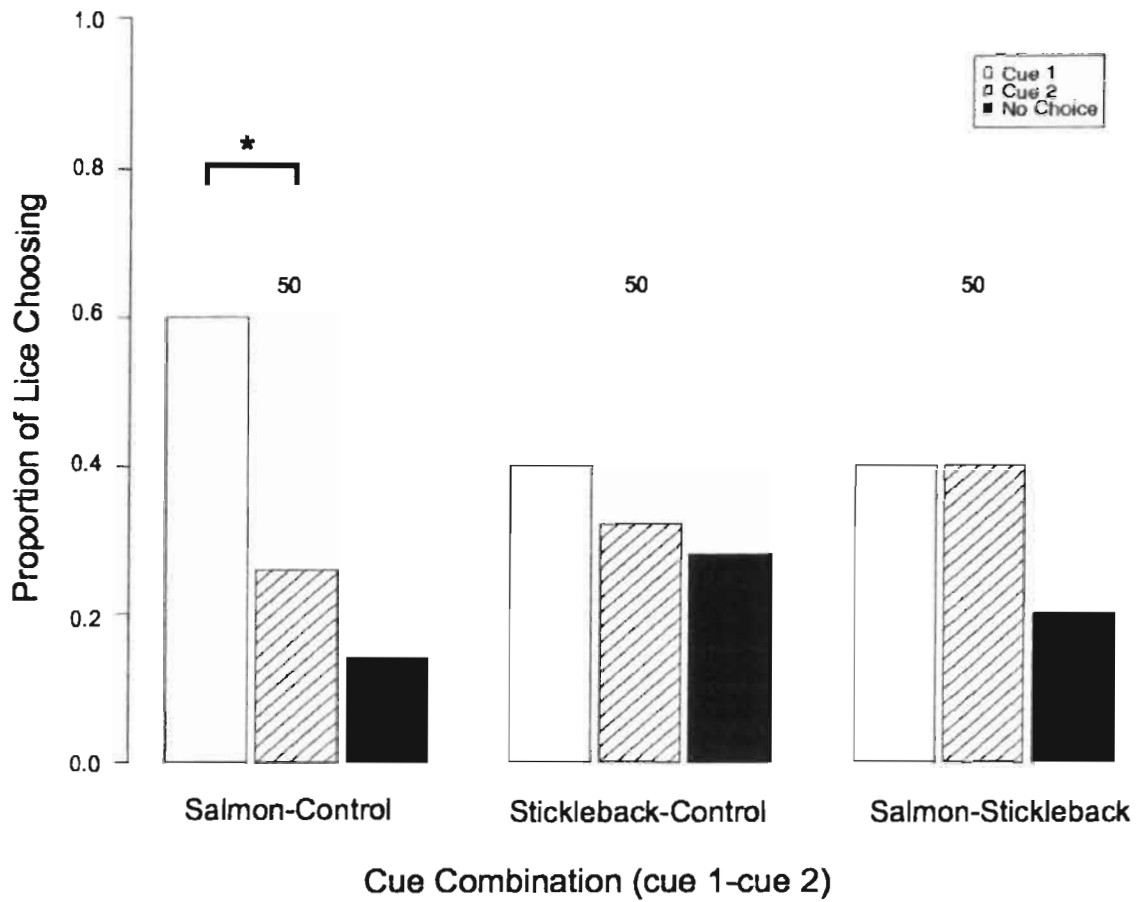


Figure 2.3 Proportions of active lice making host choices in Y-tube bioassays. “No Choice” indicates increased activity, but no arm choice made. Numbers above groups indicate total treatment sample size; asterisk indicates statistically significant difference.

water were presented to lice simultaneously, no significant preference was shown ($\chi^2=0$, $df=1$, $p=1$). Overall, there was no bias for either the left ($N=61$) or the right ($N=58$) arm of the Y-tube ($\chi^2=0.08$, $df=1$, $p=0.78$). The amount of time taken to make a choice was highly variable and did not differ statistically between cues in any cue combination treatment (ANOVA: $F_{2,111}=0.09$; $p=0.92$; Tukey post-hoc test: all three $p>0.91$).

2.4.2 Host-Specific Time to Infection

In the host-specific time to infection trials, there was no difference between the proportion of lice attaching to juvenile salmon (83.3%, $N=18$) and the proportion attaching to sticklebacks (72.2%, $N=18$) ($\chi^2=2.50$, $df=1$, $p=0.11$). However, lice attached themselves to salmon significantly more quickly than they did to sticklebacks (mean \pm 1SE; salmon: 92.17 ± 21.17 s, stickleback: 236.59 ± 55.11 s; independent sample $t_{33}=-2.22$, $p=0.03$; data were square-root transformed for normality). Figure 2.4 shows the survivorship curve for the subset of experimentally infected fish monitored for host-specific survival of lice. Lice on juvenile salmon survived significantly longer than those infecting sticklebacks (Log-Rank $\chi^2=8.33$, $df=1$, $p<0.001$).

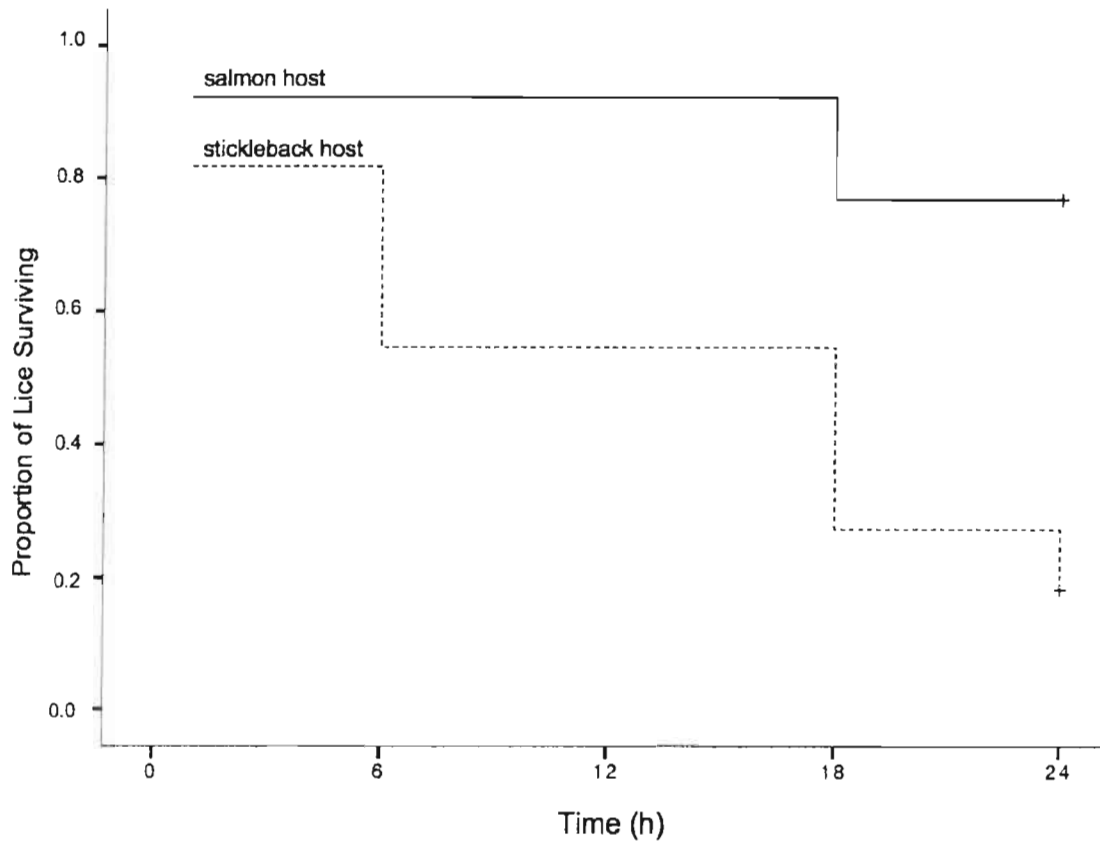


Figure 2.4 Kaplan-Meier plot of host-specific survival after infection trials. The solid line shows the survival of lice infecting juvenile pink salmon (N=13) and the dashed line shows that of lice infecting sticklebacks (N=11). The cross at the end of each line indicates censored data points of lice surviving the entire 24-hour monitoring period.

2.5 Discussion

The greater proportion of lice active in the treatments than in the controls indicates that the chemical cues elicited a behavioural response in the lice rather than upstream movement being the result of a treadmill effect of flowing water. This increased activity is an important behaviour in host location in *L. salmonis* to keep the parasite in the odour plume and increase the chance of re-encountering the odour if the signal is momentarily lost (Ingvarsdottir *et al.* 2002a). There was no difference in the proportion of lice that were active among the three treatment groups, indicating that neither salmon- nor stickleback-conditioned water was more effective at eliciting this active response. When both fish cues were presented together, there were no additive or antagonistic effects, indicating that it is simply the presence or absence of these fish-derived cues that cause the behaviour. This also confirms that water conditioned by either species contained a sufficient amount of fish-derived chemicals to elicit activity, as there is a known lower limit to concentrations detectable by *L. salmonis* (Bailey *et al.* 2006).

In terms of host preference, only the salmon-conditioned water was chosen by a significantly greater proportion of lice when presented with control water. These results are consistent with other sea louse behavioural research where both host and non-host species elicit increased activity, but only host-derived cues elicit a directional rheotaxis (Devine *et al.* 2000, Ingvarsdottir *et al.* 2002a). Here, it is important to note the salmon-host origin of all lice used in these experiments. One possible but unavoidable

confounding factor could be a prior host bias in these lice, which has been shown in another caligid copepod parasite of salmonids, *Caligus rogercresseyi* (Pino-Marambio *et al.* 2007). Avoidance behaviour has also been shown in *L. salmonis* towards non-host conditioned water (Devine *et al.* 2000). Unlike *C. rogercresseyi* and these other *L. salmonis* experiments, the lice in our experiments showed no avoidance behaviour when exposed to stickleback-conditioned water in conjunction with salmon conditioned water or control water. In the perceived absence of any other potential host (i.e., when stickleback-conditioned water was presented with control water), there was no preference for stickleback water, indicating some sort of deficiency or high cost associated with this potential host. This lack of even a temporary, default role for sticklebacks is consistent with the absence of adult lice on sticklebacks in the wild (Jones *et al.* 2006b).

If all lice receive information about the potential suitability of a host from its semiochemical signature, we would expect a marked preference for salmon-conditioned water over stickleback-conditioned water when presented simultaneously. However, this was not the case and the lack of a preference could be explained by the similar behaviour seen in *L. salmonis* copepodids where the directional responses to salmon-derived cues were no longer exhibited when a non-host cue was presented simultaneously (Bailey *et al.* 2006).

2.5.1 Host-Specific Time to Infection

Sea lice infected juvenile salmon hosts more quickly than they infected sticklebacks, which is expected from the fact that adult lice in the wild are found on salmon but essentially never (0.03% of all lice) on sticklebacks (Jones *et al.* 2006b).

There were also, however, successful infections of sticklebacks in these trials. These results are inconsistent with the lack of choice seen in the Y-tube assays, suggesting that non-chemical cues are also important for host-location and acceptance for *L. salmonis* (Genna *et al.* 2005). Sticklebacks swim and position themselves primarily by sculling with their pectoral fins, while (juvenile) salmon use their caudal fin. These full body undulations of juvenile salmon create greater disturbance in the trial containers, whereas sticklebacks are comparatively still (pers. obs.). Thus, while the decreased movement of sticklebacks may contribute to higher copepodid infection levels (Genna *et al.* 2005), it may also reduce a stickleback's suite of cues for adult lice.

Lice infecting juvenile salmon survived significantly better than did lice infecting sticklebacks. At the end of the 24-hour trial period, only 20% of the lice infecting sticklebacks remained, which further indicates that sticklebacks are an inferior host incapable of sustaining motile *L. salmonis*. The lower survival of lice on stickleback hosts suggests either that lice cannot acquire the resources they need and/or a higher immune/defense response by sticklebacks than by salmon. For *L. salmonis* to switch opportunistically from being a salmonid-specific parasite to more of a generalist one may seem adaptive in this system; however, the costs associated with infecting a new host, unrelated to the conventional one, and overcoming its immune response may be very high (Combes 1997). As a result, attempting to become a generalist parasite can ultimately result in decreased reproductive success due to this trade-off (Garamszegi 2006). Our results showing no preference by sea lice for, and decreased survival on, sticklebacks support this idea of high costs associated with host switching.

Overall, the results of these experiments illustrate differential suitability of these two fish species as hosts for sea lice. Adult lice show no tendency to choose sticklebacks as a host in response to semiochemical cues, even in the perceived absence of any other host. Also, in direct encounters, salmon are infected more quickly than are sticklebacks, and they appear to be more suitable hosts given the greater survival of sea lice on them. Although the exact role played by sticklebacks in transmission of sea lice remains to be defined, my results do not support a 'source' hypothesis but, rather, indicate that sticklebacks cannot produce viable adult parasites, which lends support to the 'sink' hypothesis.

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3 SEX-SELECTIVE PREDATION BY THREESPINE STICKLEBACKS ON SEA LICE: A NOVEL CLEANING BEHAVIOUR

3.1 Abstract

Cleaning interactions have been described in a wide range of fish species and other taxa. I discovered a novel cleaning behaviour during a study of the transmission dynamics of sea lice (*Lepeophtheirus salmonis*) between juvenile pink salmon (*Oncorhynchus gorbuscha*) and threespine sticklebacks (*Gasterosteus aculeatus*) in the Broughton Archipelago, British Columbia, Canada. Experiments showed that sticklebacks significantly reduced the number of sea lice on individual juvenile salmon. Adult female lice were preferentially consumed by sticklebacks and gravid female lice also experienced egg string cropping. Overall, 76% of gravid female lice experienced either consumption, egg string cropping, or both by sticklebacks. This preference by sticklebacks for female parasites may stem from female lice being larger than males and the added nutritional value of egg strings on gravid females. Cleaning by sticklebacks can potentially have an impact on sea louse populations on wild juvenile salmon.

3.2 Introduction

Cleaning symbioses are interspecific, mutualistic interactions in which a cleaner receives some or all of its food in the form of ectoparasites from a client, who benefits in the form of a reduction in this parasite burden (Limbaugh 1961). These relationships are widespread phenomena in nature, especially among coral reef fishes, where this behaviour has evolved independently many times in distantly related taxa (Côté 2000). These interactions are usually presumed to be mutualistic, although it has not always been possible to demonstrate this empirically. While some studies have failed to show significant, direct benefits to client populations (Cheney & Côté 2003; Grutter 1997a) or a reduction in parasite load (Grutter 1996), others have found that cleaning interactions can reduce parasite loads as much as 4.5 fold in a relatively short time (Grutter 1999). This apparent benefit to clients is further supported in nature as more heavily parasitized clients are known to visit cleaning stations more frequently than their conspecifics (Arnal *et al.* 2001).

The importance of cleaning, however, goes beyond reducing numbers of parasites on clients. Selective feeding on certain age/size classes of parasites by cleaners can alter the demographic structure of the parasite population. Preferential consumption of larger parasites can skew the parasite population size structure toward smaller body size and result in decreased biomass of parasites on client fish, even if parasite numbers are unaffected (Gorlick *et al.* 1987). For example, gnathiid parasites found in the diet of the cleaner wrasse *Labroides dimidiatus* include a greater proportion of large individuals

than do the parasites found on the body of the client – again indicating size-selective predation by the cleaner (Grutter 1997b). Opposite trends have also been seen in cleaners preferring smaller prey, likely due to throat-width limitations (Grutter & Lester 2002). Impacts of cleaning interactions, therefore, extend beyond the commonly evaluated criterion of parasite numbers.

I have discovered a cleaning interaction in the Broughton Archipelago, British Columbia, Canada, which appears to involve mutualistic benefits. The system involves a massive outmigration of juvenile pink (*Oncorhynchus gorbuscha*) and chum (*O. keta*) salmon that possess elevated sea louse (*Lepeophtheirus salmonis*) parasite loads near fish farms (Krkosek *et al.* 2005a; Morton *et al.* 2004). These artificially high parasite intensities can have dramatic negative health impacts on wild fish (Krkosek *et al.* 2006; Morton & Routledge 2005). Investigation of sea louse transmission dynamics in the region in 2006 and 2007 revealed a novel cleaning relationship between threespine sticklebacks (*Gasterosteus aculeatus*) (cleaner) and juvenile pink salmon (client). I describe this cleaning behaviour and the size-selective predation by sticklebacks involved, and its impacts on the louse load of infested juvenile pink salmon.

3.3 Methods

Wild fish were collected from the nearshore environment in the Broughton Archipelago, BC, between 1 April and 30 June, in 2006 and 2007, during the main outmigration of juvenile salmon from local streams. Juvenile pink salmon were collected

by beach seine net, and threespine sticklebacks were collected with beach seines or minnow traps. Fish were housed at the Salmon Coast Field Station on Gilford Island within the archipelago in stock tanks with flow-through seawater (28-33‰; 9-13°C) until needed for trials (0-48 hours) and were fed regularly, 3-4 times per day. All sea lice present on fish were the result of natural infestation.

Individual trials were performed in 2006 and 2007, while group trials took place only in 2007. The aim of individual trials was to understand details of the cleaning behaviour, including predictors of cleaning interactions. Group trials were used to verify the results of the individual trials in a larger arena, as both fish species exhibit shoaling behaviour.

3.3.1 Individual Trials

Juvenile salmon for individual trials were selected haphazardly from the stock tanks subject to the criterion of being infested by at least one motile (preadult or adult) louse. Each infested juvenile salmon was caught carefully using a small, soft, fine-meshed dip net and placed into a rectangular (L35 x W20 x D20 cm) plastic aquarium with a single stickleback, also chosen haphazardly from the stickleback stock tank. Louse load on these salmon was assessed once placed in the experimental aquarium as sea lice were occasionally dislodged during transfer. Accuracy of the parasite enumeration was confirmed by close inspection of all fish at the conclusion of the trials.

I created one treatment and two control setups. The treatment aquaria allowed unrestricted movement and interaction of the stickleback and infested salmon. The 2006 control setup contained one naturally infested salmon and one clean salmon, to confirm

any effect on lice was due to the presence of the stickleback (see Fig. 3.1 for the experimental set-up). For the 2007 control setup, each aquarium was divided into three sections using 6.35 mm mesh that would allow for passage of motile lice, but not of fish. This is important, as motile *L. salmonis* are able to switch hosts (Ritchie 1997) and, thus, could be consumed free-swimming in the water column.. In these 2007 control aquaria, a stickleback was placed in one end and an infested juvenile salmon in the other. The empty middle section ensured that no cleaning could occur across a single mesh layer.

All aquaria were placed in a flow-through seawater bath to buffer water temperature changes. No food was provided during most trials, however a subset of trials (N=12) was conducted with food supplementation to control for any effect of starvation over the 24-hour period. Freeze-dried krill were provided to the food-supplemented sticklebacks while they were held prior to the start of the trial and then every 2 hours during the daylight hours of the trial.

Trials took place in sets of 8-12 tanks curtained off to prevent disturbance. Trials were run for 24 hours, with regular monitoring at 6, 12, and 24 hours. Observations and video of cleaning behaviour were taken from separate tanks that were not included in the trials to ensure that conditions were identical in all experimental aquaria. At each monitoring time, juvenile salmon were inspected from above and the absence of any of the lice identified at the beginning of the trial was noted. At the end of the trial, the fork length and body depth of each juvenile salmon were measured and louse lifestage and sex were confirmed using methodology described by Krkosek *et al.* (2005b). Sticklebacks were similarly measured for total length and body depth. External gape width was also

measured using electronic calipers at the base of a closed mouth, where the upper and lower jaws meet (the posterior edge of the maxillae).

If lice were missing from a salmon at the conclusion of the 24 h trial period, the aquarium was thoroughly inspected. If no lice were found, it was assumed that the parasite had been ingested. In these cases, the stomach contents of the stickleback were examined to confirm that a cleaning event had taken place. The treatment experiment was replicated 45 times in 2006 (with 30 control replicates), and 110 times in 2007 (with 42 control replicates) (see Table 3.1a for sample sizes and lice age/sex distribution for individual trials).

3.3.2 Group Trials

For group trials, a flow-through trough (L55 x W55 x D55 cm) was separated into 3 sections, each containing approximately 120 L of seawater. Methods used to select and transfer juvenile salmon and sticklebacks were as described above.

Each treatment group consisted of 10 naturally infested juvenile salmon and 10 sticklebacks. Salmon were transferred to the experimental tanks in small containers of seawater to avoid dislodging lice. While in these containers, lifestage and sex of each motile louse were identified. Each control group consisted of 10 naturally infected juvenile salmon and 10 uninfected salmon. The sticklebacks and uninfected salmon were measured prior to each trial, as the uninfected salmon would otherwise be indistinguishable from any cleaned salmon at the conclusion of the trial. The fish were then left for 24 hours. At the completion of the trials, all fish were measured and louse

load, lifestage and sex recorded as in the individual trials. This experiment was repeated 13 times in 2007 (see Table 3.1b for sample sizes and lice characteristics).

3.3.3 Analysis

The proportion of lice of all lifestages consumed was not significantly different between 2006 (35.4%; N=79) and 2007 (37.4%; N=163) ($\chi^2=0.09$, $df=1$, $p=0.76$) in the individual trials, therefore these two years of results are combined for analysis. Control trials in 2007 saw background levels of 4.8% (individual trials, N=62 lice) and 1.2% (group trials, N=86 lice) lice lost, thus treatment louse loss is corrected by these amounts for both experimental setups. Logistic regressions were performed to identify the following potential morphometric predictors of cleaning behaviour: stickleback total length, salmon fork length, body depth of both species, stickleback external gape width, salmon louse load, and stickleback:salmon ratios of length and depth. Some data were log-transformed for normality.

3.4 Results

3.4.1 Cleaning Behaviour

Video documentation and direct observations show clear consumption by sticklebacks of lice and/or their egg strings directly from the skin of salmon (see still images from video in Fig. 3.2; see video file in Appendix B). There is no obvious posing

Table 3.1 Summary lice characteristics in treatment and control groups for (A) individual trials in 2006 and 2007, and (B) 2007 group trials.

(A)

Year		# of Trials	Total # of Lice	Adult female lice	Adult male lice	Pre-adult female lice	Pre-adult males	Pre-adult unknown sex	# trials with lice lost
2006	Treatment	45	79	49	19	2	2	7	17
	Control	30	38	18	17	0	1	2	0
2007	Treatment	110	163	77	51	13	11	11	48
	Control	42	62	10	28	14	7	3	3

(B)

Year		# of Trials	Total # Lice	Female adult lice	Male adult lice	# trials with lice lost
2007	Treatment	13	162	97	65	13
	Control	7	86	49	37	1

(i.e., immobile posture with fins held erect) by the client to solicit cleaning; however, when approached by a stickleback, salmon did not exhibit avoidance behaviour. During a cleaning interaction, the stickleback positions itself perpendicular to the salmon, and approaches the sea louse. The stickleback grabs the louse's genital complex, which is unattached to the host. The salmon can be dragged through the water during this process, indicating a great deal of force exerted by the stickleback. On several occasions, sticklebacks cropped or completely consumed the egg strings of gravid female lice without attacking the body of the louse itself. Cleaning is quantified below.

3.4.2 Cleaning Trials

A greater proportion of trials showed lice consumed from juvenile salmon when allowed direct interaction with sticklebacks (treatment) than when no interaction was permitted (2007 controls) in all individual ($\chi^2=23.43$, $df=1$, $p<0.001$) and group trials ($\chi^2=19.86$, $df=1$, $p<0.001$) (Fig. 3.1a and b). The proportions of consumed lice were not significantly different between individual (31.8%; $N=242$ lice) and group (24.7%; $N=162$ lice) trials ($\chi^2=2.40$, $df=1$, $p=0.12$). Cleaning activity resulted in a significant decrease in the mean louse load on juvenile salmon in both the individual trials (mean \pm 1SE; before cleaning: 1.56 ± 0.07 lice \cdot salmon $^{-1}$; after cleaning: 0.99 ± 0.08 lice \cdot salmon $^{-1}$; paired-sample $t_{154}=8.79$, $p<0.001$) and the group trials (mean \pm 1SE; before cleaning: 1.25 ± 0.02 lice \cdot salmon $^{-1}$; after cleaning: 0.92 ± 0.07 lice \cdot salmon $^{-1}$; paired-sample $t_{12}=4.68$, $p=0.001$). Cleaning took place within the first 6 hours in 60% of the 65 individual trials in which cleaning occurred. Stomach content analysis confirmed cleaning behaviour in all cases.

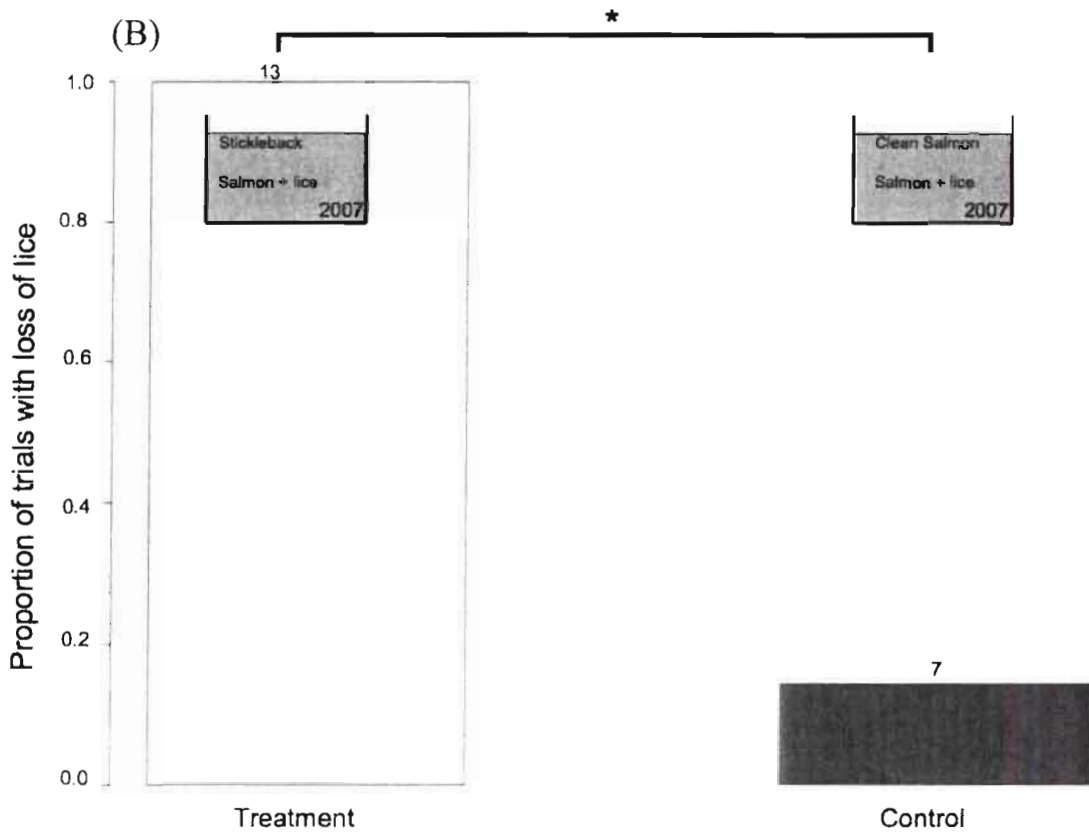
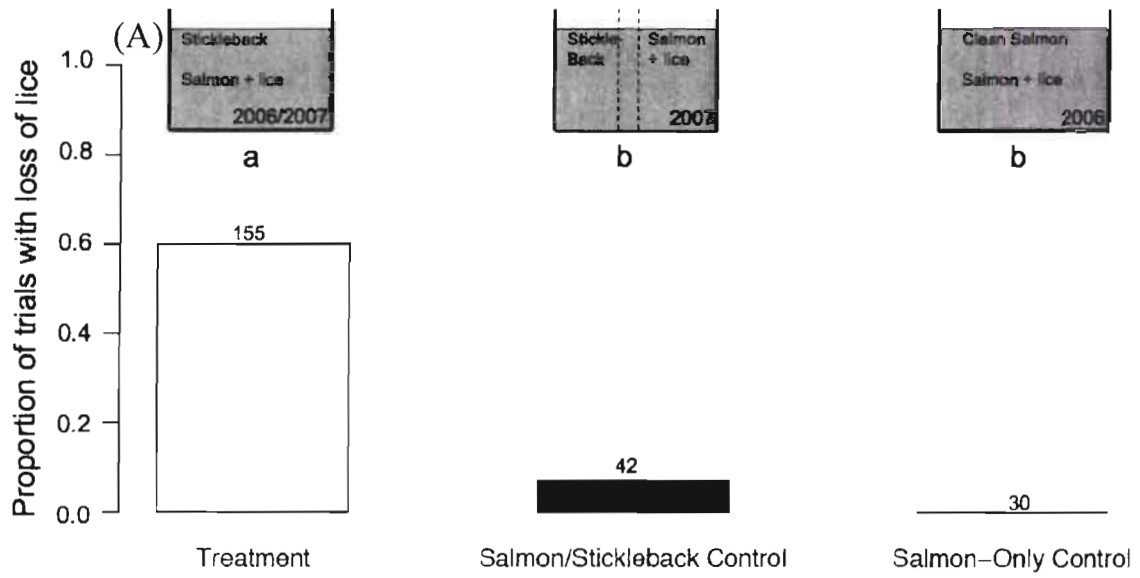


Figure 3.1 Proportion of trials in which at least one louse was lost in (A) the individual and (B) the group trials. Numbers above columns indicate sample sizes; letters above columns indicate significant differences between proportions; asterisk in (B) indicates a significant difference. Vignettes illustrate experimental setup.

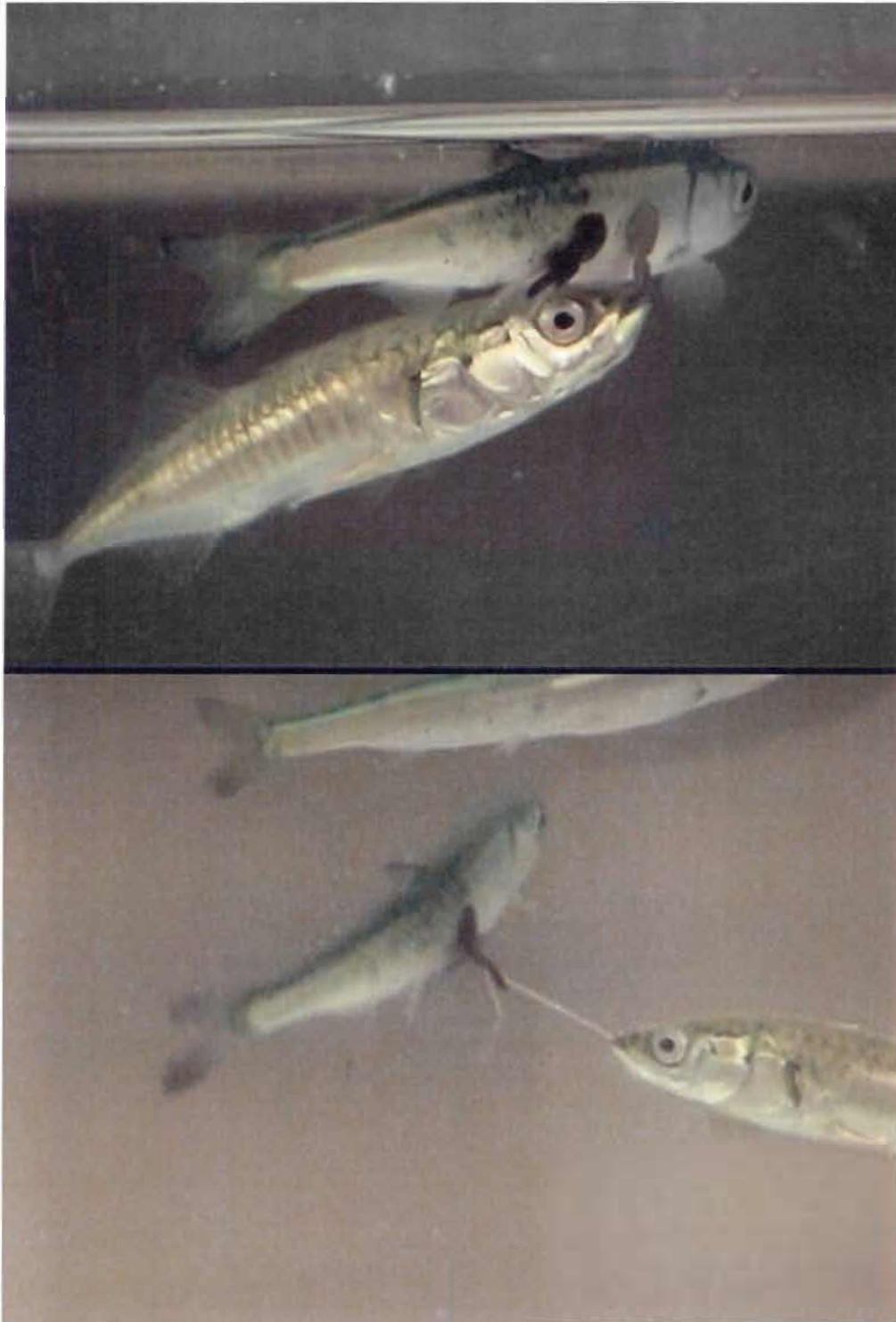


Figure 3.2 Still images of sticklebacks engaging in removal of sea lice (top) and sea lice egg string cropping (bottom) behaviours (taken from video ©Twyla Roscovich; Appendix B).

The supplementation of food had no effect on the proportion (41.7%) of trials (N=12) where cleaning took place when compared to non-supplemented trials ($\chi^2=0.01$, $df=1$, $p=0.93$).

Only the ratio of stickleback:salmon body depth showed a positive, significant relationship with the occurrence of cleaning interactions (logistic regression: $\chi^2=8.78$, $df=1$, $p=0.003$; $R^2_N=0.08$). Louse load was not a significant predictor of cleaning events (logistic regression: $\chi^2=0.75$, $df=1$, $p=0.39$; $R^2_N=0.01$; see Table 3.2 for logistic regression equation parameters).

3.4.3 Female-Biased Consumption by Sticklebacks

Sticklebacks consumed a significantly greater proportion of adult female lice than adult male lice in both the 2006 ($\chi^2=5.78$, $df=1$, $p=0.02$) and 2007 ($\chi^2=5.28$, $df=1$, $p=0.02$) individual trials (Figure 3.3a). There was no difference in the sex ratio of lice consumed between the two years ($\chi^2=1.671$, $df=1$, $p=0.20$). Similar sex-biased consumption of lice was seen in the group trials (Figure 3.3b), although it fell short of statistical significance ($\chi^2=3.15$, $df=1$, $p=0.08$).

In addition to sex-selective predation by cleaning sticklebacks on adult female lice, there was further cleaning pressure exerted on gravid female lice observed in the 2007 individual trials (2006 trials did not include gravid females). Egg string cropping occurred on 29.4% of the gravid female lice available (N=17) when sticklebacks were present, but never on gravid females (N=10) in the control groups with uninfected salmon. Gravid female lice (47.1%; N=17) were not more likely to be consumed by

Table 3.2 Parameters for logistic regression equations.

(A) Stickleback to salmon width ratio as a predictor.

Predictor	$b_0 \pm SE$	Wald, df, p	Exp(b); lower CI, upper CI
Stickleback to salmon width ratio	6.655 \pm 2.335	8.121, 1, 0.004	776.936; 7.989, 75555.275

(B) Salmon louse load as a predictor.

Predictor	$b_0 \pm SE$	Wald, df, p	Exp(b); lower CI, upper CI
Salmon louse load	0.147 \pm 0.178	0.681, 1, 0.409	1.158; 0.817, 1.642

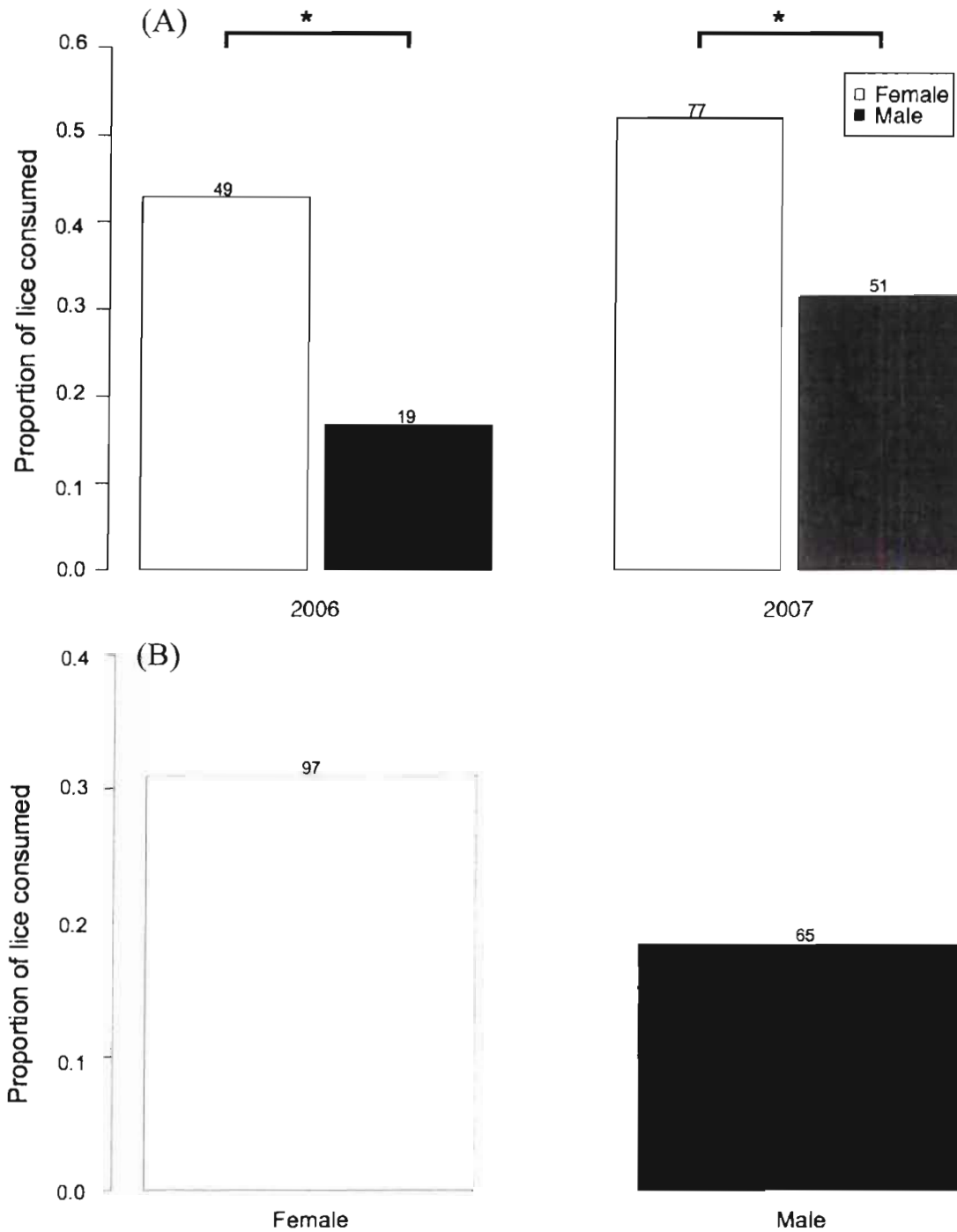


Figure 3.3 Proportion of adult female lice on juvenile pink salmon that were consumed by sticklebacks in (A) individual and (B) group trials. Numbers above columns indicate sample sizes; asterisks indicate significant differences

sticklebacks than non-gravid females (45.0%; N=60) ($\chi^2=1.33$, $df=1$, $p=0.38$); however, when combined with egg string cropping, a significantly greater proportion of gravid females (76.5%) than non-gravid females experienced at least one impact of cleaning ($\chi^2=5.26$, $df=1$, $p=0.02$) (Figure 3.4)

3.5 Discussion

3.5.1 Novel Cleaning Behaviour

The interspecific cleaning behaviour described here is the first known instance of its kind in the family Gasterosteidae or on the temperate Pacific coast of North America, widening both the taxonomic and geographic distribution of known cleaner fish (Côté 2000). Sticklebacks are primarily visual predators feeding mostly on three invertebrate groups, including copepods (which include *L. salmonis*), cladocerans, and ostracods (Wootton 1984). These characteristics lend themselves to the consumption of conspicuous ectoparasites from juvenile salmon, which are known to be seasonally sympatric with sticklebacks in the Broughton Archipelago, as they are regularly caught together in seine nets during months of salmon outmigration (Jones *et al.* 2006; B. Connors, pers. comm.; pers. obs.). Because contact with juvenile salmon infested with adult *L. salmonis* may be irregular and seasonal, this relationship may be an opportunistic behaviour resulting from the elevated parasite loads documented on juvenile salmon during this time of sympatry (Krkosek *et al.* 2005a; Morton *et al.* 2004). Similar

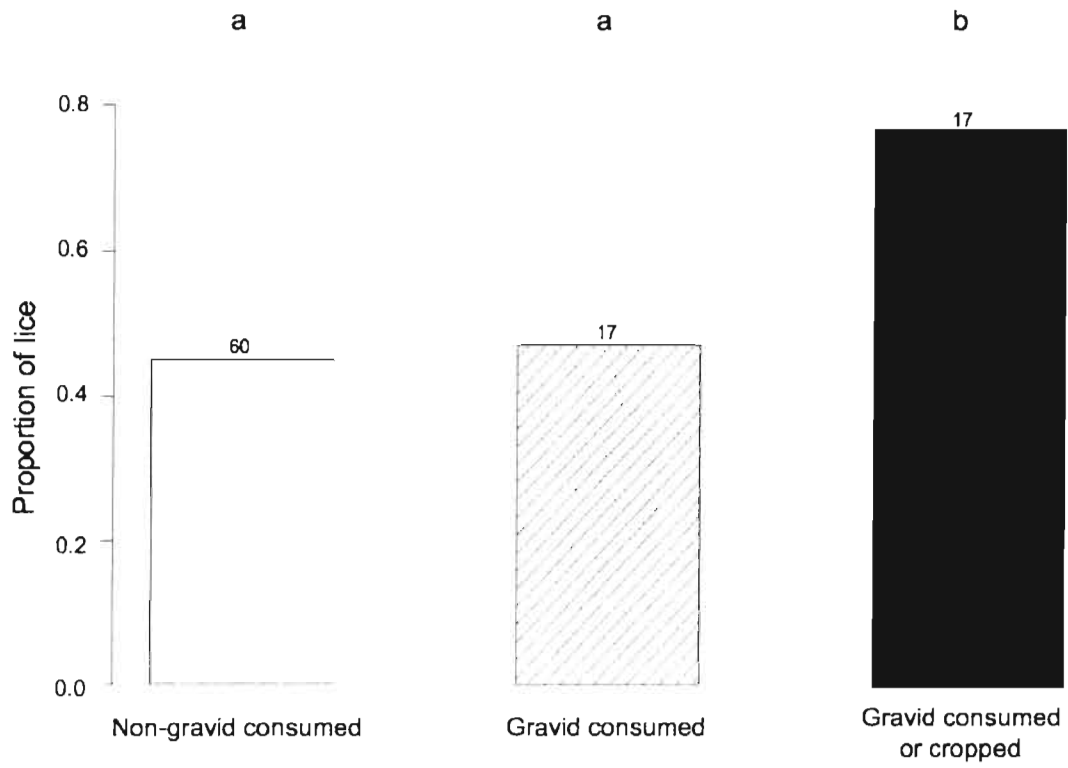


Figure 3.4 Proportion of female lice experiencing various types of cleaning behaviour in 2007 individual trials. Numbers above columns indicate sample sizes; a and b indicate significant differences between proportions.

temporal variation in diet has been noted in the cleaner wrasse, *L. dimidiatus*, which consumes more parasitic gnathiid copepods during certain months of high parasite abundance (Grutter 1997c). Threespine sticklebacks are also known to exhibit temporal changes in their diet to reflect the seasonal availability of prey items in the environment (Wootton 1984).

There are several lines of evidence that suggest the cleaning interactions studied here are not artefacts of confinement in aquaria. First, similar levels of cleaning behaviour were observed in two different experimental set-ups, including individual trials in small tanks, and group trials in much larger ones. Second, the presence or absence of food did not influence the cleaning behaviour of the sticklebacks, and most of the sea lice were consumed in the first 6 hours of the 24 h trials, suggesting that the behaviour was not the result of starvation prior to or during the trials. Finally, the absence of any avoidance behaviour by juvenile salmon indicates that this was not a coercive interaction whereby sticklebacks were taking advantage of the salmon's inability to flee while in tanks.

The absence of any formal posing behaviour by juvenile salmon could be because this is a newly developed interaction that has been facilitated by the recently elevated parasite levels on juvenile salmon in the region (Krkosek *et al.* 2005a; Morton *et al.* 2004). Posing behaviour in established tropical reef cleaning relationships is characterized by a deliberate, conspicuous body position adopted by the client that can increase the likelihood of being cleaned, but is not essential in order for cleaning to take place (Côté *et al.* 1998). In this study system, posing may not be necessary due to the conspicuous nature of these parasites. Client:cleaner body depth ratio was the only

significant predictor of cleaning behaviour, however the low R^2 value (0.075) and extremely large confidence intervals suggest a limited biological importance of this factor.

3.5.2 Effects of Cleaning

In individual trials in 2007, 4.8% of lice were lost in the control groups in which sticklebacks and salmon could not interact directly. Of these three lice lost, two were adult males, the other an early preadult whose sex could not be identified. Stomach content analysis confirmed that the sticklebacks had consumed these lice, which could only have occurred after they had left their salmonid host and strayed while free-swimming to the stickleback side of the aquaria. The sex bias in these consumed lice is consistent with sex-biased host switching behaviour previously observed in lice (Hull *et al.* 1998; Ritchie 1997), although a very small sample size limits the strength of this connection. The consumption of free-swimming lice by sticklebacks also occurred readily when lice were offered as a food item in unrelated trials (pers. obs.).

In addition to sticklebacks consuming nearly 40% of the total available lice, there was a significant trend toward consumption of adult female parasites. Removal of female lice may be particularly beneficial to juvenile salmon as females are 2-3 times larger in mass than males, and may have correspondingly greater negative impacts on their host. The reasons for the selection of adult females by sticklebacks are likely three-fold. First, the larger size of adult female lice may make them more conspicuous to visually foraging sticklebacks. Second, females have greatly reduced motility compared to males due to their larger size and *L. salmonis*' behavioural strategy of male-biased host switching,

which makes them less able to avoid predation. Finally, the larger females may also offer a greater nutritional benefit per cleaning event than males that likely outweighs any cost of increased handling time associated with this larger prey (Winfield & Townsend 1983). This benefit could be enhanced by the presence of egg strings, which may explain the elevated cleaning pressure exhibited towards gravid females.

Preferential consumption of females (and their egg strings) by sticklebacks removes the most reproductively important individuals from the louse population. For ovigerous (egg-carrying, as opposed to egg-broadcasting) non-parasitic copepods, the presence of egg masses is generally associated with increased risk of predation from visually foraging planktivorous fishes (Bollens & Frost 1991; Hairston *et al.* 1983), including sticklebacks (Vuorinen *et al.* 1983). Similar size-selective pressure on sea lice has also been documented on Atlantic salmon by cleaner wrasse (Treasurer 1994; Tully *et al.* 1996). This selective predation by planktivores contributes not only to increased mortality, but also a potential decrease in reproduction – a phenomenon that can amplify the effect of such predators (Gliwicz 1994). The combined pressure of consumption and eggstring cropping on 76.5% of the gravid female lice in our test population raises the question of how cleaning behaviour, assuming it occurs in the wild, might impact both the louse population and the population of juvenile salmon.

Cleaning by sticklebacks in the experimental enclosures significantly reduced the parasite loads of juvenile salmon. This can have important benefits to salmon, as significant mortality of juvenile salmon in this region has been seen at louse abundance levels at or below that used in our experiments (Krkosek *et al.* 2006). Juvenile salmon can sometimes succumb to infestation by a single louse (Morton & Routledge 2005).

Thus, removal of even one louse can have important survival implications for juvenile salmon and creates a strong selective pressure to reduce individual parasite loads for these fish. Conditions such as these have led to the evolution of honest cleaning relationships in established, stable systems (Freckleton & Côté 2003). The Broughton Archipelago, however, is in a state of flux due to anthropogenic disturbance and the unnaturally high levels of parasites on juvenile salmon. Nonetheless, the strong selective pressure on juvenile salmon, combined with the nutritional benefit to sticklebacks, create a set of environmental characteristics that lend themselves to the establishment of cleaning relationships.

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4 GENERAL DISCUSSION

The results of this host-parasite behavioural research begin to clarify the unique role played by threespine sticklebacks in sea louse transmission dynamics. When given the choice of host-derived cues presented in various combinations, adult male lice preferred salmon-conditioned water to control water, but showed no preference for stickleback-conditioned water in any of the treatments. The inability of sticklebacks to sustain adult lice was shown in the time-to-infection and associated adult louse survivorship trials. This finding extends across the lifecycle, as shown in the complementary chalimus survival experiment outlined in Appendix A, in which *L. salmonis* were unable to reach motile stages on sticklebacks.

The shedding and/or death of all lice from sticklebacks has been seen in previous laboratory experiments that artificially infected sticklebacks with copepodid *L. salmonis* (Jones *et al.* 2006a). In contrast to these earlier experiments, however, the results in Appendix A show motile *L. salmonis* persisting on pink salmon. This indicates that the larger salmon used by Jones *et al.* (2006a) may have had a higher immune response that allowed them to shed these lice more effectively than the smaller salmon used in my experiments, or that their cultured lice were deficient in some way. The higher intensity of sea lice (both *C. clemensi* and *L. salmonis*) on sticklebacks than on pink salmon at the time of capture is consistent with previous findings in the Broughton Archipelago, as is the lack of adult *L. salmonis* on sticklebacks (Jones *et al.* 2006b). Taken together, it is

evident that *L. salmonis* exhibits significantly decreased survivorship at every lifestage on sticklebacks compared to on pink salmon hosts. Overall, these results show that sticklebacks are very poor hosts for *L. salmonis*.

The lack of preference for and decreased survivorship on sticklebacks across all lifestages begins to clarify sticklebacks' role as sinks in this system. The sex-selective cleaning pressure on salmonid-borne sea lice described in Chapter 3 further suggests that the overall role of the stickleback in louse population dynamics may be negative, although whether this behaviour occurs at high enough levels to have population-wide effects in the wild remains to be determined.

To fully resolve this issue of sticklebacks as sources or sinks in the transmission dynamics of sea lice and juvenile salmon, behavioural and survivorship studies such as those presented here need to be combined with more studies in the wild. For example, although I have attempted to provide some justification for expecting the cleaning behaviour to be a naturally occurring phenomenon, a sampling program to analyze stickleback stomach contents would be helpful to verify the behaviour's occurrence in the wild. In addition, a study of copepodid-stage louse choice would expand the picture of host choice to both decision-making lifestages. Culturing lice and experimental infections are, however, notoriously difficult and exceeded the scope of this work. Finally, it would be helpful to scale up laboratory and wild sampling studies to the level of populations, in order to assess, for example, whether deficiencies in suitability of sticklebacks as temporary hosts are offset by sufficiently large numbers of sticklebacks carrying lice to augment infections of wild salmon from other sources.

Ultimately, in order for sticklebacks to be a source of infection for wild salmonids, lice must survive a developmental deficiency on sticklebacks, switch to a salmonid host while avoiding predation in the water column and then avoid cleaning behaviour once settled on a new host. In addition, there is no evidence to show that recruitment of lice from stickleback to salmon exceeds the mortality experienced by lice on this inferior host, which is ultimately required to consider them a source (Pulliam 1988). With this in mind, the conclusions drawn in the literature regarding sticklebacks' source role in this system (Beamish *et al.* 2006; Jones *et al.* 2006a,b) must be considered to be unsubstantiated and unlikely.

4.1 Literature Cited

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5 APPENDICES

5.1 Appendix A – Host-specific chalimus survival

5.1.1 Methods

Naturally infected sticklebacks and pink salmon were selected for trials based on the criterion of being infected with a minimum of one chalimus-stage louse. Fish were measured for fork length and body depth, and louse load was assessed visually according to a key by Galbraith (2005). The location of each louse on its host was also recorded.

Each stickleback (N=10) and salmon (N=7) was kept in its own plastic aquarium (L35 x W20 x D20 cm) for the duration of the test period (18 d). The mean length of salmon was 53.9 ± 2.32 SE mm. Sticklebacks had a mean length of 62.7 ± 1.61 mm. Louse load of fish was re-assessed visually every 2 days and lifestage of lice recorded throughout. Any disappearance of lice was noted; when lice were missing, a thorough inspection of the tank was conducted. It is assumed that the hosts consumed any lice unaccounted for. Species of louse was identified only in motile stages, as chalimi are difficult to differentiate without killing the fish host (Krkosek et al. 2005).

5.1.2 Analysis

Since live-sample species identification of chalimus-stage sea lice was not possible, background lice species ratios on fish are assumed to have been consistent with those captured by a Department of Fisheries and Oceans (DFO) sampling program carried out in the Broughton Archipelago during the same time period as these experiments (Spring 2007). *L. salmonis* made up 25.4% of lice on sticklebacks (N=1.255) and 67.7% on juvenile pink salmon (N=2.034) in 2007 (S. Jones *et al.*, unpublished data). All other sea lice were *Caligus clemensi*. These proportions were used to calculate binomial probabilities of louse distribution on test fish. See Table 5.1 for host-specific abundance and estimates of louse species abundance on test fish.

5.1.3 Results

By the end of 18 d, there were no lice remaining on any of the sticklebacks and only 2 motile lice were found in the aquaria. These were both *C. clemensi* from a single stickleback. In contrast, motile lice were found on four of the juvenile salmon during the experiment (see Fig. 5.1).

Of all motile lice (N=11) infecting sticklebacks throughout the trial, none were *L. salmonis*, which is significantly fewer than predicted by DFO's sampling program (exact binomial; $p=0.040$). In contrast, seven of the motile lice (N=10) infecting pink salmon were *L. salmonis*, as expected from the background levels (exact binomial; $p=0.498$). Table 5.1 provides a summary of background and observed infection rates.

Table 5.1 Levels of *L. salmonis* and *C. clemensii* infecting test fish.

Species	Expected* proportion of <i>L. salmonis</i> infection	Expected* proportion of <i>C. clemensii</i> infection	Expected* # motile <i>L.</i> <i>salmonis</i> on test fish	Expected* # motile <i>C.</i> <i>clemensi</i> on test fish	Observed # motile <i>L.</i> <i>salmonis</i> on test fish	Observed # motile <i>C.</i> <i>clemensii</i> on test fish
Stickleback (N=10)	0.254	0.746	2.79	8.21	0	11
Pink Salmon (N=7)	0.677	0.323	6.77	3.23	7	3

*Calculated from S. Jones *et al.*'s DFO sampling data (2007)

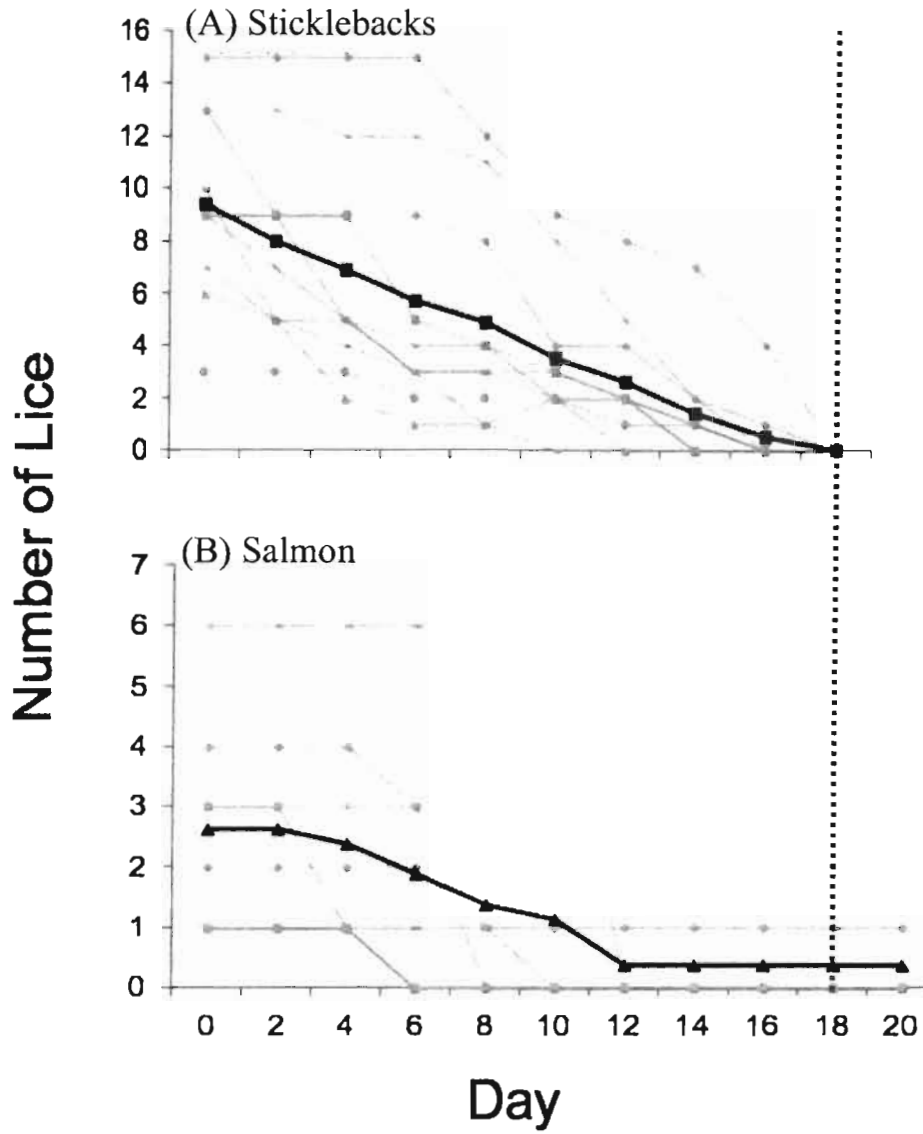


Figure 5.1 Louse loads on sticklebacks (A) and juvenile pink salmon (B) over the monitoring period. Grey lines indicate louse loads for individual test fish, black lines are the means. Vertical dashed line shows end of test period.

5.1.4 Literature Cited

Jones, S. R. M., Hargreaves, B., Morton, A. 2007. Unpublished data

Galbraith, M. 2005 Identification of larval stages of *Caligus clemensi* and *Lepeophtheirus salmonis* from the Broughton Archipelago: Canadian Technical Report of Fisheries and Aquatic Sciences, Department of Fisheries and Oceans Canada, Sidney BC.

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5.2 Appendix B: DVD-ROM

The DVD-ROM attached forms a part of this work. Video is property of Twyla Roscovich and used with her permission.

Movie file can be opened with QuickTime or other video program.

Movie File:

- Cleaning Behaviour Movie 78.4 MB