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# ENZYMES RELEASED FROM *LEPEOPHTHEIRUS SALMONIS* IN RESPONSE TO MUCUS FROM DIFFERENT SALMONIDS

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ABSTRACT: Adult and mobile preadult sea lice *Lepeophtheirus salmonis* were incubated with mucus samples from rainbow trout (*Oncorhynchus mykiss*), coho salmon (*O. kisutch*), Atlantic salmon (*Salmo salar*), and winter flounder (*Pseudopleuronectes americanus*) to determine the response of *L. salmonis* to fish skin mucus as assessed by the release of proteases and alkaline phosphatase. There was variation in the release of respective enzymes by sea lice in response to different fish. As well, sea lice collected from British Columbia responded differently than New Brunswick sea lice to coho salmon mucus. Fish mucus and seawater samples were also analyzed using protease gel zymography to observe changes in the presence of low molecular weight (LMW) proteases after *L. salmonis* incubation. Significantly higher proportions of sea lice secreted multiple bands of *L. salmonis*–derived LMW proteases after incubation with rainbow trout or Atlantic salmon mucus in comparison with seawater, coho salmon, or winter flounder mucus. Susceptibility to *L. salmonis* infections may be related to the stimulation of LMW proteases from *L. salmonis* by fish mucus. The resistance of coho salmon to *L. salmonis* infection may be due to agents in their mucus that block the secretion of these LMW proteases or factors may exist in the mucus of susceptible species that stimulate their release.

The ectoparasitic copepod *Lepeophtheirus salmonis* has a host range that includes most species of Salmonidae reared in seawater (*Oncorhynchus* spp., *Salmo* spp., and *Salvelinus* spp.) (Kabata, 1979). *Lepeophtheirus salmonis* has a direct life cycle consisting of 10 stages. These include 2 free-swimming naupliar stages, 1 free-swimming infectious copepodid stage, 4 attached chalimus stages, 2 mobile preadult stages, and a mobile adult stage (Johnson and Albright, 1991). Whereas *L. salmonis* has occasionally been reported on other hosts, this is infrequent and primarily observed in the mobile preadult stage of its life cycle (Bruno and Stone, 1990).

On Atlantic salmon *Salmo salar*, *L. salmonis* develop faster and exhibit higher intensities of infection than on other salmonids, especially coho salmon *Oncorhynchus kisutch* (Johnson and Albright, 1992; Johnson, 1993). Whether these developmental and intensity differences among hosts are due to the nutritional content of the mucus, skin, and blood on which sea lice feed, easier evasion of host defenses for certain species, or a combination of both of these factors, is not yet understood. Coho salmon have a documented resistance to *L. salmonis* (Johnson and Albright, 1992; Fast et al., 2002). In a cohabitation challenge experiment, *L. salmonis* initially infected coho salmon, Atlantic salmon, and rainbow trout at the same densities, with *L. salmonis* numbers rapidly decreasing on coho salmon within 2 wk (Fast et al., 2002). Resistance to *L. salmonis* infection is hypothesized to be nonspecific due to the rate *L. salmonis* are lost from coho salmon (Johnson and Albright, 1992; Fast et al., 2002) and the apparent lack of an antibody response against *L. salmonis* in laboratory-infected Atlantic salmon (Grayson et al., 1993). There is little difference in blood physiology between resistant (coho salmon) and susceptible species (rainbow trout and Atlantic salmon), even during rejection of *L. salmonis* by coho salmon (Fast et al., 2002). Therefore, it could be hypothesized that the differential resistance may be either due to factors in the mucus or epithelial layers (or both) or differences in the innate humoral or cellular immune responses of these host species.

The mucus layer of fishes is the first site of interaction between host and pathogen and the first potential line of defense for these hosts. *Lepeophtheirus salmonis* spends the entire parasitic portion of its life cycle in contact with the skin mucus of its fish host (Johnson and Albright, 1992). The chalimus stages feed exclusively on mucus and epidermis, but preadult and adult stages are also capable of feeding on blood (Brandal et al., 1976). Firth et al. (2000) reported that *L. salmonis* releases trypsinlike proteases in the presence of Atlantic salmon mucus, possibly to aid in feeding, or avoidance of host immune responses, or both. The source of increased alkaline phosphatase (AP) and lysozyme activities in fish mucus after *L. salmonis* infection (Firth et al., 2000; Fast et al., 2002) is not known.

In the study reported here, the response of *L. salmonis* to mucus from salmonids with differing susceptibilities to infection and a nonhost nonsalmonid species, i.e., the winter flounder *Pseudopleuronectes americanus* were assessed by measuring enzyme release. By incubating live *L. salmonis* with samples of fish mucus it becomes possible to observe their responses to differing mucus substrates in the absence of active host cells. It was hypothesized that observing the release of various enzymes by *L. salmonis* in response to fish mucus would elucidate the ability of *L. salmonis* to survive in contact with the mucus layer of different hosts. In addition, the responses of *L. salmonis* from New Brunswick and British Columbia to salmonid mucus were examined to compare responses of these geographically distinct sea lice populations.

## MATERIALS AND METHODS

### Fish maintenance and collection of mucus

Yearling (S1) Atlantic salmon (Saint John River strain) and rainbow trout (Kamloops strain) were obtained from the Department of Fisheries and Oceans (DFO) certified hatcheries in Pictou, Nova Scotia, and Murray River, Prince Edward Island, respectively. Coho salmon (S1) were obtained from the research facilities of Aqua Health Limited, Charlottetown, Prince Edward Island (original source: DFO's Big Quallicum hatchery, Vancouver, British Columbia). On arrival, fish of each species were placed in separate 1,500-L tanks and acclimated to seawater by gradually increasing the salinity with artificial seawater (Instant Ocean®, Aquarium Systems, Mentor, Ohio) over a 2-wk period. The fish were maintained in 30 ± 2 ppt (salinity) at 10 ± 1 C. Photoperiod was kept

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at 14-hr light and 10-hr dark, and fish were fed twice daily to satiation. Atlantic salmon, rainbow trout, and coho salmon had average weights (±SD) of 113 ± 23 g, 188 ± 40 g, 39.5 ± 10 g, respectively. Six fish of each species were anesthetized with tricaine methanesulfonate, MS-222 (100 mg/L), and gently placed in plastic bags filled with 10 ml of sterile seawater (28 ppt salinity) to collect mucus as described by Ross et al. (2000). Mucus from individuals of each species was then pooled, aliquoted, and stored at −80 C. Juvenile winter flounder (150–200 g each) were obtained from the Ocean Sciences Centre, Memorial University, Newfoundland, and maintained in 500-L tanks at 16 C in artificial seawater (23 ppt salinity). Photoperiod was kept at 14-hr light and 10-hr dark, and fish were fed twice daily to satiation. Six flounder were anesthetized and mucus sampled using the same procedure as for the salmonids.

### Sea lice collection and maintenance

Atlantic salmon (Saint John River strain maintained at 10 C and 30 ppt salinity in 40-L tanks) were infected with *L. salmonis* copepodids cultured from eggs obtained at an aquaculture site in the Bay of Fundy, New Brunswick. *Lepeophtheirus salmonis* were grown on salmon for 38 days under laboratory conditions as described by Bowers et al. (2000) before adult and preadult stages were removed with forceps. Sea lice were then kept off-hosts in aerated seawater (10 L) of the same temperature and salinity for 24 hr before incubation to presumably allow enough time for them to digest their gut contents.

Adult and preadult *L. salmonis* were also collected from Atlantic salmon located at an aquaculture site in Brown's Bay, Vancouver Island, British Columbia. Adult and preadult *L. salmonis* sampled in British Columbia were transported to the Pacific Biological Research Station, Nanaimo, British Columbia, in water at the same temperature and salinity as the source site. *Lepeophtheirus salmonis* were maintained in seawater for the same amount of time (24 hr) and under the same conditions (10 C) as *L. salmonis* sampled from laboratory salmon.

### Incubation conditions

All mucus samples were thawed on ice and diluted 1:1 with sterile seawater (28 ppt salinity) to a 400 μl volume in 1.5-ml centrifuge tubes. One adult or preadult *L. salmonis* was then added to each tube and incubated at 10 C for 1 hr. *Lepeophtheirus salmonis* were then removed and conditioned mucus samples frozen at −80 C until analyzed. *Lepeophtheirus salmonis* were also incubated in 400 μl seawater (28 ppt salinity) as a negative control. This procedure was carried out 30 times with pooled mucus samples from each species as well as with seawater. Before incubation, *L. salmonis* were checked for vitality to prevent using weak or dead sea lice, and only those that were actively swimming, moving, or attached to the sides of the aerated containers were used in the experiments.

### Biochemical analysis of mucus after incubation

Before analysis, conditioned mucus samples were thawed on ice and centrifuged at 9,300 *g* for 2 min, with the supernatant used for each assay. All assays were carried out using a Thermomax Microplate Reader (Molecular Devices, Sunnyvale, California). Protein concentrations of each fish species' mucus were determined using a dye binding kit (BioRad, Hercules, California) (Bradford, 1976). Protein concentrations of the rainbow trout, coho salmon, Atlantic salmon, and winter flounder mucus were 0.293, 0.151, 0.217, and 0.101 mg $ml^{-1}$, respectively. Protease activities were determined using the azocasein hydrolysis assay and zymography as described by Firth et al. (2000). The azocasein hydrolysis assay involved incubation of conditioned mucus samples (50 μL) with azocasein substrate (125 μL of 5 mg $ml^{-1}$ azocasein in 100 mM $NH_4HCO_3$ at pH 7.8) for 19 hr at 30 C. The reactions were stopped by adding trichloroacetic acid to a final concentration of 4.5% and centrifuged to pellet any nonhydrolyzed azocasein. Resultant supernatants (100 μl) were added to individual wells in a microplate containing equal volumes of 0.5 M NaOH, and absorbance at 405 nm was determined (Firth et al., 2000). One unit of activity was defined as the amount of enzyme that catalyzed an increase in absorbance (405 nm) of 0.001 OD $hr^{-1}$. 19 Mucus samples for zymography were diluted 1:1 with 4% sodium dodecyl sulfate, 20% glycerol, 125 mM Tris–HCl at pH 6.8, and equal amounts of protein added to 12% polyacrylamide gels containing 0.1% gelatin (Firth et al., 2000). After electrophoresis, gels were washed 3 times at 4 C with 2.5% Triton X-100, 50 mM Tris–HCl at pH 7.5, and incubated (30 C) for 19 hr with the same buffer containing 50 mM $MgCl_2$ and 6.25 mM $CaCl_2$. After the incubation gels were stained in 0.1% amido black in MeOH:$H_2O$:AcOH (45:45:10) for 1 hr and destained with MeOH:$H_2O$:AcOH (50:48:2) until the desired contrast was obtained. Alkaline phosphatase activity was determined by incubating mucus samples with 4 mM *p*-nitrophenol phosphate in 100 mM $NH_4HCO_3$ at pH 7.8, 1 mM $MgCl_2$ according to the methods described by Ross et al. (2000). The change in optical density at 405 nm was measured over a 30-min period at 30 C, and the initial rate of activity was determined. One unit of activity was defined as the amount of enzyme required to release 1 μmol of *p*-nitrophenol product in 1 min. The extinction coefficient of *p*-nitrophenol in the microplate wells was experimentally determined. Lysozyme activity was determined using a turbidometric assay (Ross et al., 2000). Briefly, mucus samples were lyophilized, resuspended in an equal volume of 40 mM $NaH_2PO_3$ (pH 6.2) and then incubated in a 96-well plate with lyophilized *Micrococcus lysodeikticus* at 30 C for 30 min. The initial rate of reaction was used to calculate the activity, with 1 U of activity being defined as the amount of enzyme that catalyzed a decrease in absorbance (450 nm) of 0.001 OD $min^{-1}$.

### Statistical analysis

Statistical analyses were performed using SigmaStat for Windows, Version 2.0 (SPSS Inc., Chicago, Illinois). Enzyme activity data were analyzed using 2-way ANOVA to compare *L. salmonis*–treated versus nontreated mucus and to compare differences in enzyme activity between host species. Multiple comparisons were conducted using Tukey's test analysis. Differences were considered significant at $P < 0.05$. Data were checked for normality, and nonnormal data were transformed using ($\sqrt{x}$) or log (x + 1) transformations. All numerical values shown in this report are mean ± SEM, except in the case of salmonid population weights, from which samples were taken, where SDs are presented. Enzyme data were analyzed as a positive or negative response from *L. salmonis*. If the enzymatic activity in a *L. salmonis*–treated incubation was significantly greater than the mean activity of nontreated mucus controls, this was considered a positive response. This provided information on the fraction of responders in the *L. salmonis* population as apposed to the degree of response. Results were then analyzed using a chi-square test. Zymography measurements of protease activity were assessed as the presence or absence of *L. salmonis*–derived low molecular weight (LMW) protease bands. A positive response was assigned when the series of LMW protease bands were present in the zymogram (Ross et al., 2000). Results were analyzed using a Chi-square test.

## RESULTS

*Lepeophtheirus salmonis*–derived LMW proteases were present as multiple bands on a zymogram in contrast to a single protease band in coho salmon or rainbow trout mucus (Fig. 1). There was no significant difference in the number of New Brunswick *L. salmonis* secreting multiple bands of LMW proteases in the presence of coho salmon (20%) or flounder mucus (37%) when compared with the seawater control (23%) (Fig. 2A). In comparison, multiple bands of LMW proteases were produced by 87 and 73% of New Brunswick *L. salmonis* in response to Atlantic salmon and rainbow trout mucus samples, respectively (Fig. 2A). Zymography gels did not show differences among the protease profiles released by British Columbia and New Brunswick *L. salmonis* in the presence of fish mucus from the 4 fish species, and the numbers of British Columbia *L. salmonis* secreting LMW proteases in response to coho, Atlantic salmon, and rainbow trout (Fig. 2B) were similar to the response of New Brunswick sea lice (Fig. 2A).

Mucus protease activity assessed by azocasein hydrolysis showed no significant changes after New Brunswick *L. salmonis* incubation for all species except rainbow trout (Fig. 3). A significantly higher percentage of New Brunswick *L. sal-*

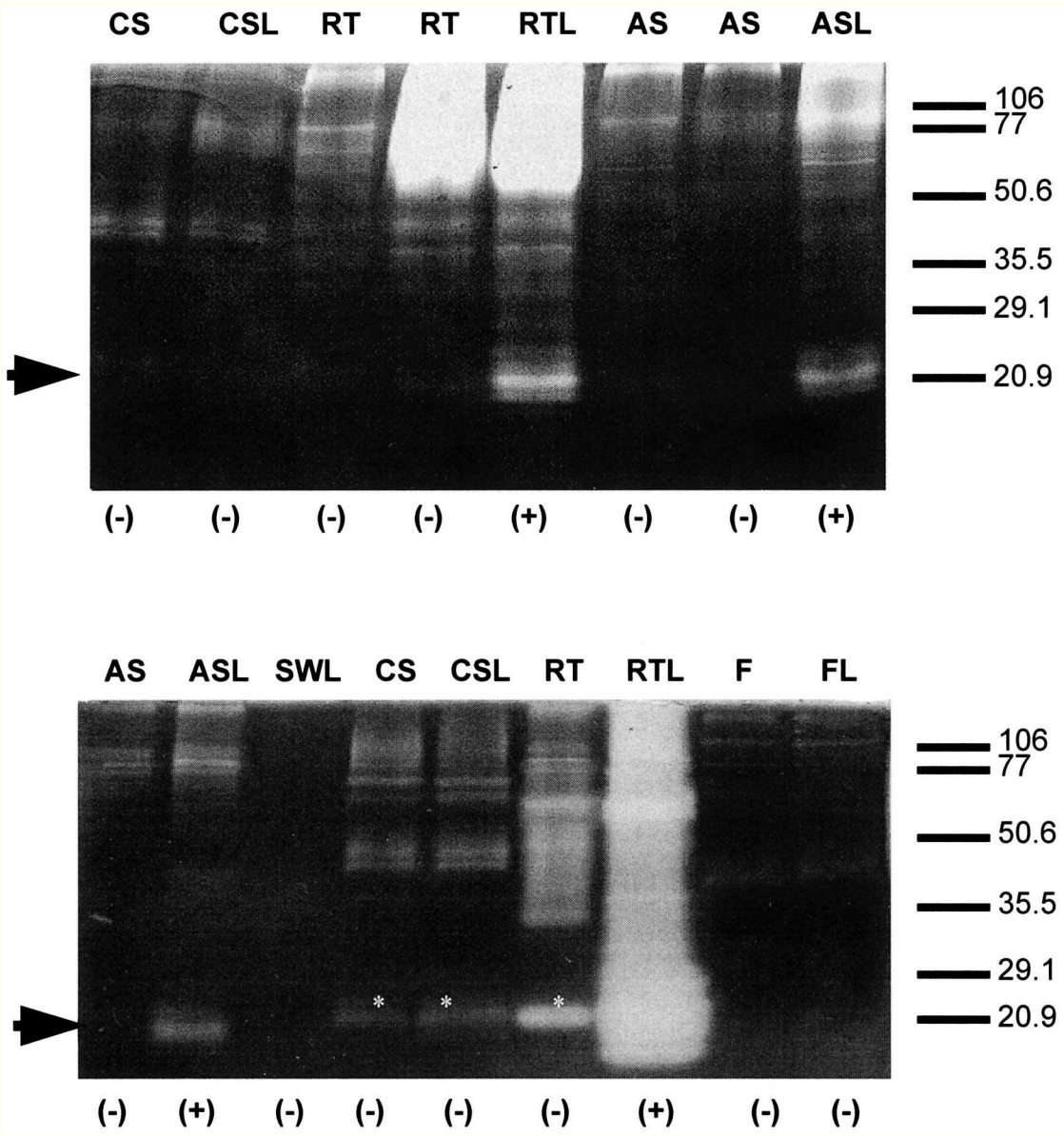


FIGURE 1. Zymograms of protease activity in mucus samples of coho salmon (CS), rainbow trout (RT), Atlantic salmon (AS), winter flounder (F) and seawater (SW) incubated for 1 hr without or with (L) live New Brunswick *Lepeophtheirus salmonis*. Each well contains 3 μg of mucus protein and samples chosen are representative of each species. Numbers along the side of the gel indicate molecular weights (kDa). Indicates sea lice LMW proteases, (*) indicates single bands of endogenous protease, (+) indicates a positive response, (−) indicates a negative response. All fish samples were analyzed using protease zymography, and representative samples chosen exhibited the most common mucus protease patterns for each species.

*monis* also released proteases into rainbow trout mucus than those that did not (Fig. 2A). However, in the azocasein assay, protease activity was significantly higher in coho mucus samples incubated with British Columbia *L. salmonis* than with New Brunswick *L. salmonis*–treated or mucus-only controls (Fig. 3). This corresponded with a significantly higher percentage of British Columbia *L. salmonis* releasing proteases in coho salmon mucus samples than those that did not (Fig. 2B). Interestingly, this was not accompanied by an increase in the number of British Columbia sea lice releasing LMW proteases. British Columbia *L. salmonis* appeared to have higher protease activities versus control mucus after incubation with Atlantic salmon or rainbow trout mucus; however, these differences were not significant (Fig. 3). There was a significant increase in the number of British Columbia *L. salmonis* having increased protease activity in response to rainbow trout mucus (Fig. 2B). There was no distinct relationship between the life stage or gender of New Brunswick *L. salmonis* and the LMW proteases released into the fish mucus.

There was no significant difference in AP activity in the mucus of rainbow trout, coho salmon, or flounder regardless of whether the mucus was incubated with New Brunswick *L. salmonis* or not (Fig. 4). Only Atlantic salmon mucus exhibited a significantly higher AP activity (vs. controls) after 1-hr incu-

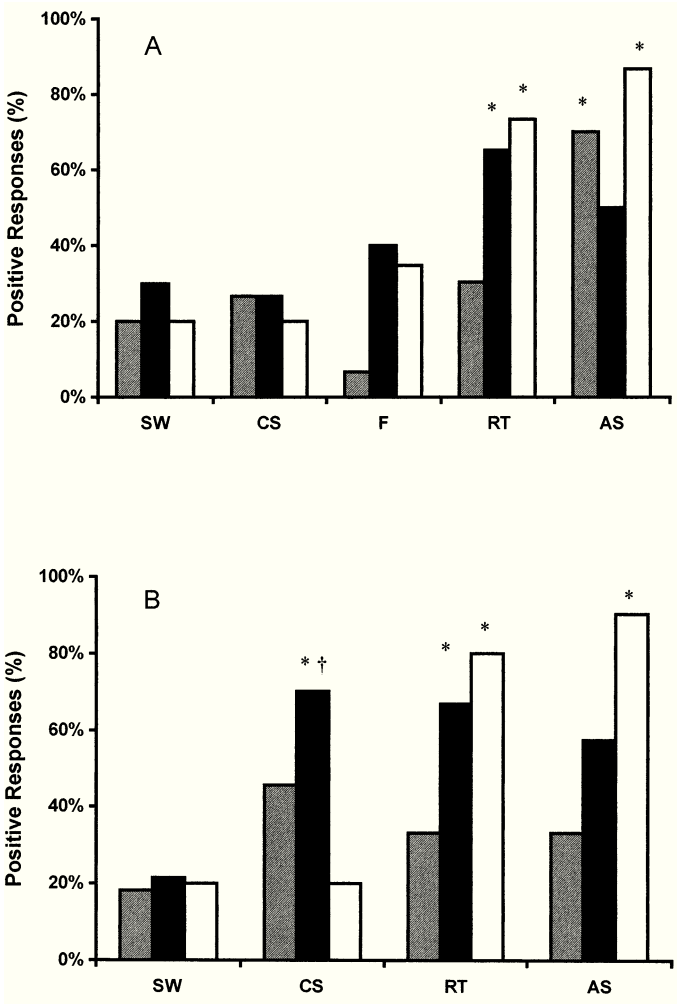


FIGURE 2. Percentage of *L. salmonis* producing AP , proteases , and multiple LMW proteases after 1 hr of incubation in the presence of seawater (SW), coho salmon mucus (CS), winter flounder mucus (F), rainbow trout mucus (RT), and Atlantic salmon mucus (AS). (**A**) New Brunswick *L. salmonis*, (**B**) British Columbia *L. salmonis*. Increases in AP and protease (as assessed by azocasein hydrolysis) activities above mean mucus levels and the presence of multiple LMW protease bands in zymograms were considered positive responses for each parameter, and the results are displayed as a percentage of the total number of replicates in which a positive response occurred (n = 30). * Indicates significant differences from SW controls ($P < 0.05$).

bation with New Brunswick *L. salmonis* (Fig. 4). Atlantic salmon control mucus also had significantly lower AP activity than that of all other species. *Lepeophtheirus salmonis* also produced AP activity in a significantly higher percentage of Atlantic salmon mucus samples than those that did not (Fig. 2A). Alkaline phosphatase activities were not significantly different from Atlantic salmon, rainbow trout, and coho salmon mucus samples incubated with British Columbia and New Brunswick *L. salmonis*. The percentage of British Columbia *L. salmonis* that produced AP activity in fish mucus was also not significantly different from the seawater controls (Fig. 2B).

Lysozyme activity was low in the mucus of all pooled samples before and after incubation with New Brunswick *L. salmonis*. There were no significant differences in mucus lysozyme activity in any pooled mucus samples after New Brunswick *L.*

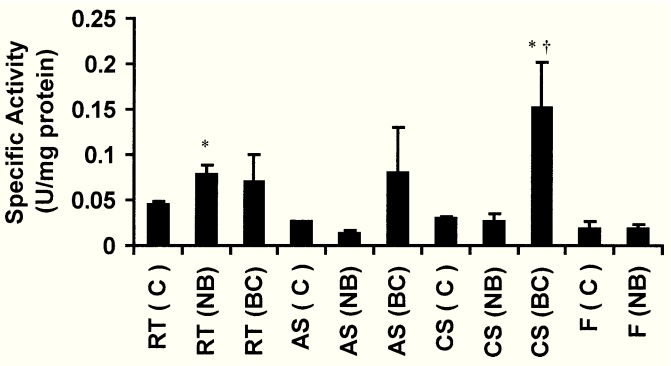


FIGURE 3. Mean (±SEM) specific activity of proteases (as measured by azocasein hydrolysis) in mucus of rainbow trout (RT), Atlantic salmon (AS), coho salmon (CS), and winter flounder (F) after 1 hr of incubation with live New Brunswick *L. salmonis* (NB) and British Columbia *L. salmonis* (BC). C = mucus incubated without lice. * Indicates significant differences from control ($P < 0.05$) (n = 30 for New Brunswick sea lice and n = 20 for British Columbia sea lice), † indicates significant differences between British Columbia and New Brunswick sea lice.

*salmonis* incubation, and New Brunswick *L. salmonis* did not release lysozyme into a significant percentage of any species' mucus.

## DISCUSSION

Proteases are salivary constituents of several types of parasites, including helminths and arthropods (Hotez and Cerami, 1983; Kerlin and Hughes, 1992; Berasain et al., 1997; Rosenfeld and Vanderberg, 1998). The consistent release of LMW proteases by *L. salmonis* in the presence of Atlantic salmon and rainbow trout mucus indicated a preference for host substrate. The multiple bands of LMW proteases observed in 70–80% of the rainbow trout and Atlantic salmon incubations, however, is lower than the 100% prevalence in Atlantic salmon mucus incubations observed previously (Firth et al., 2000). The greater time between removal of *L. salmonis* from the host, incubation with mucus (96–144 hr), and higher incubation temperature (21 C) may account for the higher prevalence of these bands in the

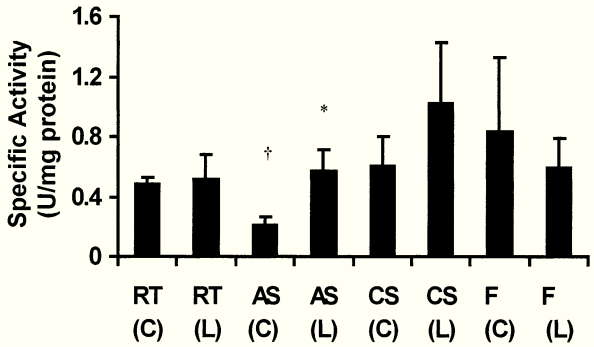


FIGURE 4. Mean (±SEM) specific activity of AP in mucus samples of 4 fish species incubated for 1 hr with live New Brunswick *L. salmonis*. Rainbow trout (RT), Atlantic salmon (AS), coho salmon (CS), and winter flounder (F). C = control mucus and L = mucus incubated with New Brunswick *L. salmonis*; *indicates significant differences from control ($P < 0.05$, n = 30), † indicates significantly lower activity than all other species.

study of Firth et al. (2000). *Lepeophtheirus salmonis* LMW proteases, however, may constitute little of the total increase in proteases observed in fish mucus as measured by azocasein. For instance, the increases in LMW protease activity observed by zymography may not correspond to increases in overall azocasein hydrolyzing activity as they may represent insignificant amounts as compared with other proteases present in fish mucus. Alternatively, trypsin (a LMW protease) activity may also be underestimated using azocasein as a substrate because azoproteins have modified lysine groups that may decrease the number of trypsin cleavage sites (Charney and Tomarelli, 1947).

Previous work characterized the LMW proteases released by *L. salmonis* in the presence of salmon mucus as trypsin (Firth et al., 2000). Trypsin is found in the guts and saliva of several other types of arthropod parasites (Kerlin and Hughes, 1992), which also inhibits phagocytosis in monocytes (Huber et al., 1968). Thus, trypsin derived from *L. salmonis* may decrease host phagocytic activity and immune responses after infection. A reduced phagocytic and respiratory burst response was observed in *L. salmonis*–infected rainbow trout and Atlantic salmon at the same time that the multiple bands of LMW proteases occurred in the mucus (Fast et al., 2002). This occurred in the absence of an integrated stress response. Saliva from other arthropod parasites has also shown the ability to decrease macrophage function in their respective hosts. The tick *Rhipicephalus sanguineus* has been shown to impair host T-cell proliferation and γ-interferon (IFN-γ)–induced macrophage microbicidal activity in mice (Ferreira and Silva, 1998). Saliva from the sand fly *Phlebotomus papatasi* has shown the ability to inhibit host IFN-γ–induced macrophage activation (Hall and Titus, 1995). The multiple bands of LMW proteases (trypsin) may be linked to virulence of sea lice either through immunomodulation or in their capacity as digestive enzymes. Zuo and Woo (2000) observed that metalloprotease secretions from the hemoflagellate *Cryptobia salmositica* directly lysed fish leukocytes and was one of the causes of anemia in salmonid cryptobiosis. Although immunosupression of the host caused by *L. salmonis* secretions is more likely to occur at the individual sites of *L. salmonis* attachment and feeding, an accumulative effect of several sea lice may eventually cause immunosuppression of the entire host. Rainbow trout infected with *L. salmonis* have exhibited decreased macrophage function and an increased susceptibility to the microsporidian *Loma salmonae* again in the absence of an integrated stress response (Mustafa et al., 2000). Nolan et al. (1999) also showed indirect effects of short-term *L. salmonis* infection on Atlantic salmon such as increased apoptosis, necrosis, and decreased numbers of mucous cells in the skin and gill epithelia distal to infection sites, and they attributed these effects to an integrated stress response. However, later work by Nolan et al. (2000) showed that cortisol administration had no effect on numbers of epithelial mucous cells of rainbow trout over the short term, suggesting that factors such as *L. salmonis*–derived immunosuppressive agents may be responsible for the observed effects as opposed to an integrated stress response by the host.

Despite variation among individual sea lice, differences were observed in *L. salmonis* obtained from different sources. The significantly higher mean protease activity and positive protease responses, as measured by azocasein hydrolysis may be indicative of an evolutionary difference between British Columbia and New Brunswick *L. salmonis* populations. Genetic differentiation has been observed previously in populations of *L. salmonis* on farmed salmon in Scotland (Todd et al., 1997; Shinn, Banks et al., 2000; Shinn, Bron et al., 2000). *Lepeophtheirus salmonis* has previously been reported on wild and farmed coho salmon in the Pacific Ocean, albeit at much lower densities than on rainbow trout and Atlantic salmon (Nagasawa, 1987, 1991; Johnson and Albright, 1992; Ho and Nagasawa, 2001).

Alkaline phosphatase has been observed in the salivary extracts of arthropod parasites such as the cattle tick *Boophilus microplus* and the buffalo fly *Haematobia irritans exigua* (Kerlin and Hughes, 1992), and our results suggest AP may also be a constituent of *L. salmonis* saliva. The increased AP activities in Atlantic salmon mucus after *L. salmonis* incubation is similar to results observed in the mucus of *L. salmonis*–infected Atlantic salmon (Ross et al., 2000; Fast et al., 2002). Observing higher mean AP activity and positive AP responses from *L. salmonis* in Atlantic salmon mucus suggests that the increase in AP activity observed in Atlantic salmon mucus after infection may partially be derived from the *L. salmonis* (Ross et al., 2000; Fast et al., 2002).

Lysozyme is an enzyme that has been shown to increase in the mucus of rainbow trout and Atlantic salmon immediately after *L. salmonis* infection (Fast et al., 2002). Lysozyme, however, does not appear to accompany protease and AP release from *L. salmonis* when incubated with fish mucus. Conversely to AP, the lack of changes in lysozyme activity after *L. salmonis* incubation of fish mucus suggest that the observed increases in mucus lysozyme activity in rainbow trout and Atlantic salmon after infection are a host response to *L. salmonis* infection (Fast et al., 2002).

The variation observed in the activities of these enzymes after incubation with mucus of different fish species could be due to differences in the health of the different *L. salmonis* or other factors, such as preference for host substrate. Marinotti et al. (1990) showed that the type of meal being ingested by female mosquitoes *Aedes aegypti* altered their salivation. Particular secretions have been linked to pathogenicity (Zuo and Woo, 1997a, 1997b) and shown preferences for substrate (Cupp et al., 1995). Because the incubations were carried out for only 1 hr, it is possible that some *L. salmonis* did not need to feed or had recently released their secretions (24 hr previously on the host), resulting in *L. salmonis* not releasing enzymes into the sample. Although it is assumed that *L. salmonis* feeds continuously, other types of arthropod parasites, such as black flies, horn flies, and buffalo flies, exhibit intermittent feeding within a 24-hr period (Kerlin and Hughes, 1992; Cupp et al., 1995, 1998).

It has been suggested that chemoattractants are involved in host recognition by *L. salmonis* (Ingvarsdottir et al., 2002). Species of fish not expressing these chemicals in high enough concentrations will not attract *L. salmonis* to settle and attach (Bron et al., 1993). These sea lice, however, infect coho salmon at similar densities (Fast et al., 2002) to rainbow trout and Atlantic salmon but are rapidly reduced in number within 10–14 days (Johnson and Albright, 1992). This occurs in the absence of any obvious systemic response (Fast et al., 2002). These observations combined with the current results suggest that although *L. salmonis* readily attach to coho salmon their removal

within 7–14 days may be due to blockage of sea lice secretions by coho salmon mucus, thus preventing parasitic feeding, or immunomodulation, or both. This hypothesis may explain why coho salmon experimentally infected with *L. salmonis* mount an acute inflammatory response as early as 1 day postinfection (dpi) and a well-developed epithelial hyperplasia at 10 dpi in the gills and skin (Johnson and Albright, 1992).

The overall results suggest that there are factors in rainbow trout and Atlantic salmon that identify these species as acceptable hosts to sea lice, whereas *L. salmonis* identify winter flounder and coho salmon as unacceptable hosts and hence do not secrete material or are unlikely to release the same suite of enzymes into the mucus of these fish. The ability of coho salmon to reduce *L. salmonis* infection quickly may result from a combination of factors in the mucus that block *L. salmonis* from secreting key digestive enzymes or immunomodulatory agents, thus allowing a well-developed innate immune or inflammatory response against sea lice to occur. Further work into fractionation of coho salmon mucus could be useful in isolating a possible agent that inhibits sea lice secretions.

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