Physiology and immunology of *Lepeophtheirus salmonis* infections of salmonids

Glenn N. Wagner[1], Mark D. Fast[2] and Stewart C. Johnson[3]

[1] Rescan Environmental Services, 1111 Hastings Street, Vancouver, BC V6E 2J3, Canada
[2] School of Marine and Atmospheric Sciences, Stony Brook University, Stony Brook, NY 11794, USA
[3] National Research Council of Canada, Institute for Marine Biosciences, 1411 Oxford Street, Halifax, NS, B3N 2Z1, Canada

'Sea lice' is a common name for a large number of species of marine ectoparasitic copepods, many of which are widespread and important disease-causing agents that infect both cultured and wild fish. Of these copepods, the salmon louse *Lepeophtheirus salmonis* is the most extensively studied because of its economic impact on the salmonid aquaculture industry and its possible impacts on wild salmonid populations. Different levels of infection by this parasite can affect the long-term survival and viability of its hosts. In this article, we review the nature of the interactions between *L. salmonis* and it hosts to identify crucial areas that warrant further research to aid understanding of the impact of infection with *L. salmonis*.

The biology and ecology of *Lepeophtheirus salmonis*

When present in high numbers [>0.5–0.75 adult (see Glossary) parasites g^{-1} fish], the ectoparasitic copepod *Lepeophtheirus salmonis* causes disease in both farmed and wild susceptible salmon species; without intervention, such infection can have devastating effects on host populations [1–5]. Consequently, *L. salmonis* is the most widely studied of all of the parasitic copepods. Much of this interest stems from the view that *L. salmonis* derived from aquaculture sources is having a negative impact on wild host populations. A great deal is known about the biology and ecology of this parasite – especially its responses to changes within the environment, including changes that occur on salmon farms. However, when comparing knowledge about *L. salmonis* to knowledge about other economically important parasites, it is obvious that gaps exist in several key areas. More research is required to understand fully the physiological, immunological and behavioural effects of *L. salmonis* on its hosts.

There are several reasons for the lack of knowledge of these areas, including: (i) difficulties associated with conducting experimental challenges in both laboratory and field conditions; (ii) difficulties in maintaining the health of experimental hosts, especially those captured from the natural environment; and (iii) a lack of tools and reagents with which to study the physiological and immunological responses of fish and the biology of *L. salmonis*. In this review, we summarize recent information about the

physiological and immunological interactions between *L. salmonis* and its hosts (for a recent review of *L. salmonis* biology and epizootiology, see Ref. [5]). Much of the information available regarding salmon lice is obtained primarily from laboratory-based studies, among which direct comparisons are not always possible.

Limitations of laboratory-based studies

The majority of information about the interactions between *L. salmonis* and its hosts has been obtained from laboratory studies. These studies have used different host species and/or strains, host and *L. salmonis* life-history stages, and levels of infection. Furthermore, there are large variations in the experimental conditions under which

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Glossary

Adult: the final stage of the parasite life cycle, whereupon it becomes reproductively active. This life stage is also mobile and uses the suction of its cephalothorax to maintain its attachment to the host.

Chalimus: there are four chalimus stages in the salmon louse; these refer to the stages at which the parasite is attached to its host via the frontal filament.

Copepodid: mobile infective stage of the parasitic copepod. This life stage is the first obligate parasitic stage of the louse, and if it does not encounter a suitable host it will expire within 40–50 degree days.

Cortisol stress response: cortisol is a stress hormone released by the interrenal cells of the kidney within minutes of an animal undergoing a stressful event. This hormone can be maintained at high levels by chronic or acute stressors. If maintained at high levels for extended periods of time, cortisol can have deleterious effects on host reproduction, immunology, ionic balance and many other processes.

Degree days post-infection (ddpi): the number of days multiplied by the temperature, in Celsius, following infection. This method of measuring accrued temperature and time in concert is commonly used in invertebrate parasite studies in which the parasite life cycle is temperature dependent. Because laboratory infection trials are carried out at different temperatures, they can be directly compared using this approach owing to louse development correlating with temperature.

Disease-inducing threshold: parasite infection level at which pathogenic signs related to infection begin to emerge (i.e. skin ulceration, lethargy and morbidity). Infection levels below this threshold are considered to be subclinical.

Limited tissue response: this subjective and relative terminology refers to the timing and amplitude of the inflammatory response to injury. Limited indicates a delayed or slow response and a small or minimal area of occurrence.

Mortality events: refers to multiple host deaths occurring at the same time and as a result of parasite infection.

Pre-adult: there are two pre-adult stages in the salmon louse. The pre-adult stage is the first at which both sexes can be identified and distinguished. This life stage is mobile and uses the suction of its cephalothorax to maintain its attachment to the host.

Single-pulse infections: infection trials carried out in a laboratory setting in which fish are exposed only once to parasites before measurements are taken.

Subclinical: infection level below the disease-inducing threshold.

Box 1. Reducing laboratory artefacts by using appropriate experimental systems

Studies require the use of reliable host-infecting systems to enable researchers to control the number and distribution of *Lepeophtheirus salmonis* on the hosts. The goal is the establishment of an infection that is similar to that seen under natural conditions [12,13]. To study stress-induced effects on the host(s), fish must first be allowed to adapt to the laboratory conditions. The appropriate environmental conditions and food resources must be supplied. In addition, the following factors must be taken into consideration.

(i) Appropriate host variables. Many studies of *L. salmonis* have used gross pathology and/or histology as the starting point in determining the effects of infection. With increased interest in the effects on physiology and stress response, common indicators of physiological condition and stress (e.g. host condition factor, plasma cortisol, plasma glucose, concentration of plasma ions, plasma protein levels and gill Na^+/K^+-ATPase activity) are commonly used, either alone or in combination with a measure of immune function or physiological performance [15,16,18–27]. With regard to immune function, measured variables include cellular immune function, as determined by macrophage respiratory burst and phagocytic activity, and real-time PCR studies of immune-related gene expression [13,17,39,40].

(ii) The 'normal' condition of the host. To assign biological significance to the measured host variables, detailed information is required about how these variables change in the absence of *L. salmonis*. Such baseline studies must be conducted for the hosts at different ages and maturation statuses over the normal range of environmental conditions to which the hosts are normally exposed.

(iii) The reporting of parasitic infection must be consistent and have biological meaning. Another problem for study comparisons is the method used to categorize infection by sea lice. To assign biological significance to estimates of pathogenic impact in wild populations, infection variables such as abundance and intensity should be standardized with subsequent effects known for the particular host species (Box 2).

(iv) The age structure of *L. salmonis* and its distribution on the host. Part of the difficulty in comparisons between studies arises from the presence of *L. salmonis* lice of different ages and their variable distribution on the host body at the time of sampling. Both the intimacy of the attachment and the distribution on the host change during development (Box 3).

Box 2. Problems with current laboratory estimates of sea louse pathogenicity

Some confusion exists in the literature with regard to laboratory estimates of the pathogenicity of salmon lice, beginning with the suggestion that >30 louse 'larvae' can cause death in 40-g Atlantic salmon post-smolts [22]. In their study, Grimnes and Jakobsen [22] refer to pre-adult lice as larvae (non-adult stages) because their study was stopped before the appearance of adults (31 days at 10.4 °C). However, <10% of the moribund fish appeared before the moult to pre-adult lice, and the minimum number of chalimus larvae on these fish was 51 (mean of 89). For moribund fish appearing after the parasite moult to pre-adults, the minimum number of lice was 59, 39, 28 and 29, as suggested by the authors' estimated mortality threshold. These results have since been inappropriately cited, probably because of the use of the term 'larval lice'.

Finstad *et al.* [48], who reference the study by Grimnes and Jakobsen [22], suggest that 30 chalimus larvae, or a relative intensity of 0.75 chalimus larvae g^{-1} fish, might kill a post-smolt when the parasites become pre-adults. Either the authors assume the same effects across differing parasite stages, which is not consistent with the rest of the literature, or they disregard natural mortality between stages. However, there is a 30–50% natural decrease in *Lepeophtheirus salmonis* numbers over time due to mortality and dislodging in laboratory investigations between chalimus and pre-adult moults [15,22,49]. In other words, suggesting a threshold of 30 or more pre-adult lice causing mortality in 40-g post-smolt Atlantic salmon would be closer to 60 chalimus larvae infecting size-matched Atlantic salmon, as observed by Grimnes and Jakobsen [22].

Furthermore, Heuch *et al.* [44] state that 'dose–response' studies of wild-salmon smolts (referencing Ref. [48]) have estimated that 11 lice per fish would cause wild-salmon smolts to die. The authors proceed to confirm the data using wild-salmon survey observations of no more than ten adult lice per salmon smolt. The initial problem with these statements is that dose–response studies were not conducted by Finstad *et al.* [48], but rather the figure of 11 total lice, or 0.75 lice g^{-1}, was taken from Grimnes and Jakobsen [22] and applied to a 15-g fish. For people not familiar with the differences between the life stages of the parasite, these seemingly similar statements can have drastic effects on their interpretation of the current literature. This point is illustrated in Figure 1 in the main text by the varying clinical responses of fish that exceed the morbidity limits listed above. Therefore, it is imperative for authors to take care to attribute pathogenicity within the confines of louse life-stage (see Box 3) and particular host species.

these studies were conducted and the tools used to study the interactions. These factors make it extremely difficult for comparisons between studies and hinder the extrapolation of these results to situations observed outside of the laboratory. The requirements that must be satisfied to attribute observed changes in the host to the presence of *L. salmonis* are presented in Box 1.

Most laboratory studies use relatively large numbers (e.g. >0.3 lice g^{-1} fish) of *L. salmonis*, which often creates situations that are indicative of the disease state. Such studies provide little information about the nature of the interactions that take place when parasites are present at lower abundances (e.g. <0.1 lice g^{-1} fish), in the absence of disease (i.e. lice levels below a disease-inducing threshold) – as seen more commonly for fish in the natural environment (Figure 1).

Many mammalian–tick models have shown that host responses to successive infections differ from single or primary infections, even in situations in which resistance to infection does not develop [6–9]. Evidence that this occurs in fish includes the differing responses of rainbow trout (*Oncorhynchus mykiss*) to primary and secondary infections

with the ectoparasitic monogenean *Gyrodactylus derjavini* [10]. In the case of *L. salmonis*, wild populations of fish typically acquire infections over relatively long periods of time (months to years), as evidenced by the presence of different developmental stages [11]. However, the majority of laboratory studies used single-pulse infections of *L. salmonis* (>100 copepodids per fish). Laboratory infections can also result in a large proportion of *L. salmonis* on the gills [12] compared with the less vascularized and less physiologically active tissues (e.g. skin and fins), which are typical sites of natural infection. Further limitations have been encountered when trying to determine louse pathogenicity in a laboratory setting (Box 2). These differences between laboratory and natural infections must be considered when attempting to predict the physiological and immunological effects of natural infections of hosts.

Additional refinement of laboratory methods and reporting of infection trials with *L. salmonis*, as discussed by Fast *et al.* [13], is necessary. Perhaps the most important consideration is obtaining the appropriate sample sizes and replication to enable robust statistical analysis. Owing
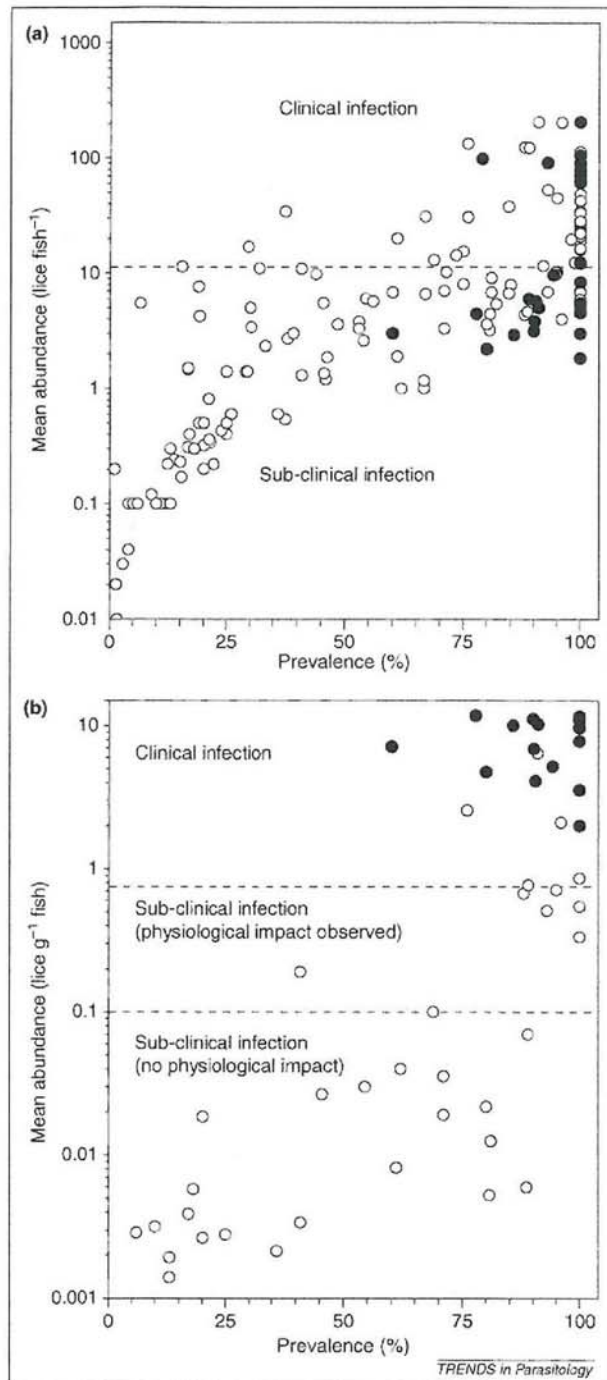
Figure 1. Different ways of measuring mean abundance of salmon lice on wild salmon. (a) Using the mean number of lice per fish. Broken line indicates the suggested level above which a salmon louse infection can become clinical in nature. (b) Using the number of lice g^{-1} fish. Regions divided by broken lines indicate the physiological impact of salmon lice based on laboratory studies. Solid circles denote reported morbidity or epizootics. Data include all feeding stages of lice. The sources of data used to compile the figure are presented as online supplementary material.

because small differences in tank environments can lead to markedly different parasite settlement and host stress responses. Another consideration is the availability of sufficient copepodids to infect enough fish for proper statistical analysis. This number changes depending on the relative susceptibility and size of the hosts. For example, an infective dose of 150 copepodids per fish is required to establish an infection level of 5–10 lice per 10 g on pink (*Oncorhynchus gorbuscha*) or chum (*Oncorhynchus keta*) salmon [14], whereas the same dose would result in almost 20–30 lice per 60 g on Atlantic salmon (*Salmo salar*) or rainbow trout [15,16]. Depending on the factorial design, these values would conservatively result in the need for 20 000 copepodids for a study. Based on an assumed number of eggs per egg string (∼350) and hatch survival (25%) to infective stage, the need for >200 egg strings at one time could be a logistical problem.

Host–parasite physiological interaction

Host species differ in their susceptibility to infection

Initial work on the interactions between *L. salmonis* and its hosts used microscopy to observe host tissue responses to louse attachment and feeding (for reviews, see Refs [2,3]). Attachment and feeding can have varying effects depending on the species of host and the parasite life-history stages that are present (Box 3). Based on parasite loss and histological examination of the tissue responses, it was proposed that naïve Atlantic salmon are more susceptible to infection than are naïve intermediate chinook salmon (*Oncorhynchus tshawytscha*) and naïve coho salmon (*Oncorhynchus kisutch*). There is little evidence of a host tissue response in Atlantic salmon at the sites of feeding and/or attachment, regardless of developmental stage. However, inflammation and hyperplasia of the epithelium have been reported around the periphery of these sites and around frontal filaments to which chalimus larvae are no longer attached [17,18]. By contrast, coho salmon show strong tissue responses to *L. salmonis*, with sites of attachment and feeding characterized by the presence of well-developed epithelial hyperplasia and inflammatory responses. This inflammatory response [18,19] seems to be the main mechanism by which most *L. salmonis* lice are rejected by coho salmon within the first week of infection.

Recently, a series of laboratory infection trials demonstrated that pink salmon and, to a lesser extent, chum salmon could be similar to coho salmon in their ability to reject *L. salmonis* following a single-pulse infection [14]. Histological examinations of parasite attachment and feeding sites on pink salmon revealed nonspecific tissue responses, which were similar to those reported for coho salmon [20].

Subclinical physiological effects on the host

The feeding and attachment activities of mobile *L. salmonis* result in changes to host skin mucus consistency, physical damage, and in most cases a generalized stress response mediated through cortisol release [15,18,21–24]. The production of cortisol by the hypothalamic–pituary–interrenal (HPI) axis of fish has an important role in *L. salmonis*–salmon physiological interactions. Cortisol influences hydromineral balance and energy metabolism and

to the level of variability between individual salmon physiological and immunological responses, large sample sizes at each time-point are required to demonstrate significant trends. Replication is particularly important

Box 3. *Lepeophtheirus salmonis* life history and pathogenicity

Copepodids are the first life-stage at which *Lepeophtheirus salmonis* seeks out a host to infect. Once attached, the copepodid will feed for a short period of time (3–4 days, more at low temperatures), before undergoing a moult into a first chalimus louse [1]. Sea louse chalimus stages attach to the host by a frontal filament that adheres to hard structures such as scales and cartilage (Figure I). Development from chalimus to pre-adult and adult louse signifies a key modification to the host–parasite interaction because an individual louse is no longer affixed to a localized site. At this point, it becomes mobile, maintaining contact with the host by using its cephalothorax as a 'suction cup'.

The pathogenic impact of *L. salmonis* differs greatly depending on the age structure and abundance of the parasite on a particular host species. For example, Atlantic salmon show little initial tissue response to the early stages (copepodid and chalimus) of *L. salmonis* [12,19,21] after initial attachment. However, pre-adult and adult lice are associated with a significant increase in cortisol and glucose levels [21], leading to chronic stress during prolonged infection with large numbers of lice [50,51]. In fish with critical levels of chalimus infection (3 lice g^{-1} fish [24]), rapid shock-like mortalities can occur in the absence of lesions as the sea lice moult into pre-adults. Nonetheless, only prolonged infections by mobile pre-adult and adult lice lead to chronic stress, anaemia and eventual mortality due to the loss of osmotic and ionic balance caused by skin lesions and blood loss from feeding [21,22,45,46].
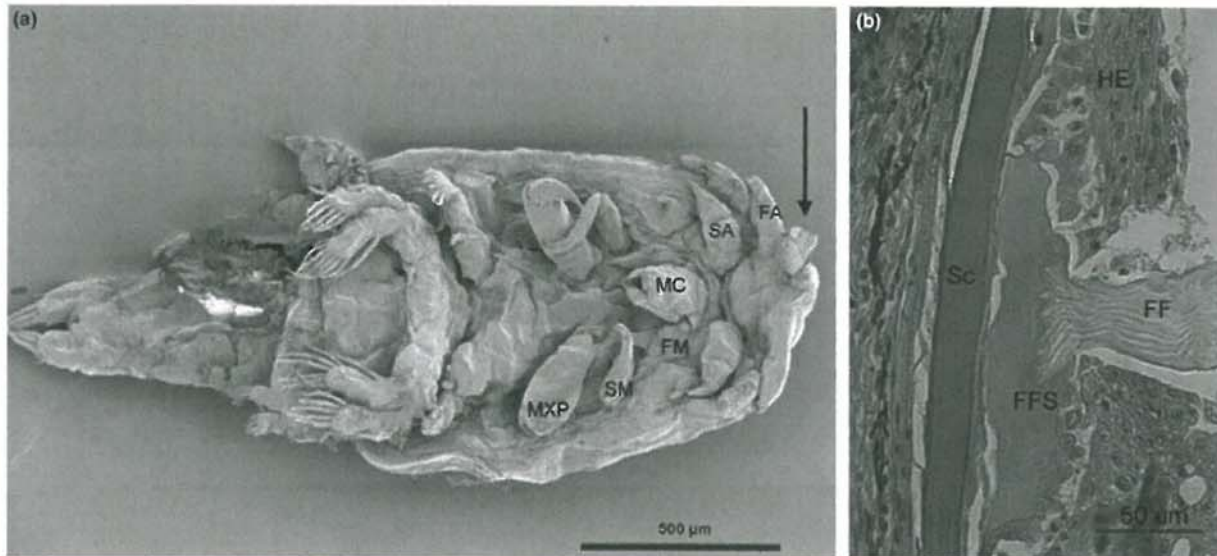

Figure I. *Lepeophtheirus salmonis* chalimus. (a) Ventral surface, with arrow indicating frontal filament. (b) Frontal filament secretion attaching the chalimus to the host. Abbreviations: FA, first antennae; FF, frontal filament; FFS, frontal filament secretion; FM, first maxilla; HE, host epidermis; MC, mouth cone; MXP, maxilliped; SA, second antennae; Sc, scale; SM, second maxilla.

suppresses immune function [2,25], which impact both the host and *L. salmonis* (Figure 2). In fact, even short-term (5–10 days) infection with as few as ten pre-adults or adults per fish (0.04 lice g^{-1} fish) causes a stress response in Atlantic salmon [23].

There is a great deal of disagreement with respect to the effect *L. salmonis* infections have on individual hosts and host populations at subclinical (i.e. below the disease-inducing threshold) levels. Part of the problem lies in determining the appropriate host variables to measure and the assignment of biological significance to measured values. Another issue is how to measure the level of infection accurately to link the physical presence of lice to the observed effects (Box 2). Subclinical levels of infection can cause changes in host physiology, biochemistry and immunology in both the presence and the absence of a cortisol stress response [3,16]. Studies of Atlantic salmon have shown that 10–30 *L. salmonis* of varying life-stages per juvenile fish ($\sim \leq 0.2$ lice g^{-1} fish) can cause changes in host mucus cell discharge, mucus biochemistry, macrophage function and inflammatory gene regulation [2,3,16]. The presence and absence of cortisol increases observed under different experimental infections could be caused by an attenuated cortisol response in salmonids under chronic stress [26]. Regardless of changes in cortisol during clinical infection, other changes occur that compromise the physiological and immunological status of the host. Changes in head kidney macrophage populations in Atlantic salmon eventually lead to a decrease in the ability of macrophages to respond to bacterial challenge, despite attenuation of the cortisol response during chronic stress [26].

Recent swimming performance studies [27,28] have shown that low levels of *L. salmonis* infection can have major effects on the physiology of salmonids, indicating possible ecological repercussions for the host. Infection levels, averaging only 0.1 lice g^{-1} fish, altered the cardiac performance of adult Atlantic salmon during exercise and led to 19–22% reductions in swimming performance compared with uninfected fish (Figure 3). Short-term exposure of similarly infected fish to freshwater restored the reduction in swimming performance to uninfected levels [28]. In addition to alleviating much of the systemic stress caused by *L. salmonis*, exposing salmon to freshwater would also be beneficial because the parasites cannot osmoregulate in fresh water, and therefore eventually detach [29,30]. These physiological benefits of fresh water
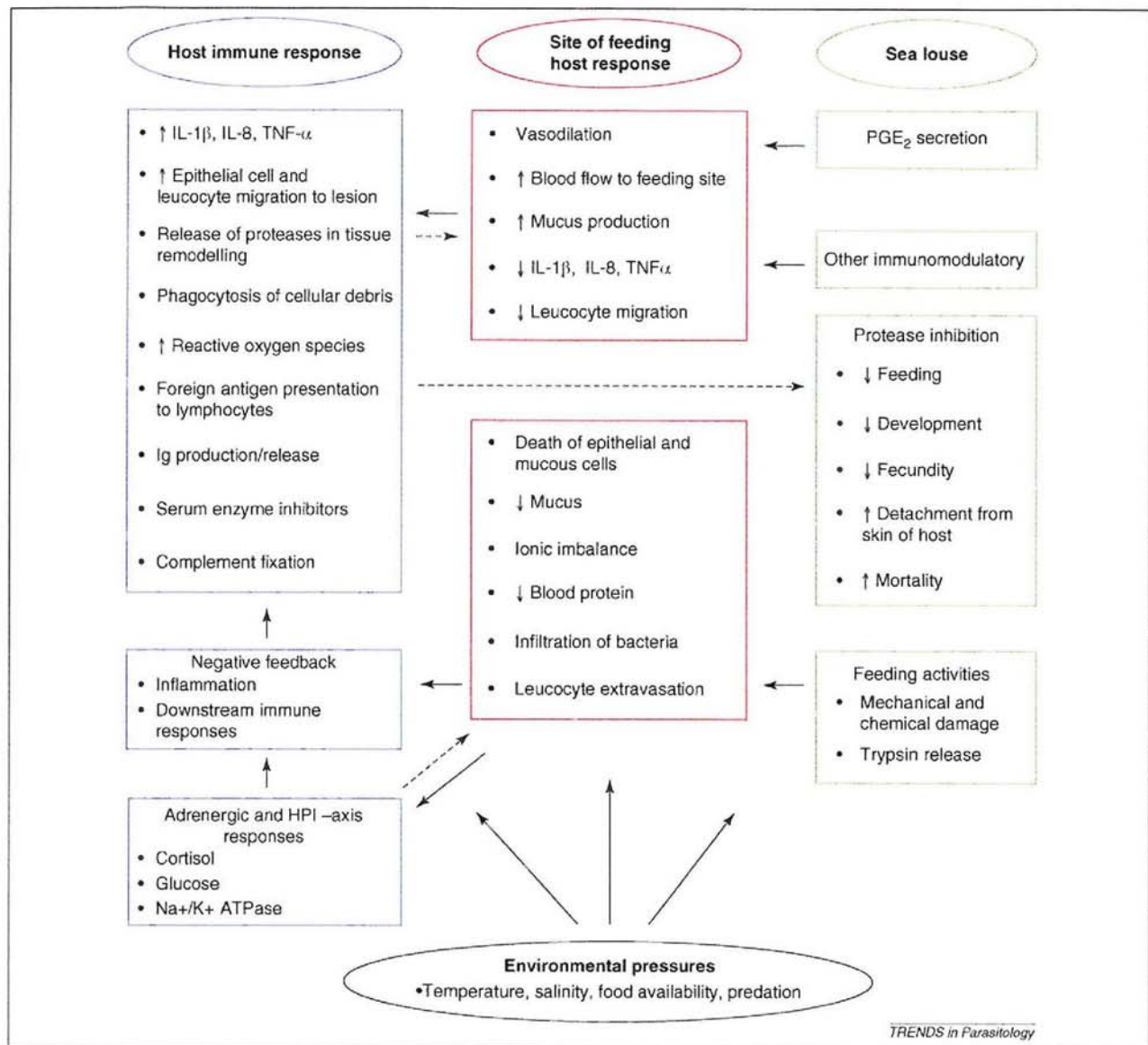
Figure 2. Schematic representation of the *Lepeophtheirus salmonis* and salmonid interaction. Arrows within the text boxes indicate net effects. Arrows between text boxes indicate the targets of these effects.

re-exposure help to explain the early return of heavily infected wild salmonids to native streams in Europe, as observed since the early 1990s [31,32]. Although re-entry into freshwater delays their seawater feeding time and subsequent growth, infected fish benefit from loss of lice and restoration of their ionic and osmotic balance.

Immunological effects on the host

The observation of limited tissue responses to *L. salmonis* in Atlantic salmon led to the suggestion that salmon lice, as with other arthropod parasites, might secrete substances to aid feeding and to avoid host immune responses [20,33]. Trypsin has been identified in the secretions of *L. salmonis* and in the mucus of infected Atlantic salmon [20,33]. The midgut has been identified as the site of trypsin production [33,34]. Trypsin-like proteases are present in the

secretions of other arthropod parasites, where they have a role in the invasion of host tissues and the evasion of host immune responses (reviewed in Ref. [3]). More recently, prostaglandin E_2 (PGE_2) has been identified in *L. salmonis* secretions [35]. At physiologically meaningful levels, PGE_2 downregulates Atlantic salmon inflammatory gene expression and might increase the availability of blood where lice are feeding [35,36]. Immunomodulatory activity also has been observed recently in fractions of *L. salmonis* secretions that do not contain trypsin or PGE_2 [37]. However, the question remains as to how resistant hosts overcome these secretions. In the case of coho salmon, mucus does not stimulate *L. salmonis* secretory release to the same degree as does the mucus of susceptible species [38]. This inhibition might help to explain the ability of coho salmon to mount a vigorous tissue response, which results
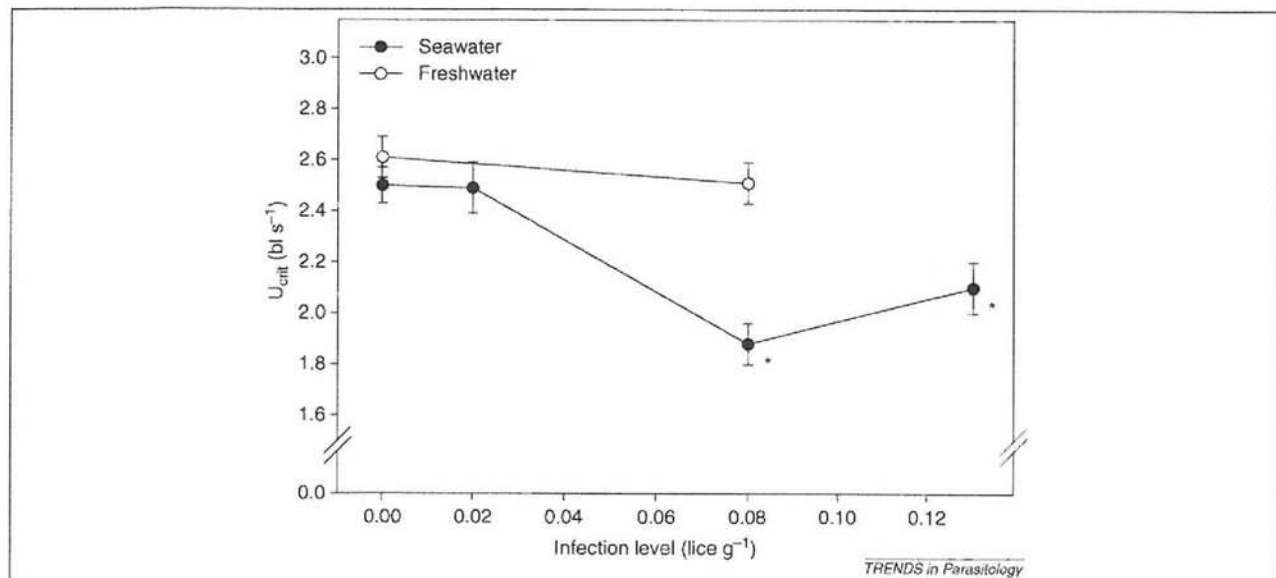
Figure 3. The effect of sea lice infection on salmon swimming ability. Low-level sea lice infection decreases the critical swimming performance [U_{crit}, measured in body lengths (bl) s^{-1}] of Atlantic salmon in seawater and in freshwater. Asterisks indicate statistically lower ($P<0.05$) U_{crit} for infected fish in seawater. Modified, with permission, from Refs [26,27].

in the loss of parasites. Leukocyte migration to the infection site, reactive oxygen species (ROS) release, serum enzyme inhibition, complement fixation, antigen presentation to lymphocytes and immunoglobulin (Ig) production are all expected to lead to decreased feeding and development in parasites (Figure 2); these physiological processes can combine to increase louse mortality. It is currently being investigated whether *L. salmonis* produces and/or releases immunomodulatory secretions on pink and chum salmon.

At the whole-animal level, infection of Atlantic salmon with *L. salmonis* has adverse effects on immune function in both the presence and the absence of a cortisol stress response (reviewed in Ref. [3]). For example, Fast *et al.* [15] reported a significant reduction (20%) in respiratory burst and phagocytic activity (10%) of head kidney macrophages isolated from infected Atlantic salmon at 140 and 210 degree days post-infection (ddpi). A similar reduction was observed for infected rainbow trout at 210 ddpi. Both of these observations occurred in the absence of a cortisol response. Furthermore, *L. salmonis* infections inhibit lipopolysaccharide-induced macrophage expression of immune-related genes at these same time-points [16]. Expression levels of inflammation-related gene products such as interleukin (IL)-1β, tumor necrosis factor (TNF)-α and the cyclooxygenase (COX)-2 enzyme increase significantly (100-fold) shortly after attachment (104 ddpi) in the head kidneys of Atlantic salmon in response to *L. salmonis* infection [15]. However, expression of these gene products usually subsides as *L. salmonis* develops through the four attached chalimus stages [16,39]. With the appearance of large numbers of pre-adult lice (e.g. >0.3 lice g^{-1} fish or >80 per fish), the expression of some of these inflammatory markers (IL-1 β and TNF-α) increases again (>20-fold) [39]. This is another example of changes in the host–parasite interaction occurring at or shortly after the moult

to pre-adult lice, as seen in cortisol responses, macrophage responses and mortality events [15,24,40]. Such changes in the host immune response might not only assist in or result from *L. salmonis* maintaining itself on the host but also increase the likelihood of the development of secondary infections [41].

As mentioned, both pink and chum salmon quickly rid themselves of *L. salmonis* following a single-pulse infection [14]. The importance of the nonspecific immune response seems to be supported by an increased expression of inflammatory genes (e.g. those encoding IL-8 and TNF-α) in the head kidneys of infected pink salmon [20]. Chum salmon, which are relatively more susceptible (i.e. larger parasite numbers and greater stress response) than pink salmon, do not exhibit this response [20]. In response to a single-pulse infection, resistant host species lose the majority of *L. salmonis* before lice develop into pre-adults [18]. It is possible that the physical attachment to the host by the frontal filament makes chalimus lice more susceptible to inflammatory processes. Motile pre-adult and adult lice might be able to avoid these responses simply by switching their position on the body. This behaviour would be consistent with the observation that pre-adult and adult *L. salmonis* lice are more resistant on wild salmonids in laboratory examinations [11,42].

Pathogenicity

It is well recognized that large numbers of mobile *L. salmonis* (>0.75 lice g^{-1} fish) can cause host morbidity and death [1–3]. The stress of initial entry into seawater has recently been shown to exacerbate the physiological impact of salmon louse infection on sea trout (*Salmo trutta*) smolts, so that even lower levels of infection (13 lice per fish, ~0.35 lice g^{-1} fish) can increase the chance of morbidity [43]. In most cases, morbidity can be attributed directly to physical damage caused by the attachment

and feeding activities of lice, leading to elevated levels of plasma cortisol and subsequent loss of ionic and osmoregulatory capability [1,2,44]. Anaemia caused by direct blood feeding and blood loss through lesions might further exacerbate morbidity at high levels of infection (>0.5 lice g^{-1} fish) [45]. In the laboratory, infection with similarly large numbers of *L. salmonis* commonly results in high levels of host mortality following the moult into pre-adults, without the development of open lesions [22,24,46]. Fast *et al.* [35] suggested that this mortality event is similar to toxic shock in mammals and might be mediated by PGE_2. Serum PGE_2 levels increased twofold in *L. salmonis*-infected Atlantic salmon following initial infection and after the moult of lice into pre-adults [39]. Furthermore, exogenous PGE_2 administration at high doses (1×10^{-6} and 1×10^{-8} M) exponentially increases the expression of the gene encoding COX-2 in a salmonid head kidney cell line [36]. Because COX-2 is responsible for the production of prostaglandins, it is possible that, at pathogenic burdens of lice, the secretion of PGE_2 by *L. salmonis* stimulates the elevation of host-derived PGE_2 to toxic levels. These mortality events might be distinguishable from mortality caused by prolonged exposure to elevated cortisol levels and ionic imbalance in that they occur over a shorter period of time: within 24–48 h of the moult of lice into pre-adults, rather than in a week or longer.

Future perspectives

The dynamic parasite–host interaction between salmon lice and salmon is understood at a basic level. We have stressed some of the limitations of the data obtained from wild studies. In the future, controlled laboratory experiments or small-scale field experiments are needed to confirm observations made from natural infections. This is especially true with regard to the Pacific Ocean; knowledge is needed about host–parasite interactions in this area between *L. salmonis* and multiple salmonid species that probably have varying responses to infection. Furthermore, generalizations involving different host and parasite species, and the use of different developmental stages of the host and parasite must be eliminated. These steps will help to prevent confusion for those attempting to model the epidemiological impacts of salmon lice or trying to develop louse management strategies [43,47].

Making conclusions from data biased towards high levels of parasite infection limits the ability to understand which effects can be attributed to the parasite and which can be attributed to the stress response of the host. Therefore, it is important that additional biochemical and immunological research into *L. salmonis*–salmon interactions be carried out at both clinical (i.e. disease-inducing) and subclinical levels. This is particularly true for most of the Pacific salmon species because much remains to be discovered about their physiological interactions. Research into whether susceptible host species have factors that stimulate parasitic secretions could help to elucidate parasite immunomodulation of the host, in addition to possible vaccine targets and breeding strategies for the aquaculture industry. The current construction of salmon and other teleost genomes will, no doubt, lead to these possibilities in the near future. Combined with available microarray and real-time PCR techniques, unlocking the intricacies of the interactions of ectoparasitic copepods with their fish hosts will certainly become more attainable.

Another issue concerning salmon lice is their impact on early marine mortality and the return of adult salmon to freshwater. Understanding this relationship remains a key management problem for salmon fisheries because any additional source of mortality further complicates return estimates. A combination of laboratory and field studies examining the physiological impact of salmon lice on their hosts would complement physical and population models of louse dispersal [47]. This two-pronged approach is important for helping to determine the effective geographic area for salmon louse populations and their effect on salmon at the population level.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.pt.2007.12.010.

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