Title: **Differential Infection of Juvenile Pacific Salmon by Parasitic Sea Lice**

**Introduction:**

Many parasites are known to infect more than one host, with multi-host dynamics often involving a reservoir host (Haydon et al. 2002). These reservoir hosts can allow parasites to maintain high levels of abundance as the critical community size, or the minimum number of individuals in a population needed for a parasite to persist (Bartlett, 1960), will be smaller for the focal host species. By facilitating the maintenance of high parasite abundance and subsequent infection pressure even in the face of a decline in the focal host population, reservoir hosts effectively remove the typical density-dependent transmission dynamics that usually exist between a single host and parasite (Anderson & May 1979). This allows said parasites to pose a threat to the long-term viability of the focal host population (Cleaveland et al. 2002). The effects of the parasite on the reservoir host span the range of potential virulence (Brunner et al. 2004), but if the size of the reservoir population is large enough, the parasite will be maintained at high abundance despite high virulence to the reservoir host. Reservoir hosts, which can be both domestic and/or wild populations (e.g. Quinnell & Courtenay 2009), often allow spillback infection, re-infecting the population(s) that originally infected the reservoir (Daszak et al. 2001). Reservoirs thus can facilitate a type of apparent competition when the reservoir is a wild population (Power & Mitchell 2004; Kelly et al. 2009) by negatively affecting the focal host species simply due to the reservoir’s presence and the subsequent increase in abundance of the shared parasites (Holt 1977; Holt & Lawton, 1994).

Spillback from reservoir hosts (and subsequent apparent competition) has been shown in multiple aquatic systems (Arkush et al. 1998; Krkošek 2010; Hartigan et al. 2011; Adlard et al. 2015), and is commonly associated with invasive species (Adhard et al. 2015). However, spillback from domestic populations has been shown to be a concern, particularly with economically valuable wild populations wherein a native parasite flourishes in a domestic reservoir population, subsequently increasing the infection pressures on the native hosts.

Parasitism has been known for decades to play a strong role in fish recruitment, with wide ranging effects, notably including well-studied behavioural and reproductive effects (Williams 1964; Barber et al. 2000; Longshaw et al. 2010; Krkošek et al. 2013). Understanding how parasitism affects even one population of fish can be difficult, but understanding the dynamics of a multi-host, multi-parasite system can prove challenging from not only a scientific perspective, but more importantly from a conservation and management perspective. Such is the case on the Pacific coast of British Columbia, Canada, where five species of Pacific salmon, and numerous other commercial fish species are infected by multiple parasites whose infection pressures have been shown to increase with the growth of industrial fish farms in the region (Krkošek et al. 2005; Krkošek et al. 2007a).

Pacific salmon make up some of the most important fisheries in Canada, not only with respect to their economic importance but also from a cultural and historical perspective (Cooke et al. 2004. Eliason et al. 2011; FAO 2015). Yet despite the importance placed on them, these fisheries have seen significant declines in recent decades. Indeed, the Fraser River sockeye salmon (*Oncorhynchus nerka*) in particular have seen large population declines, resulting in a federal judicial inquiry by the Canadian government into the causes of this decline (Cohen 2012). The current consensus is that multiple intertwined factors impact the survival and growth of these populations (Peterman et al. 2010). It has been posited that threats specific to these fish during their juvenile life stage might be most important in determining the number of fish that return as adults and are subsequently available to fisheries and natural predators that rely on them (Godwin et al. 2017). Fraser River sockeye salmon in British Columbia begin their lives in freshwater spawning environments, and subsequently migrate out to the marine environment where they traverse up through the Strait of Georgia, the Discovery Islands and Johnstone Strait, and then out through Queen Charlotte Sound to the open ocean (Fig. 1). When the juveniles leave their freshwater spawning areas, they encounter a whole host of stressors including a lack of sufficient food, as well as numerous parasites and pathogens (Hunt et al. 2018).

The Hakai Juvenile Salmon Marine Survival Program was set up in 2015 and is centred around the Fraser River sockeye salmon, as well as two other species of Pacific salmon, pink (*O. gorbuscha*), and chum salmon (*O. keta*). The goal of the program is to quantitatively answer questions regarding juvenile salmon condition, relevant threats to their survival, and ultimately the mortality rates of juvenile salmon during their migration from natal spawning grounds to the open ocean (Hunt et al. 2018). One of the most important factors affecting juvenile salmon survival, and a focus of this program, is parasitism of these juvenile salmon by two species of sea lice, *Caligus clemensi* a generalist parasite infecting multiple fish species, and *Lepeophtheirus salmonis,* a salmon specialist (Johnson & Albright 1991)*.* These ectoparasitic copepods are native to the region, and feed on the soft tissues and musculature of numerous species of marine fishes (Costello 1993; Krkošek 2009). Unable to survive in freshwater environments (Bricknell et al. 2006), sea lice naturally infect juvenile pink, chum, and sockeye salmon once they move out of their natal streams and rivers and into the marine environment. Because the parasites here are marine only, the abundances observed on juveniles are a result of transmission by other fish species in the environment, and not a maintenance of the parasite populations on the juvenile salmon themselves as not enough time has elapsed for the population of lice to actually establish on the juveniles.

Sea lice infect juvenile chum, pink, and sockeye from a variety of sources, primarily from farmed Atlantic salmon (*Salmo salar*) with respect to the specialist *L. salmonis* (Morton et al. 2004; Krkošek et al. 2005; Peacock et al. 2016) but likely less so for the generalist louse, *C. clemensi*. In a form of apparent competition, wild Pacific herring (*Clupea pallasii*) likely play a large role in transmitting *C. clemensi* to juvenile salmon (Groner et al. 2016), and these generalists also host-switch from sympatric subadult coho (*O. kisutch*) and chinook (*O. tshawytscha*) salmon (Krkošek 2009). Typically, adult pink, chum, and sockeye reside offshore during spring/early summer as the juvenile salmon migrate northwards up the coast. The juveniles become sympatric with their in-migrating adult conspecifics at the northern end of their coastal migration towards the end of summer, when the juveniles are typically 10-100 times larger than when they first emerged into the marine environment, and have developed scales (Krkošek et al. 2007b). At this point in their migration, juveniles are prepared to deal with the increased infection pressure that will come with adult salmon who have been harbouring significant parasite loads out at sea (Nagasawa 2001). However, research has shown that with the addition of fish farms along the western coast of mainland British Columbia and the eastern coast of Vancouver island, this whole dynamic has changed. These farms, which hold large numbers of Atlantic salmon, can act as reservoirs for both species of lice, thereby eliminating the spatio-temporal allopatry between the adult salmon with their high levels of infection, and the vulnerable juvenile salmon. This elimination vastly increases the infection pressure on the juveniles during their northern migration, likely contributing significantly to population decline (Krkošek et al. 2005; Krkošek et al. 2007a; Krkošek et al. 2007b; Costello 2009; Price et al. 2011).

While evidence suggests that population-level declines are directly related to increased infection pressure from these parasites, there are still many unknowns surrounding the host-pathogen dynamics in this system (Hunt et al. 2018). Indeed, there is little known about the patterns of infection across the different species of fish and lice. Questions still remain as to whether or not different species of salmon show higher infection loads of *C. clemensi* vs. *L. salmonis* as well as what environmental and life history traits affect parasite loads in these focal salmon species. While some previous work has suggested that sockeye juveniles are infected primarily by *C. clemensi* (Price et al. 2011; Godwin et al. 2015), estimates for pink and chum salmon do not exist, and information regarding what other factors affect parasite load for these three species is still relatively sparse. The present study attempts to shed some light on the relationships between pink, chum, and sockeye juvenile salmon and their sea lice parasites using data from the Hakai Juvenile Salmon Marine Survival program, collected between 2015 and 2018. Specifically, the goals of this study are to 1) determine the parasite loads of both *C. clemensi* and *L. salmonis* on pink, chum, and sockeye salmon and investigate what factors contribute to the differences in infection across the different salmon species, and 2) explore the spatial differences in infection patterns between the Discovery Islands and Johnstone Strait to lend further insight into which possible infection pathways could be most important to determining lice load for these juvenile salmon.

**Methods:**

*Field Observations*

In order to obtain data on species-specific lice abundances on the three focal species of juvenile salmon, an extensive sampling regime was maintained over the course of 4 years (2015-2018) as part of broader work on juvenile Pacific salmon survival (Hunt et al. 2018). Individuals were sampled during the peak of juvenile salmon outwards migration from May to July, at entry points to the Discovery Islands from the Strait of Georgia, and exit points from Johnstone Strait to Queen Charlotte Sound (Fig. 1). Sites were visited every 4-7 days in 2015/16, and then weekly in 2017/18. Purse seine nets (bunt: 27m x 9m with 13mm mesh; tow: 46m x 9m with 76mm mesh) were deployed from small, 6-8m twin-outboard research vessels to capture samples of heterospecific schools comprised of juvenile pink chum and sockeye salmon. The purse seine netting often also inadvertently captured some sub-adult coho and chinook salmon, as well as Pacific herring. During sampling, observers on the research vessel would conduct a visual survey transect to assess the surface activity of juvenile salmon. If no surface activity was observed after 20 minutes, the seine net was not deployed at that location.

Once the seine had been deployed, it was used to corral the fish beside the boat, and juveniles were scooped up individually using a 4L plastic jug and transferred to a sampling bag. Some individuals were euthanized using a 250 mg • L-1 concentration of tricaine methanesulfonate, and retained for laboratory analysis, while the other captured fish underwent examination using a hand lens on the boat and then were released. Information regarding fish body size, as well as number, life stage, and species of any sea lice present were recorded, along with relevant environmental data. During field observations *in situ*, two methods were used to identify sea lice. The ‘Motile’ method, comprised of identifying only the motile lice (pre-adult females, females, gravid females, and males for *C. clemensi,* and pre-adult females, females, gravid females, pre-adult males, and males for *L. salmonis*  lice), and the ‘All Stages’ method that also categorized the copepodite- and chalimus-staged lice as *C. clemensi*, *L. salmonis,* or ‘unidentified species’. Fish that were sacrificed for lab analysis were dissected at the Hakai Institute Quadra Island Field Station, where they were also examined for the presence, number, species, and life stage of sea lice, corresponding to either ‘Fine-Scale’ (chalimus- and copepodite-stage lice identified as either *C. clemensi* or *L. salmonis,* as well as the motile lice being identified aspre-adult females, females, gravid females, and males for *C. clemensi* lice, and pre-adult females, females, gravid females, pre-adult males, and males for *L. salmonis*  lice) or ‘Motile’ (identical to fine-scale classification, but with all chalimus/copepodite lice ignored) methods. Fish were also weighed to the nearest 0.1g and it’s fork length and standard length were taken to the nearest mm. This resulted in a dataset of over 1,800 individual fish, collected at eight different sites, across 52 individual collections, with a collection representing a unique sampling site/date combination, where at least five individuals of pink, chum, and sockeye juvenile salmon were sampled. In addition, a less species-balanced dataset was compiled, totalling almost 6,000 observations divided between the two sampling regions, the Discovery Islands, and Johnstone Strait.

*Statistical Analyses*

To investigate potential differences in lice parasitism between the three salmon species, we fit a series of generalized linear mixed-effects models (GLMMs) to our data using the glmmTMB package (Brooks et al. 2017) in R statistical software (R Core Team, 2018), with salmon species, sampling year, and fish fork length as predictive variables, along with collection as a random effect. These models were run separately for *C. clemensi* and *L. salmonis,* so as to more firmly parse out drivers of abundance of the two lice species in our study system. Due to different lice identification regimes used at different points in the study, motile life stages were the only ones easily identifiable by species on each and every fish, and as such, only motile data were retained for the purposes of our analyses. While the initial model set included all three predictor variables, including fork length resulted in a dataset with fewer observations, since fork length measurements were not taken for every fish. As such, this the variable was removed in favour of including a larger number of observations. Therefore, the full model set we used in our analysis incorporated salmon species and sampling year, as fixed effects (Table 1, 2).

The models employed a negative binomial error for the response distribution, as well as a log link function. The GLMMs were fit such that the number of lice per fish was treated as the response variable, and Akaike Information Criterion (AIC) was used to perform model comparison and selection. The results of these models were used to determine the predicted effect of each of the fixed effects variables on the number of lice per fish for each species of louse. This was done by back-transforming the coefficients from the models, and creating a 95% confidence interval using the ‘ggeffects’ package (Lüdecke, 2018), representing the estimated number of lice per fish between the two species.

In addition to our models relating louse infections to the three salmon species, we wanted to determine the effect that sampling region had on infection levels. The two distinct sampling regions (Fig. 1), are traversed successively, moving from south to north by the majority of the juvenile salmon in the region. As such, the Johnstone Strait represents a region wherein fish sampled will have typically had longer exposure to lice from all potential sources. To investigate any relevant differences, we ran a separate set of GLMMs, using the lice species as the response variable, with the site regions and years as fixed effects, and the week of the year (with 1 delineating the first week in January and counting numerically forwards from there) as a random effect. This resulted in two models, one for *L. salmonis* and one for *C. clemensi* for each of the three species of salmon.*.* Akaike Information Criterion (AIC) was again used to perform model comparison and selection.

In addition, we used hierarchical bootstrapping to pair our model-driven estimates with data-based measurements of the data to confirm that our model predictions were appropriate given the data for our region-level models. For each unique salmon species, lice species, year, and site region combination (48 separate combinations, ex. Sockeye salmon, *L. salmonis,* 2015 and Discovery Islands), we bootstrapped the average number of lice per fish in an individual collection 10,000 times. This involved sampling all of the collections that each unique combination occurred in, with replacement, and calculating the mean number of lice per fish for that collection, allowing us to garner a better understanding of the spread of our data.

**Results:**

The average number of lice per individual fish varied from more than 4 lice per fish in some collections, to less than 0.5 in other collections (Fig. 2), with each collection showing at least some lice present, though not each collection showed lice on each species of salmon (Fig. 3). When looking separately at the two louse species, it is clear that not only is *C. clemensi* present at many more collections than *L. salmonis* but also generally present at higher numbers (Fig. 3). Additionally, the highest average numbers of lice per collection for *C. clemensi* were just under three lice per fish, while for *L. salmonis*, the highest average number of lice per fish was just under 2.5 fish. In addition, there were very few *L. salmonis* lice present on sockeye salmon, compared to both pink and chum salmon.

Our species-level results indicated that both *C. clemensi* and *L. salmonis* infections were shown to be predicted most strongly by the full model with the additive combination of both fixed effects (Tables 3 & 4), indicating a clear signal of species-level differences in terms of salmon infection for both species of sea lice. The model results show estimates that *C. clemensi* was present at higher per-fish averages across the whole dataset (Fig. 3), with the highest frequencies being shown on pink and sockeye salmon. *L. salmonis* lice were present at lower numbers on each of the three salmon species, but were highest on pink salmon. These results are contrary to previous field observations which indicated that sockeye salmon exhibit the highest levels of *C. clemensi*, however they do confirm field observations that suggested pink salmon carry the highest levels of *L. salmonis.* Infection of the two lice species are notably more similar in 2015 and 2016 than they are in 2017 and 2018, with the levels of infection for *L. salmonis* being comparatively closer to that of *C. clemensi* during 2015 and 2016 than for the later two year. Sampling year differences are marked between both *C. clemensi* and *L. salmonis*, with estimated per-fish infections being highest in 2015, then seeing a relative decline in 2016/17 for both, with a small up-tick in 2018 for *C. clemensi*.

For the region-level models, AIC scores showed that the full model (both year and site region) provided the best fit for five out of the six models, with the model for *L. salmonis* and sockeye salmon showing a better fit model when only site region was included (Tables 5-10). The results of these models did lend insight into region-level differences, specifically showing that the much more abundant *C. clemensi* was estimated to be present at substantially higher infection rates in the Johnstone Strait than in the Discovery Islands for all three salmon (Table 6, Figs. 5 & 6). The less abundant *L. salmonis* was present at slightly lower levels in Johnstone Strait than in the Discovery Islands for both pink and chum salmon, but was present at marginally higher levels in Johnstone Strait than in the Discovery Islands for sockeye salmon. These region-level estimates confirm the low abundance of *L. salmonis* on sockeye salmon. For *C. clemensi* estimates, it was also apparent that within each salmon species the ratios of lice in the two regions stayed fairly consistent across the four years sampled, despite fluctuations in lice abundances overall, the relative abundances between the two regions stayed more or less consistent throughout the sampling periods.

Bootstrap results showed that our model estimates were well within the 95% confidence limits displayed by our data (Fig. 7). These results confirmed the relatively low abundance estimates of *L. salmonis* in the system, with all median values for *L. salmonis* being equal to zero with only three exceptions. General infection patterns were present for the bootstrapped data as well, with *C. clemensi* showing high prevalence on pink salmon, and intermediate prevalence on the other two species. Generally, these results confirmed the appropriate estimates of our models with respect to the data themselves.

Overall, we show here that in fact species-level differences are present between our three species of salmon with respect to the abundance, and species of lice causing the infections. Site region also appears to play an important role in determining the infection levels for both species of sea lice, indicating that the overall patterns of infection likely fluctuate from year to year, but that some general patterns remain through time.

**Discussion:**

The first notable result from this present study is the relative contribution of each species of louse to the overall sea lice burden on juvenile Pacific salmon. We show that *C. clemensi* exists at much higher levels across nearly all collections and across all three salmon species (Figs. 3 & 4) in our data, indicating that this generalist parasite is likely more important in terms of determining the overall infection pressure from sea lice on juvenile Pacific salmon. It is likely that a number of reasons contribute to why *C. clemensi* shows higher abundances. Notably, there are vastly more host opportunities for *C. clemensi* in this system, as it is a generalist parasite with multiple known hosts in this region, including the abundant Pacific herring (Beamish et al. 2009). Although outbreaks of *L. salmonis* from salmon farms are known to result in negative consequences for juvenile salmon populations (Krkošek et al. 2006), these specialist lice have fewer hosts on which they can maintain high levels of abundance in the absence of suitable conditions on fish farms. It is therefore possible that the generalist louse can maintain a relatively constant level of infection, in absence of high prevalence on salmon farms, albeit with some fluctuations throughout the years. Our data support this, with less variation shown in the yearly, species-level and region-level differences in *C. clemensi* compared to *L. salmonis* (Figs. 5 & 6).

It is clear from a species-level perspective with regards to Pacific salmon, that pink salmon are likely the most competent hosts for both species of louse (Figs 3 & 5). The fact that pinks show the highest levels of infection for both *C. clemensi* and *L. salmonis* indicates that this single salmon species likely takes the brunt of baseline infections. However, and perhaps more importantly, this species of salmon is also most likely to feel the greatest effects from an outbreak of either or both lice simultaneously. This potentially offers an explanation for previous work that has linked declines in pink salmon population abundance with spikes in infestations (Morton & Williams 2003; Krkošek et al. 2007a ). In addition, Fig. 5 clearly shows that sockeye salmon experience the largest difference between the two lice species with respect to infection pressure. These results, while not conclusive, indicate that it is possible that *C. clemensi* has a larger role to play than previously thought in terms of determining the effect of sea lice infections on sockeye population abundance. That is to say, higher levels of *C. clemensi* infections could be driving sockeye population declines to a higher degree than *L. salmonis* infections*.* However, it has been shown that *L. salmonis* is a larger and potentially more lethal parasite to juvenile salmon compared to it’s generalist counterpart (Morton & Routledge 2005).

With respect to *C. clemensi* infections, we ought to consider the possibility that perhaps baseline levels of infection with regards to this generalist louseare higher than earlier estimates given our results here. In addition, previous work by Krkošek et al. (2005) showed that a small increase in the number of lice per fish could have serious consequences for Pacific salmon populations. Therefore, perhaps the increase in louse abundance relative to baseline infections needed to instigate a population decline is not only lower overall than previously thought, but that the increase needed to cause a decline varies between salmon species and lice species in a predictable way. Particularly with regard to pink and sockeye salmon who show high levels of *C. clemensi* infection through all four years of our data, if these already lice-laden fish were to come under pressure from an outbreak of the more lethal *L. salmonis,* this, in combination with their existing infection load, could have serious consequences for mortality. Furthermore, since pink salmon already harbour higher loads of *L. salmonis*, perhaps the increase in infections needed to cause a decline in pinks would be even lower than the increase needed to cause a decline in sockeye, assuming that the effect of adding a single louse is equivalent in the two species.

The large differences we see in terms of abundance between the two lice species is of interest and merits further discussion. Previous work has shown that *C. clemensi* are smaller and less fecund on farmed Atlantic salmon (they exhibited fewer eggs per string) (Byrne et al. 2018), which suggests that *L. salmonis* might in fact have higher transmission success/settlement and survival rates on juvenile Pacific salmon than its generalist counterpart. If we expect lice coming from salmon farms to thus be majority specialist lice, then when taken with the fact that we see *C. clemensi* in much higher abundances than *L. salmonis,* it stands to reason that there is a huge reservoir of *C. clemensi* unaccounted for somewhere else in the environment. Pacific herring pose the most logical source for this unaccounted reservoir (Morton et al. 2008; Beamish et al. 2009; Godwin et al. 2017). While Pacific herring are common in the environment and are known to carry infections of *C. clemensi* (Krkošek et al. 2007; Morton et al. 2008; Krkošek 2017), some sources have reported very high abundances of the generalist louse species on salmon farms (Di Cicco et al. 2017) and thus indicate that salmon farms themselves are the major source of *C. clemensi* infections to juvenile Pacific salmon. However, we believe that such high abundances are aberrations rather than the norm, as confirmed by a comparison of louse species abundance by Godwin et al. (*In Prep*). In addition, Pacific herring have been reported at record high abundances during the years corresponding with this present study (DFO, 2019), indicating a large reservoir host for *C. clemensi* was indeed present during those years. More data are needed regarding levels of infection on herring to confirm these assumptions, but given our conclusions here in conjunction with the previous work described, Pacific herring seem the most likely major source of transmission of *C. clemensi* to juvenile salmon.

Should Pacific herring in fact be the primary source of *C. clemensi* infections to juvenile Pacific salmon, then our results indicate that apparent competition between Pacific herring and juvenile Pacific salmon is indeed taking place. Particularly with regard to sockeye salmon, as they show the highest infection levels of *C. clemensi* combined with the lowest levels of the specialist louse. Thus, the effects on sockeye salmon from sea lice could largely be the results of apparent competition in this system between sockeye and Pacific herring. While both Pacific herring and Pacific salmon likely contributed to the initial infection of farmed Atlantic salmon and the subsequent spillback of both lice species, apparent competition in this system takes place between Pacific salmon and Pacific herring only. When viewing the Pacific salmon as the focal hosts in this system, both Pacific herring and farmed Atlantic salmon can be viewed as reservoir hosts, but apparent competition by definition includes the possibility that either of the indirectly competing populations can drive the other to extinction (Holt 1977, reviewed by: Dunn et al. 2012; Holt & Bonsall 2017). In this system, since Atlantic salmon are being actively added to the environment via fish farming, the two-way extinction pathway is removed, thus eliminating the possibility for apparent competition between the domestic and the wild populations. Other so-to-speak domestic populations have been considered a part of apparent competition (i.e. free-ranging domesticated dogs and wolves such as in Perry et al. 2018), but this type of example describes an introduced population, not one being actively managed. As such, this type of example is categorically different than the actively managed farmed Atlantic salmon population discussed here, and can be considered apparent competition while not classifying the present system as such.

The potential of Pacific herring providing a primary source of *C. clemensi* infections is reinforced via our region-level results. Fig. 6, which displays the results of our region-level model clearly shows that the generalist louse is present at higher levels in Johnstone Strait relative to the Discovery Islands. These model-driven results are confirmed by our hierarchical bootstrapping (Fig. 8) which shows the same patterns, albeit with one exception (chum salmon in 2015). However, this disagreement between the model and the data is likely irrelevant not only because just one estimate differed, but furthermore that our species-level results indicate that chum salmon are likely the least impacted with regards to overall infection pressure from these sea lice and thus the differing conclusion is less important with regards to inference for the maintenance of Pacific salmon populations. The reason that higher *C. clemensi* abundances in Johnstone Strait implicate Pacific herring as a primary source is simply that it differs from our estimates for the specialist louse. Since it is reasonable to assume that a large proportion of *L. salmonis* are in fact coming from farmed Atlantic salmon, this region-level pattern should persist if this source is also the most important for *C. clemensi*. The fact that these region-level patterns differ indicates the source pathway also differs, and when combined with the fact that spawning biomass of Pacific herring has been reaching record highs (DFO, 2019) these results further suggest that Pacific herring are the likely culprit.

An important take-home our results indicate is that something more is going on here deterministically that is structuring the patterns of infection in this multi-host parasite system. These data are describing a sympatric outmigration of three species of Pacific salmon, all of comparable sizes, migrating in heterospecific schools through the same region, and experiencing the same infection pressures from various transmission sources. A null hypothesis regarding the infection dynamics in this system might be that there are no differences between Pacific salmon species or between site regions with respect to the average number of lice per fish nor the ratio of louse species per fish. However, this is the opposite of what we see. Pink salmon clearly show the highest levels of infection from both species of louse, indicating that they are likely bearing the brunt of the infection pressure from both louse species, and further that they are likely the most competent hosts for both species of louse. This is logical given that they are the most abundant species of juvenile Pacific salmon in this system, but the ratio of louse species on the other species of Pacific salmon is not consistent, indicating pink salmon abundance does not explain these trends.These results speak to some sort of deterministic driver(s) that shape the infection landscape of this multi-host parasite system. There is clearly some sort of difference playing out here in terms of host preference or host specificity between these two lice, or perhaps this is hinting at some differing underlying mechanism of susceptibility in the three species of Pacific salmon. Some previous work has explored this issue (Peacock et al. 2014), but has not fully described the system.

This last conclusion merits further study with regards to teasing apart the potential explanations for these differences in infection. Further data are also needed to solidify our hypothesis with respect to the role of Pacific herring in this multi-host parasite system. In addition, our present study has not taken into account all of the various abiotic factors that are known to influence sea lice infections. An important note of further interest is that *L. salmonis* has been reported to show higher rates of settlement and higher rates of survival on the host fish with increased water temperatures (Tucker et al. 2000; Stien et al. 2005). This indicates that warming climates and subsequently warming water temperatures are likely to have a negative impact with regards to infection pressure on migrating juvenile salmon, particularly during spring and early summer when juvenile salmon are at their smallest and most vulnerable. Further work is also needed in this regard to gain a more complete understanding of this multi-host parasite system not only as it currently stands, but how further anthropogenic change will alter its dynamics in the future. However, the present study is a valuable step forward in terms of using temporally and spatially explicit data to derive quantitative results that shed light on species-level and region-level differences in infection in this system as well as hinting at potential mechanisms that are driving them.

**Table 1:** AIC table for *L. salmonis* and *C. clemensi* full model set.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Model Fixed Effects** | **Lice Species** | **Negative Log Likelihood** | **AIC value** | | **Delta-AIC value** | |
| **Null** | *C. clemensi* | -1509.7 | 3025.5 | | 27.9 | |
| **Year** | *C. clemensi* | -1502.8 | 3017.7 | | 20.1 | |
| **Species** | *C. clemensi* | -1497.3 | 3004.7 | | 7.1 | |
| **Species, Year** | *C. clemensi* | -1490.7 | 2997.6 | | 0.0 | |
| **Null** | *L. salmonis* | -461.3 | | 928.7 | 75.8 |
| **Year** | *L. salmonis* | -452.4 | | 916.7 | 63.9 |
| **Species** | *L. salmonis* | -427.8 | | 865.7 | 12.8 |
| **Species, Year** | *L. salmonis* | -418.4 | | 852.8 | 0.0 |

**Table 2:** Model results for the highest ranked models according to the AIC results

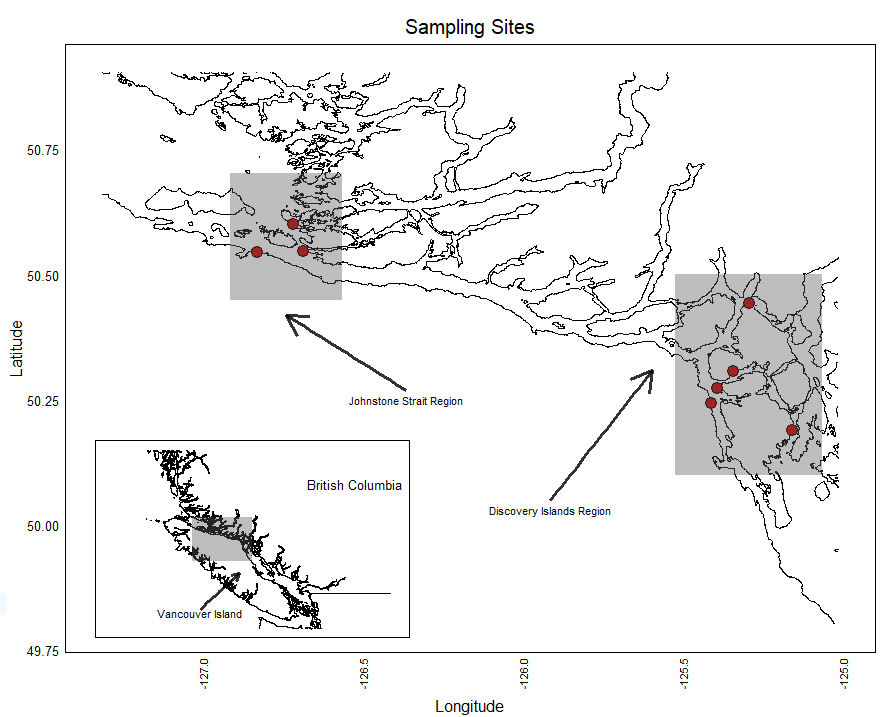
|  |  |  |  |
| --- | --- | --- | --- |
| **Model Output** | **Lice Species** | **Coefficient** | **Standard Error** |
| **Chum** | *L. salmonis* | -2.0747 | 0.3620 |
| **Pink** | *L. salmonis* | -1.2833 | 0.3241 |
| **Sockeye** | *L. salmonis* | -3.5723 | 0.4034 |
| **2016** | *L. salmonis* | -0.6621 | 0.3923 |
| **2017** | *L. salmonis* | -2.1076 | 0.9000 |
| **2018** | *L. salmonis* | -2.1381 | 0.5178 |
| **Chum** | *C. clemensi* | -0.9367 | 0.1667 | |
| **Pink** | *C. clemensi* | -0.3512 | 0.1508 | |
| **Sockeye** | *C. clemensi* | -0.4703 | 0.1467 | |
| **2016** | *C. clemensi* | -0.6012 | 0.1735 | |
| **2017** | *C. clemensi* | -0.8461 | 0.3117 | |
| **2018** | *C. clemensi* | -0.5259 | 0.1935 | |

**Table 3:** AIC table for region level effects of data for *C. clemensi* and chum salmon

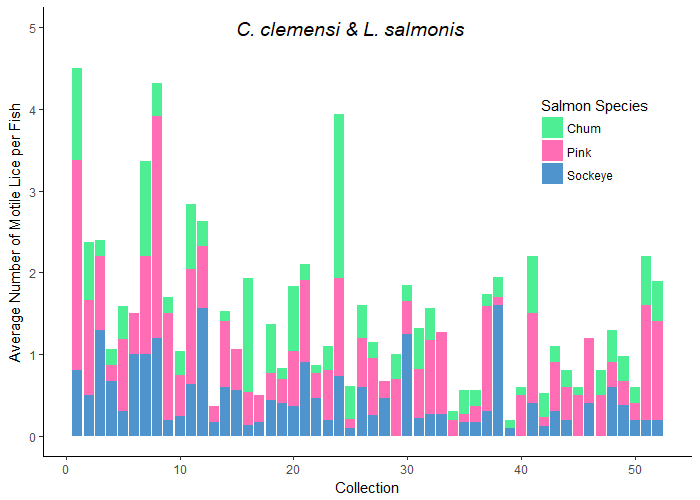
|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Fixed Effects** | **Lice Species** | **Salmon Species** | **Negative Log Likelihood** | **AIC value** | **Delta-AIC value** |
| **Null** | *C. clemensi* | Chum | -1062.0 | 2130.0 | 9.2 |
| **Site Region** | *C. clemensi* | Chum | -1060.2 | 2128.4 | 7.6 |
| **Year** | *C. clemensi* | Chum | -1055.8 | 2123.6 | 2.8 |
| **Site Region, Year** | *C. clemensi* | Chum | -1053.4 | 2120.8 | 0.0 |
| **Null** | *C. clemensi* | Pink | -907.8 | 1821.6 | 26.0 |
| **Site Region** | *C. clemensi* | Pink | -894.3 | 1796.5 | 1.0 |
| **Year** | *C. clemensi* | Pink | -907.0 | 1819.9 | 24.4 |
| **Site Region, Year** | *C. clemensi* | Pink | -890.8 | 1795.5 | 0.0 |
| **Null** | *C. clemensi* | Sockeye | -3281.3 | 6568.5 | 101.1 |
| **Site Region** | *C. clemensi* | Sockeye | -3275.3 | 6558.5 | 91.1 |
| **Year** | *C. clemensi* | Sockeye | -3229.9 | 6471.7 | 4.3 |
| **Site Region, Year** | *C. clemensi* | Sockeye | -3226.7 | 6467.4 | 0.0 |
| **Null** | *L. salmonis* | Chum | -258.7 | 523.5 | 12.9 |
| **Site Region** | *L. salmonis* | Chum | -256.8 | 521.5 | 11.0 |
| **Year** | *L. salmonis* | Chum | -251.1 | 514.2 | 3.7 |
| **Site Region, Year** | *L. salmonis* | Chum | -248.3 | 510.6 | 0.0 |
| **Null** | *L. salmonis* | Pink | -385.5 | 777.1 | 48.9 |
| **Site Region** | *L. salmonis* | Pink | -384.6 | 777.2 | 49 |
| **Year** | *L. salmonis* | Pink | -359.1 | 730.2 | 2.0 |
| **Site Region, Year** | *L. salmonis* | Pink | -357.1 | 728.2 | 0.0 |
| **Null** | *L. salmonis* | Sockeye | -392.3 | 790.5 | 6.7 |
| **Site Region** | *L. salmonis* | Sockeye | -387.9 | 783.8 | 0.0 |
| **Year** | *L. salmonis* | Sockeye | -390.1 | 792.3 | 8.5 |
| **Site Region, Year** | *L. salmonis* | Sockeye | -386.7 | 787.5 | 3.7 |

**Table 6:** Model results for the region-level models for all three species of salmon

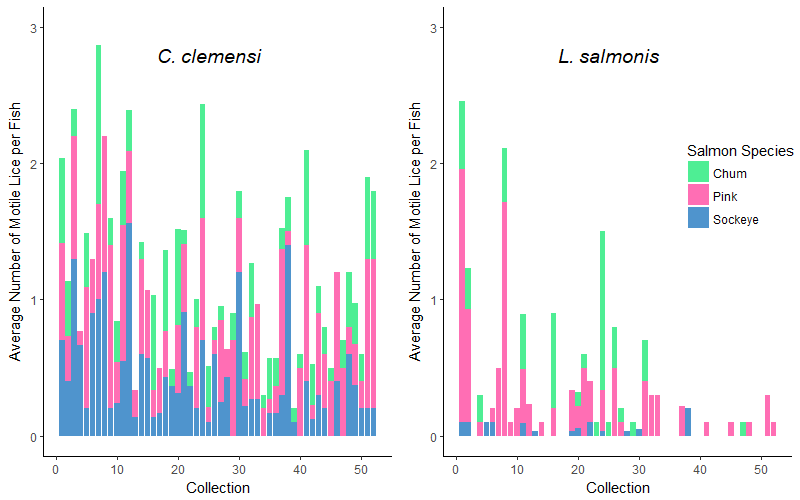
|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Fixed Effect** | ***Lice Species*** | Salmon Species | Coefficient | Standard Error | |
| **Discovery Islands** | ***C. clemensi*** | Chum | -0.8537 | 0.1846 | |
| **Johnstone Strait** | ***C. clemensi*** | Chum | -0.6314 | 0.1926 | |
| **2016** | ***C. clemensi*** | Chum | -0.5131 | 0.1637 | |
| **2017** | ***C. clemensi*** | Chum | -0.2677 | 0.1306 | |
| **2018** | ***C. clemensi*** | Chum | -0.3880 | 0.1352 | |
| **Discovery Islands** | ***C. clemensi*** | Pink | -0.7243 | 0.1773 | |
| **Johnstone Strait** | ***C. clemensi*** | Pink | -0.1891 | 0.1751 | |
| **2016** | ***C. clemensi*** | Pink | -0.4151 | 0.1591 | |
| **2017** | ***C. clemensi*** | Pink | -0.0865 | 0.1647 | |
| **Discovery Islands** | ***C. clemensi*** | Sockeye | -0.5722 | 0.0929 | |
| **Johnstone Strait** | ***C. clemensi*** | Sockeye | -0.4152 | 0.0926 | |
| **2016** | ***C. clemensi*** | Sockeye | -0.5296 | 0.0710 | |
| **2017** | ***C. clemensi*** | Sockeye | -0.7348 | 0.1012 | |
| **Discovery Islands** | ***L. salmonis*** | Chum | -2.3025 | 0.2821 | |
| **Johnstone Strait** | ***L. salmonis*** | Chum | -3.1487 | 0.3838 | |
| **2016** | ***L. salmonis*** | Chum | 0.4227 | 0.4203 | |
| **2017** | ***L. salmonis*** | Chum | -1.2784 | 0.4857 | |
| **Discovery Islands** | ***L. salmonis*** | Pink | -0.8806 | 0.1975 |
| **Johnstone Strait** | ***L. salmonis*** | Pink | -1.3489 | 0.2128 |
| **2016** | ***L. salmonis*** | Pink | -0.6860 | 0.2576 |
| **2017** | ***L. salmonis*** | Pink | -1.6262 | 0.4798 |
| **2018** | ***L. salmonis*** | Pink | -2.2009 | 0.3248 |
| **Discovery Islands** | ***L. salmonis*** | Sockeye | -4.5724 | 0.322 |
| **Johnstone Strait** | ***L. salmonis*** | Sockeye | -3.8232 | 0.296 |



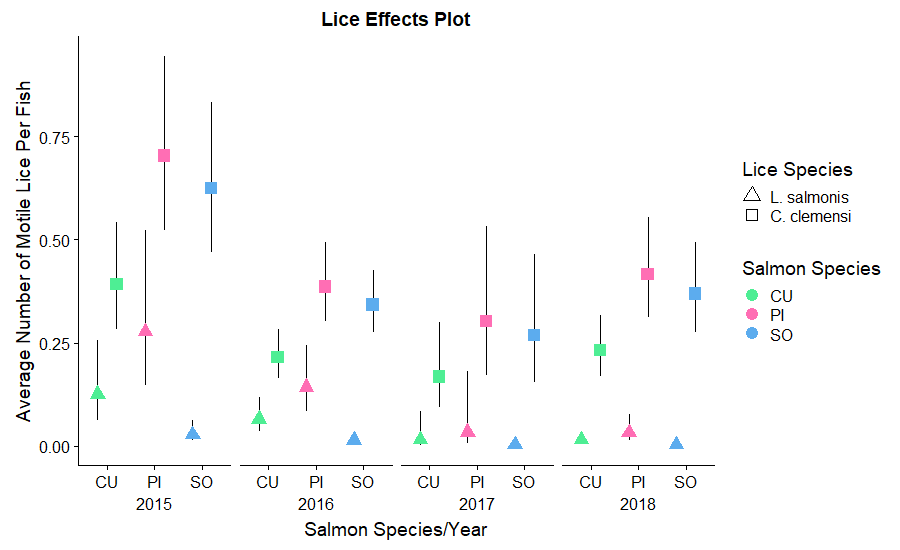
**Figure 1:** The study region, on the west coast of British Columbia, Canada. All sites lie in the Discovery Islands or Johnstone Strait regions, wedged between the western coast of the mainland, and the east coast of Vancouver Island. This is the region that is most heavily developed for fish farming.

****

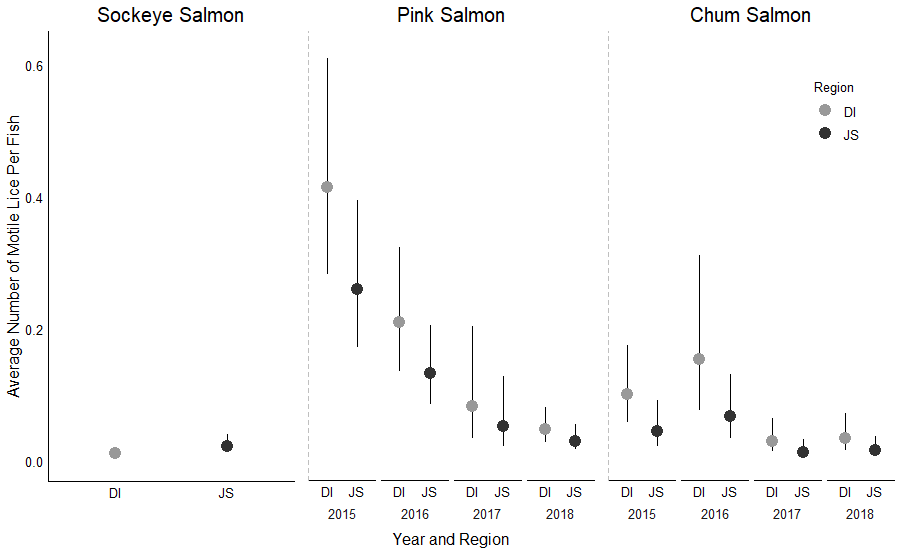
**Figure 2:** Here we see the average number of lice per fish (both lice species as well as individuals at unidentified stages combined), for each collection across the data. The colours in each collection represent the species of salmon**.**



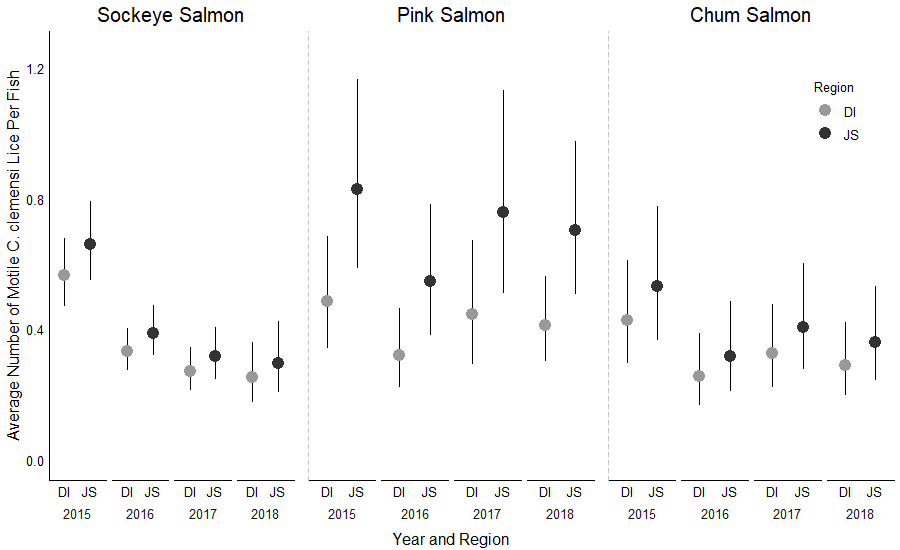
**Figure 3:** Here we see the average number of lice per fish in each collection, divided into two plots to separate by louse species, and divided by colour to separate by fish species.. Visually, pink salmon seem to have higher average numbers of *L. salmonis*.



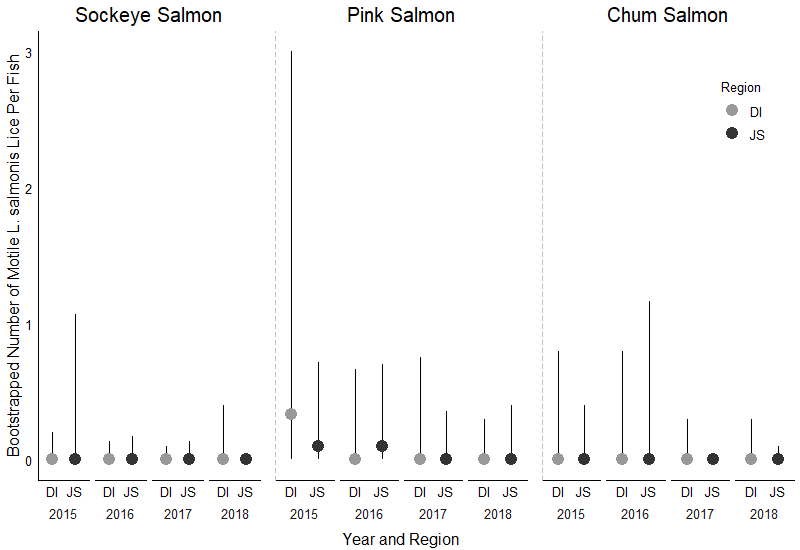
**Figure 4:** Estimated number of lice per fish for *C. clemensi* & *L. salmonis,* divided by sampling year, as well as by salmon species, and grouped by site region. These estimates were derived from the model output of our best model according to AIC analysis, and thus give estimates relative to each of the significant predictor variables.



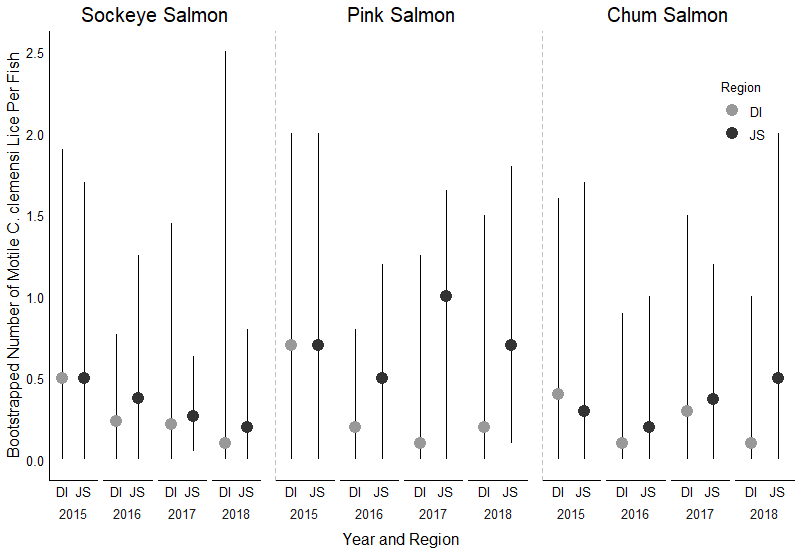
**Figure 5:** Estimated number of lice per fish for the two study regions, Johnstone Strait and the Discovery Islands, compared within salmon species for *L. salmonis* lice only. These estimates area a result of our region-level models. The sockeye-*L. salmonis* data combination AIC scores indicated that a model with only site region effects fit better than a model with site region and year, whereas all other AIC scores indicated that the full model with both fixed effects was preferred.

****

**Figure 6:** Estimated number of lice per fish for the two study regions, Johnstone Strait and the Discovery Islands, compared within salmon species for *C. clemensi* lice only, and across the four sampling years.

****

**Figure 7:** Bootstrapped median and 95% confidence intervals for the number of *L. salmonis* per fish for the two study regions, Johnstone Strait and the Discovery Islands, compared within salmon species.

****

**Figure 7:** Bootstrapped median and 95% confidence intervals for the number of *C. clemensi* per fish for the two study regions, Johnstone Strait and the Discovery Islands, compared within salmon species.

**References:**

1. Adlard RD, Miller TL, Smit NJ. 2015 The butterfly effect: Parasite diversity, environment, and emerging disease in aquatic wildlife. *Trends Parasitol.* **31**, 160–166. (doi:10.1016/j.pt.2014.11.001)

2. Brooks ME, Kristensen K, van Benthem KJ, Magnusson A, Berg CW, Nielsen A, Skaug HJ, Mächler M, Bolker BM. 2017 glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *R J.* **9**, 378–400. (doi:10.3929/ETHZ-B-000240890)

3. Brunner JL, Schock DM, Davidson EW, Collins JP. 2004 Intraspecific Reservoirs: Complex Life History and the Persistence of a Lethal Ranavirus. *Ecology*. **560**.

4. Byrne AA, Pearce CM, Cross SF, Jones SRM, Robinson SMC, Hutchinson MJ, Miller MR, Haddad CA, Johnson DL. 2018 Planktonic and parasitic stages of sea lice (Lepeophtheirus salmonis and Caligus clemensi) at a commercial Atlantic salmon (Salmo salar) farm in British Columbia, Canada. *Aquaculture* **486**, 130–138. (doi:10.1016/j.aquaculture.2017.12.009)

5. Cleaveland S, Hess GR, Dobson AP, Laurenson MK, McCallum HI, Roberts MG, Woodroffe R. 2002 The role of pathogens in biological conservation. In *The Ecology of Wildlife Diseases*, pp. 139–150.

6. Cohen BI commissioner. 2012 Commission of Inquiry into the Decline of Sockeye Salmon in the Fraser River (Canada) The uncertain future of Fraser River sockeye Final Report – October 2012.

7. Cooke SJ, Hinch SG, Farrell AP, Lapointe MF, Jones SRM, Macdonald SJ, Patterson DA, Healey MC, Van Der Kraak G. 2004 Abnormal migration timing and high en route mortality of Sockeye Salmon in the Fraser river, British Columbia. *Fisheries* **29**, 23–32. (doi:10.1577/1548-8446(2004)29[22:AMTAHE]2.0.CO;2)

8. Costello MJ. 1993 Review of methods to control sea lice (Caligidae: Crustacea) infestations on salmon (Salmo salar) farms. *Pathog. wild farmed fish sea lice.* , 219–252.

9. Costello MJ. 2009 How sea lice from salmon farms may cause wild salmonid declines in Europe and North America and be a threat to fishes elsewhere. *Proc. R. Soc. B Biol. Sci.* **276**, 3385–3394. (doi:10.1098/rspb.2009.0771)

10. Daszak P, Cunningham AA, Hyatt AD. 2001 Anthropogenic environmental change and the emergence of infectious diseases in wildlife. *Acta Trop.* **78**, 103–116. (doi:10.1016/S0001-706X(00)00179-0)

11. DFO. 2015 Stock Assessment and Management Advice for BC Pacific Herring: 2015 Status and 2016 Forecast. *Can. Sci. Advis. Secr. Sci. Response*. **2015/038**.

12. Eliason EJ *et al.* 2011 Differences in Thermal Tolerance Among Sockeye Salmon Populations. *Science (80-. ).* **332**, 109–112. (doi:10.1126/science.1198767)

13. FAO. 2017 Global capture production 1950–2013. *United Nations, Fish. Aquac. Inf. Stat. Branch.* **91**. (doi:10.1111/jfb.13325)

14. Godwin SC, Dill LM, Reynolds JD, Krkošek M. 2015 Sea lice, sockeye salmon, and foraging competition: lousy fish are lousy competitors. *Can. J. Fish. Aquat. Sci.* **72**, 1113–1120. (doi:10.1139/cjfas-2014-0284)

15. Hartigan A, Fiala I, Dyková I, Jirků M, Okimoto B, Rose K, Phalen DN, Šlapeta J. 2011 A suspected parasite spill-back of two novel myxidium spp. (myxosporea) causing disease in Australian endemic frogs found in the invasive cane toad. *PLoS One* **6**, e18871. (doi:10.1371/journal.pone.0018871)

16. Haydon DT, Cleaveland S, Taylor LH, Laurenson MK. 2002 Identifying reservoirs of infection: a conceptual and practical challenge. *Emerg. Infect. Dis.* **8**, 1468–73. (doi:10.3201/eid0812.010317)

17. Holt RD, Lawton JH. 2003 The Ecological Consequences of Shared Natural Enemies. *Annu. Rev. Ecol. Syst.* **25**, 495–520. (doi:10.1146/annurev.es.25.110194.002431)

18. Holt RD. 1977 Predation, Apparent Competition, and the Structure of Prey Communities. *Theor. Popul. Biol.* **12**, 197–229.

19. Hunt BPV, Johnson BT, Godwin SC, Krkošek M, Pakhomov EA, Rogers LA. 2018 The Hakai Institute Juvenile Salmon Program : Early Life History Drivers of Marine Survival in Sockeye , Pink and Chum Salmon in British Columbia. *North Pacific Anadromous Fish Comm.*

20. Johnson SC, Albright LJ. 1991 Development, growth, and survival of lepeophtheirus salmonis (Copepoda: Caligidae) under laboratory conditions. *J. Mar. Biol. Assoc. United Kingdom* **71**, 425–436. (doi:10.1017/S0025315400051687)

21. Kelly DW, Paterson RA, Townsend CR, Poulin R, Tompkins DM. 2009 Parasite spillback: A neglected concept in invasion ecology? *Ecology*. **90**.

22. Krkošek M. 2010 Sea lice and salmon in Pacific Canada: Ecology and policy. *Front. Ecol. Environ.* **8**, 201–209. (doi:10.1890/080097)

23. Krkošek M, Ford JS, Morton A, Lele S, Myers RA, Lewis MA. 2007 Declining Wild Salmon Populations in Relation to Parasites from Farm Salmon. *Science (80-. ).* **40**, 1772–1775. (doi:10.1126/science.1149887)

24. Krkošek M, Gottesfeld A, Proctor B, Rolston D, Carr-Harris C, Lewis MA. 2007 Effects of host migration, diversity and aquaculture on sea lice threats to Pacific salmon populations. *Proc. R. Soc. B Biol. Sci.* **274**, 3141–3149. (doi:10.1098/rspb.2007.1122)

25. Krkošek M, Lewis MA, Volpe JP, Morton A. 2006 Fish farms and sea lice infestations of wild juvenile salmon in the Broughton Archipelago - A Rebuttal to Brooks (2005). *Rev. Fish. Sci.* **14**, 1–11. (doi:10.1080/10641260500433531)

26. Krkošek M, Lewis MA, Volpe JP. 2005 Transmission dynamics of parasitic sea lice from farm to wild salmon. *Proc. R. Soc. B Biol. Sci.* **272**, 689–696. (doi:10.1098/rspb.2004.3027)

27. Krkosek M, Revie CW, Gargan PG, Skilbrei OT, Finstad B, Todd CD. 2013 Impact of parasites on salmon recruitment in the Northeast Atlantic Ocean. *Proceedings. Biol. Sci.* **280**, 20122359. (doi:10.1098/rspb.2012.2359)

28. Lüdecke D. 2018 ggeffects: Tidy Data Frames of Marginal Effects from Regression Models. *J. Open Source Softw.* **3**. (doi:10.21105/joss.00772)

29. Morton A, Routledge RD, Williams R. 2005 Temporal Patterns of Sea Louse Infestation on Wild Pacific Salmon in Relation to the Fallowing of Atlantic Salmon Farms; Temporal Patterns of Sea Louse Infestation on Wild Pacific Salmon in Relation to the Fallowing of Atlantic Salmon Farms. *North Am. J. Fish. Manag.* **25**, 811–821. (doi:10.1577/M04-149.1)

30. Morton A, Routledge R, Peet C, Ladwig A. 2004 Sea lice ( Lepeophtheirus salmonis ) infection rates on juvenile pink ( Oncorhynchus gorbuscha ) and chum ( Oncorhynchus keta ) salmon in the nearshore marine environment of British Columbia, Canada. *Can. J. Fish. Aquat. Sci.* **61**, 147–157. (doi:10.1139/f04-016)

31. Morton A, Routledge R, Krkoš M. 2008 Sea Louse Infestation in Wild Juvenile Salmon and Pacific Herring Associated with Fish Farms off the East-Central Coast of Vancouver Island, British Columbia. *North Am. J. Fish. Manag.* **28**, 523–532. (doi:10.1577/M07-042.1)

32. Nagasawa K. 2001 Annual changes in the population size of the salmon louse Lepeophtheirus salmonis (Copepoda: Caligidae) on high-seas Pacific Salmon (Oncorhynchus spp.), and relationship to host abundance. *Hydrobiologia* **453**–**454**, 411–416. (doi:10.1023/A:1013154403992)

33. Peacock SJ, Bateman AW, Krkošek M, Connors B, Rogers S, Portner L, Polk Z, Webb C, Morton A. 2016 Sea-louse parasites on juvenile wild salmon in the Broughton Archipelago, British Columbia, Canada. *Ecology* **97**, 1887. (doi:10.1002/ecy.1438)

34. Perry LR, Marino J, Sillero-Zubiri C. 2018 Going to the Dogs: Free-Ranging Domestic Dogs Threaten an Endangered Wild Canid through Competitive Interactions. *J. Biodivers. Endanger. Species* **6**. (doi:10.4172/2332-2543.1000211)

35. Peterman RM, Marmorek D, Beckman B, Bradford M. 2010 Synthesis of evidence from a workshop on the decline of Fraser River sockeye. *A Rep. to Pacific Salmon Comm. Vancouver, B.C.* , 123pp.

36. Price MHH, Proboszcz SL, Routledge RD, Gottesfeld AS, Orr C, Reynolds JD. 2011 Sea louse infection of juvenile sockeye salmon in relation to marine salmon farms on Canada’s west coast. *PLoS One* **6**, e16851. (doi:10.1371/journal.pone.0016851)

37. Williams HH. 1964 Some observations on the mass mortality of the freshwater fish Rutilus rutilus (L.)\*. *Parasitology* **54**, 155–171.

38. Tucker CS, Sommerville C, Wootten R. 2000 The effect of temperature and salinity on the settlement and survival of copepodids of Lepeophtheirus salmonis (Krøyer, 1837) on Atlantic salmon, Salmo salar L. *J. Fish Dis.* **23**, 309–320. (doi:10.1046/j.1365-2761.2000.00219.x)

39. Stien A, Bjorn PA, Heuch PA, Elston DA. 2005 Population dynamics of salmon lice Lepeophtheirus salmonis on Atlantic salmon and sea trout. *Mar. Ecol. Prog. Ser.* **290**, 263–275.

40. Quinnell RJ, Courtenay O. 2009 Transmission, reservoir hosts and control of zoonotic visceral leishmaniasis. *Parasitology* **136**, 1915–1934. (doi:10.1017/S0031182009991156)

41. Longshaw M, Frear PA, Nunn AD, Cowx IG, Feist SW. 2010 The influence of parasitism on fish population success. *Fish. Manag. Ecol.* **17**, 426–434. (doi:10.1111/j.1365-2400.2010.00741.x)

42. Holt RD, Bonsall MB. 2017 Apparent Competition. *Annu. Rev. ofEcology, Evol. Syst.* **48**, 447–471. (doi:10.1146/annurev-ecolsys-110316)

43. Anderson RM, May RM. 1979 Population biology of infectios diseases: Part I. *Nature* **280**, 361–367.

44. Beamish R, Wade J, Pennell W, Gordon E, Jones S, Neville C, Lange K, Sweeting R. 2009 A large, natural infection of sea lice on juvenile Pacific salmon in the Gulf Islands area of British Columbia, Canada. *Aquaculture* **297**, 31–37. (doi:10.1016/J.AQUACULTURE.2009.09.001)

45. Peacock SJ, Connors BM, Krkosek M, Irvine JR, Lewis MA, Krkos M. 2014 Can reduced predation offset negative effects of sea louse parasites on chum salmon ? *Proc. R. Soc. B Biol. Sci.*

46. Krkošek M. 2017 Population biology of infectious diseases shared by wild and farmed fish. *Can. J. Fish. Aquat. Sci.* **74**, 620–628. (doi:10.1139/cjfas-2016-0379)

47. Bricknell IR, Dalesman SJ, O’Shea B, Pert CC, Mordue Luntz AJ. 2006 Effect of environmental salinity on sea lice Lepeophtheirus salmonis settlement success. *Dis. Aquat. Organ.* **71**, 201–212.

48. Godwin SC, Krkošek M, Reynolds JD, Rogers LA, Dill LM. 2017 Heavy sea louse infection is associated with decreased stomach fullness in wild juvenile sockeye salmon. *Can. J. Fish. Aquat. Sci.* **75**, 1587–1595. (doi:10.1139/cjfas-2017-0267)

49. Groner ML *et al.* 2016 Lessons from sea louse and salmon epidemiology. *Philos. Trans. R. Soc. B Biol. Sci.* **371**. (doi:10.1098/rstb.2015.0203)