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Sensitivity towards low salinity determined by bioassay in the salmon louse, *Lepeophtheirus salmonis* (Copepoda: Caligidae)

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A B S T R A C T

Sea lice, *Lepeophtheirus salmonis* (Krøyer, 1837), are ectoparasitic crustacean parasites responsible for economic losses in the Atlantic salmon aquaculture industry in the northern hemisphere. Numerous chemical and non-chemical control methods have been developed, including freshwater bathing. Freshwater bathing is regarded as an environmentally friendly treatment; however, reports of variable treatment efficacies have raised concerns regarding the general applicability of this treatment method. Our study aimed to determine the salinity in parts per thousand (‰) at which median survival (EC_{50}) was obtained at 24 h for the copepodid and pre-adult II stages of *L. salmonis* from geographically separated populations and to develop a bioassay method for on-site pre-treatment tests. Parasites were separated into four different geographically separated populations which are referred to as population A, B, C, and D. Using a stepwise method, parasites were exposed to a range of salinities (35‰ to 0‰) for 24 h, survival was observed after 24 h and results were classified as unaffected/affected. *L. salmonis* copepodids illustrated significant differences in tolerance among populations. Population A was the least tolerant, with an EC_{50} of 17.5‰, whereas population C had an EC_{50} of 11.3‰. No significant population difference in tolerance was observed among the pre-adult II stages. Pooling the data from pre-adult II *L. salmonis* from three populations yielded an EC_{50} of 2.8‰ for both sexes combined, 2.4‰ for males, and 2.6‰ for females. All stages of *L. salmonis* examined in this study exhibited higher tolerances to low salinity than observed in previous studies. We suggest regular monitoring of freshwater sensitivity in areas where this control option is regularly used to detect possible deviations from baseline sensitivity at an early stage.

1. Introduction

Salmon lice, *Lepeophtheirus salmonis* (Krøyer, 1837), also called sea lice, are ectoparasites of wild and cultured salmonid fish in the northern hemisphere (Costello, 2006). *L. salmonis* mainly occur on the skin and fins of the fish host, where they feed on blood, mucus and skin tissue (Johnson and Albright, 1991; Pike and Wadsworth, 1999; Wagner et al., 2008). Negative impacts resulting from infestations have been recorded when parasite numbers increase without appropriate control measures being implemented (Morton and Routledge, 2016; Torrissen et al., 2013). High densities of *L. salmonis* have been associated with the development of skin lesions, secondary infections, decreased growth rates, increased treatment needs, production costs, and mortality (Costello, 2009; Torrissen et al., 2013; Wagner et al., 2008).

Various chemical and non-chemical treatment methods have been developed to control *L. salmonis* populations on farmed fish (Aaen et al., 2014; Jones et al., 2012; Ritchie et al., 2002; Torrissen et al., 2013;). Treatments may be applied through either bathing (cypermethrin, deltamethrin, azamethiphos, hydrogen peroxide) or in-feed (emamectin benzoate, diflubenzuron, teflubenzuron) methods (Aaen and Horsberg, 2016; Poley et al., 2016; Sevatdal and Horsberg, 2003). In Norway, such treatments can be legally applied only after receiving a prescription from the local fish health services, a measure aimed at limiting drug use and the development of parasites' resistance to the treatments. Nevertheless, issues arising from the development of drug resistance exist throughout salmon-production regions (Aaen et al., 2015; Fallang et al., 2005; Helgesen et al., 2015; Ljungfeldt et al., 2014; Treasurer et al., 2000). Integrated pest management approaches using non-chemical treatments, such as the use of cleaner fish, warm water, and freshwater bathing have been implemented to reduce the use of chemicals and prevent the development of treatment resistance (Boxaspen, 2006; Brooks, 2009; Imsland et al., 2014; Stien et al., 2018; Wright et al., 2016; Wright et al., 2017).

To monitor the possible changes in sensitivity of the parasites to a particular treatment over generations, a robust assay method is necessary. Bioassays are valuable tools when monitoring sensitivity levels by allowing for the quantification of a response (unaffected/affected) of a target organism, in this case *L. salmonis*, to increasing treatment doses or concentrations (Brogdon, 1989; Westcott et al., 2008). Bioassays are often used as a monitoring tool but can also indicate whether a treatment will provide the desired results (Sevatdal and Horsberg, 2003). Conducting such bioassays would provide information on sensitivity towards the planned treatment at a population level for *L. salmonis*, providing an opportunity to change to a more effective treatment method (Denholm, 1990). This in turn would reduce the probability of the development of treatment resistance (Denholm et al., 2002). In

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Fig. 1. Map of Norway, including the locations of *L. salmonis* populations A-D, and the testing site (star) where the bioassays were performed [Norway administration location map, 2019].

order for such bioassays to be useful in the field, they need to be simple yet robust, and repeatable, and provide a clear endpoint (Denholm et al., 2002). In the case of *L. salmonis,* treatment bioassays conducted on the host fish are time- and resource-consuming, thus shifting the focus to conducting *in vitro* bioassays using pre-adult and adult *L. salmonis* (Sevatdal and Horsberg, 2003). Since the early 1990s, bioassays have been effectively used to identify resistance to the commonly used chemotherapeutants (Jones et al., 1992; Lees et al., 2008; Roth et al., 1996; Treasurer et al., 2000). The accuracy of such *in vitro* bioassays has resulted in them being routinely used in field sensitivity testing throughout the production cycle to monitor the efficacy of commonly used chemical treatment compounds on *L. salmonis* populations (Helgesen and Horsberg, 2013). In addition, bioassays are conducted for regional surveillance purposes (Helgesen et al., 2018; Taranger et al., 2015). These bioassays can indicate treatment efficacy and often provide a median effective concentration (EC_{50}) (Westcott et al., 2008). Ideally, such bioassays are simple to perform and easily replicable, allowing the bioassays to be conducted on site prior to pre-planned treatments. In this way, they can allow for adjustments in treatment plans to counter treatment resistance development (Sevatdal et al., 2005). Furthermore, when the same method is applied in different laboratories and different areas over years, the data can be used to follow spatial and temporal trends in sensitivity development towards treatments, as described in the Norwegian national monitoring program for resistance in salmon lice (Helgesen et al., 2018).

Freshwater bathing has long been used to combat ectoparasites on cultured fish and is conducted using either the well-boat method or the tarpaulin method (Powell et al., 2015; Reynolds, 2013). The well-boat method entails pumping fish into a well-boat carrying oxygenated freshwater. The fish are then held for up to 8 h and thereafter returned to their netpens (Powell et al., 2015). The tarpaulin method entails lining an empty cage with a tarpaulin and filling it with freshwater; the fish are then pumped into the tarpaulin and held for 8 h, and then the tarpaulin is removed (Pironet and Jones, 2000; Powell et al., 2015). Variable results following freshwater treatment have been reported over the years (Connors et al., 2008; Stone et al., 2002); this led us to re-examine current methods and attempt to develop a low-cost bioassay protocol that can be used on-site.

Freshwater bathing has been identified as a method to combat *L. salmonis*. Short-time exposures (1 h) have been evaluated as not effective (Stone et al., 2002), while holding times up to 4 h in a well boats demonstrated an efficacy of 96%, without correction for handling (Reynolds, 2013). An added benefit is having a much lower impact on the environment surrounding the farm site when compared to other chemical treatments (Burka et al., 2003; Burridge et al., 2010; Jackson et al., 2017; Taranger et al., 2015). However, this treatment method does have its negative aspects which should be recognised. Aside from the danger of the development of treatment tolerance, these include the increased labour costs, high well boat maintenance costs, and increased fish handling that may result in injuries or loss of fish. As there is normal variation in the parasite's tolerance to fresh water (Ljungfeldt et al., 2017), there is also a possibility that *L. salmonis* could develop a

tolerance to the treatment, which in turn introduces the need to monitor the parasite's sensitivity using bioassay methods.

The aim of this study was to develop a simple, on-site bioassay protocol to rapidly identify the salinity level at which a local salmon louse population experiences reduced survival (50%) and to provide baseline sensitivity information that is necessary for interpreting results and avoiding the development of treatment tolerance. The stepwise method used here is more representative of the natural conditions experienced by the salmon louse when moving through different salinity gradients whilst attached to the host fish. This bioassay will allow authorities and salmon farmers to monitor the sensitivity towards fresh water over time to identify possible shifts in sensitivity. They can also be used by the fish health worker to evaluate whether a freshwater bath might be effective at a specific time point.

2. Materials and methods

2.1. Copepodid L. salmonis bioassays

A total of 17 bioassays were conducted using four geographically distinct populations of *L. salmonis* (Fig. 1). In this study, even though they all belong to the same species we refer to them as separate populations as they are prevented from mixing with each other due to physical barriers such as long distance and extensive fjord systems. The populations include one originally collected in 2010 in northern-Norway (Population A) and one originally collected in 2013 in mid-Norway (Population B). These populations have since been reared in the laboratory. In addition, two field-collected populations from western-Norway (Population C) and mid-Norway (Population D) were included. Egg strings were either hatched directly upon arrival (field populations) or collected from reservoir adult *L. salmonis* (laboratory population) cultured on Atlantic salmon (AquaGen strain). Once they had reached the copepodid stage, the parasites were either tested directly or used to infect new fish to produce pre-adult II (PA II) and adult parasites. The fish were held in 1000 L fibreglass tanks at the NIVA Marine Research Station, Solbergstrand, Drøbak, Norway (Fig. 1). They were supplied with through-flow seawater piped in from a depth of 60 m which ensured that the water temperature remained between 7 and 10 °C and the salinity remained near constant at 34.5‰ throughout the year. The cultivation protocol used was that of Hamre et al. (2009), following the guidelines provided in the animal ethics statement. Water temperature affected the development time, which is approximately two weeks from collection of egg strings to copepodids during the winter months. The copepodids used in the bioassays were transported in 1 L containers of seawater to the laboratory and placed in a temperature-controlled cabinet set at 12 °C where they were allowed to acclimate prior to use.

The bioassays were performed in 50 mL containers, two per salinity level, each containing an average of 16.6 actively swimming copepodids (range 10–27 copepodids). The containers were each assigned to one the following 15 salinities: a seawater control (35‰), 31, 29, 27, 25, 23, 21, 19, 17, 15, 13, 11, 9, 7, and 5‰ (g/L). The stepwise exposure method was employed as it was more representative of the natural conditions experienced by the salmon louse when moving through different salinity gradients whilst attached to the host fish. This was achieved by removing seawater and replacing with deionised freshwater was used to gradually achieve the assigned salinity. The exact volume to be removed depends on the salinity of the source seawater, for example the salinity of the seawater used in these bioassays remained consistent at 34.5‰. Using an online salinity calculator (Target salinity calculator, 2019) we calculated that in order to reach 23‰ we had to remove 15 ml seawater and replace it with 15 ml deionised water, followed by removing 3 ml seawater and adding 3 ml deionised water. The lower salinities required more steps to reach the target salinity than the higher salinities, however we ensured that we used the same amount of time between steps throughout the bioassays allowing all copepodids the same amount of time to respond to each change in salinity. The containers were then returned to the temperature-controlled cabinet (12 °C) and remained undisturbed for 24 h. Once 24 h had elapsed, each container was examined by emptying its contents into a petri dish and recording the numbers of unaffected and affected copepodids. Using methods described by Hamre et al. (2009) status of the copepodids was determined by agitating the water around each copepodid and observing it for signs of normal swimming movement (unaffected); animals exhibiting abnormal movement or lack of movement were classified as affected. Abnormal movement may be classified as erratic swimming behaviour, an inability to hold position in the water column, and delayed reaction to external stimuli.

2.2. Pre-adult II and adult L. salmonis bioassays

Thirteen PA II bioassays and four adult bioassays were conducted. The parasites originated from three populations collected in the field, named populations A, B and C. Copepodids were cultured in the laboratory as described above. For each bioassay, a 1000 L tank was prepared, into which 8 Atlantic salmon (approximately 500 g each) were introduced and allowed to acclimate for 1–2 days. The water was reduced to a level just above the dorsal fin, the water was aerated to maintain sufficient oxygen levels, and approximately 40 copepodids/fish were introduced. The fish remained undisturbed for 1 h, after which the water flow was returned to normal levels as set by the research facility. Approximately 5 weeks following infection, the majority of *L. salmonis* had reached the PA II stage at the average water temperature at the experimental station. The fish were lightly anaesthetised with metacaine (100 mg/L) (Finquel™, Scanvacc, Norway), and all of the parasites were removed with forceps, placed in a 1 L container of seawater and transported to a temperature-controlled cabinet (12 °C) in the laboratory. The same procedures were followed for the adult stage, however it took 1–2 weeks longer for them to reach maturity.

The following methods were the same for both the PA II and adult bioassays. All bioassays were performed in 1 L beakers containing 500 mL seawater and 10 PA II *L. salmonis*. A beaker was randomly designated one of the following salinities: seawater control (35‰), 26, 20, 15, 10, 5 and 0‰. Due to limitations in acquiring large numbers of parasites, we used one replicate beaker per salinity for each bioassay. As described in the previous section, a stepwise method of removing seawater and replacing it with deionised freshwater was used to gradually achieve the assigned salinity. The containers were then returned to the temperature-controlled cabinet (12 °C), provided aeration, and left undisturbed for 24 h. After the 24 h had elapsed, the containers were vigorously stirred, allowed to settle, and stirred again before being emptied over a sieve. If a salmon louse remained attached to the wall of the container or exhibited normal swimming behaviour, it was considered unaffected; those parasites that did not attach were placed in a petri dish and observed. Using forceps we gently disturbed them, if they swam away from the stimulus and were able to attach they were unaffected. If they attempted to swim away from the stimulus, however the swimming movements were erratic and they were unable to attach, they were classified as affected. These observations were conducted for approximately 5 min in order to ensure that all the parasites were observed equally.

2.3. Statistical methods

The mortality data presented in the results were log-transformed and analysed *via* probit regression to determine the median immobilising concentrations (EC_{50}) for each life stage. Bivariate ANOVA tests of survival as a function of salinity were conducted for the copepodids; combined PA II males and females; PA II males *vs* females; combined adult male and females; and adult males *vs* females. Pairwise *t*-tests were conducted to compare survival between populations in the copepodid stage. Comparisons in survival between the PA II males and

females, and the adult males and females, at the different salinities were conducted using multivariate pairwise correlations. Population differences in the response to different salinity levels were examined by performing ANOVAs and regression analyses. All statistical analyses were conducted using JMP Pro 12.1 (SAS Institute Inc., Cary, North Carolina, USA).

2.4. Animal ethics statement

The cultivation of *L. salmonis* on Atlantic salmon was approved by the Norwegian Food Safety Authority on 22 March 2016, ID 8490, and 2 May 2018, ID 15454, in accordance with the guidelines set by the authority. The NIVA Marine Research Station is approved as an animal research facility by the Norwegian Food Safety Authority, in accordance with guidelines set by the authority. All fish were anesthetised with metacaine prior to the collection of parasites for the experiments. Research on *L. salmonis* does not require a special permit from the Norwegian Food Safety Authority.

3. Results

Analysis of the four populations of *L. salmonis* revealed population differences in tolerance to low salinity levels at the copepodid stage (Fig. 2). Observing the survival curves for each population across the salinity gradient revealed that population C (Wild, West-Norway, 2016) maintained high survival rates (> 90%) until 15‰, after which survival rapidly dropped (Fig. 2), whereas populations B (Lab, Mid-Norway, 2013) and D (Wild, Mid-Norway, 2016) experienced a gradual reduction in survival below 90% at salinities below 19‰, and population A (Lab, North-Norway, 2010) showed a decline in survival below 90% at salinities below 23‰ (Fig. 2). In addition, the dose-response curve shows a significant difference in treatment tolerance when comparing the EC_{50} values for populations A (17.5‰) and C (11.3‰), however populations B (13.9‰) and population D (13.7‰) were very similar (Fig. 3). Paired sample *t*-test was conducted between the survival curves of population A and C indicated a statistically significant difference in survival between populations (T: 2.14, $P < .018$).

When examining the PA II stage, all three populations of *L. salmonis* showed a high tolerance to 24 h exposure to reduced salinity, with survival being above 68% in all populations down to a salinity of 5‰, but thereafter declining rapidly to below 20% (Fig. 4). An ANOVA test of survival as a function of salinity was conducted using the pooled male PA II ($F_{1,71} = 29.6$; $P < .0001$) and pooled female PA II data ($F_{1,72} = 39.9$; $P < .0001$). These results suggest that salinity does affect the survival rates of both male and female PA II, however there is no significant difference in survival rates between the sexes (Fig. 5). No significant difference in PA II EC_{50} value was observed among the populations with values of 2.3‰ for population A, 3.3‰ for population A, and 3.5‰ for population C, this is supported by analysis of the dose-response curve indicating that there is no significant difference in tolerance between populations (Fig. 6).

Adult *L. salmonis* (males and females) from populations A and C exhibited high rates of survival across all salinities, thus resulting in no significant differences between the two populations (Fig. 7) or between adult males and females when the populations were combined (Fig. 8). ANOVA analyses of the effect of reduced salinity on survival were conducted on adult males ($F_{1,21} = 0.33$; $P = .57$), adult females ($F_{1,21} = 1.44$; $P = .24$), and combined males and females ($F_{1,21} = 1.86$; $P = .19$) supported this. It is not possible to produce high numbers of adult lice in our facilities, which is why PAII is the preferred stage for conducting bioassays. In this case we had a smaller pool of adults from population A, resulting in the need to drop the 0‰ group in favour of having enough parasites for the remaining exposures. Survival between the controls (35‰) were significantly different with population A having a mean survival of 60% compared to population C with 90%. Adults are older, and more sensitive to handling which could have resulted in weaker individuals being used in this group.

4. Discussion

In this study, we focused on the copepodid and PA II stages *in vitro*. A few bioassays using adults were conducted, however it is more complicated obtaining enough adult lice in the lab to conduct a full bioassay, thus only adults from two laboratory populations were included in this study. The PA II stage is though very relevant for bioassays being conducted on site, as the parasites in this stage can be collected directly from the fish in the pens, are often present in high numbers and are of the same age. The copepodid assay is simple and robust but requires capability of hatching and cultivating the parasites. This assay provides additional information on a different life stage of *L. salmonis*, for comparison with PA II assays and with other studies (Arriagada et al., 2016; Wright et al., 2016).

Exposure to low-salinity water is a widely accepted natural deterrent to the settlement of *L. salmonis* copepodids on Atlantic salmon (Connors et al., 2008; Finstad et al., 1995; Hahnenkamp and Fyhn, 1985; Wright et al., 2016). Previous studies have found that copepodids die following 1–3 h of exposure to freshwater when attached to the fish host (Bricknell et al., 2006; Stone et al., 2002; Wright et al., 2016). Free-swimming copepodids are reported to die after 3 h at a salinity of 4‰ (Bricknell et al., 2006). Our findings support the claim that there is

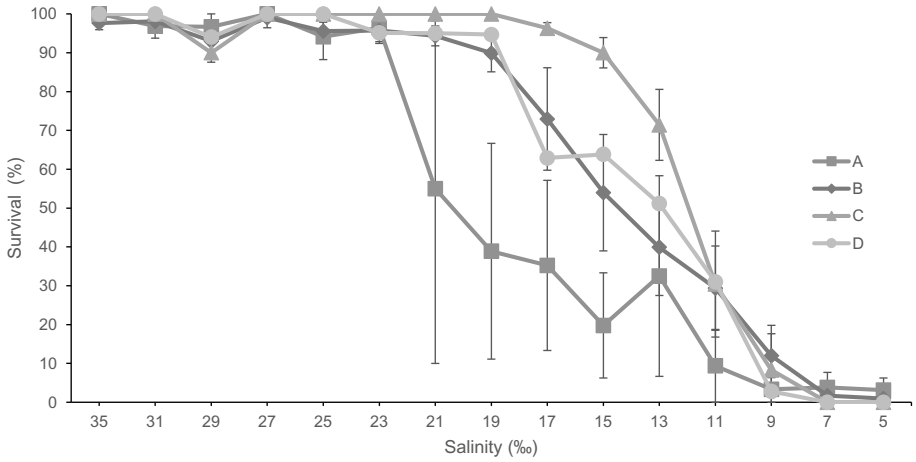

Fig. 2. Survival (%) for copepodids from four *Lepeophtheirus salmonis* populations following 24 h exposure to a range of salinities (‰), standard error bars (SE) are also shown.

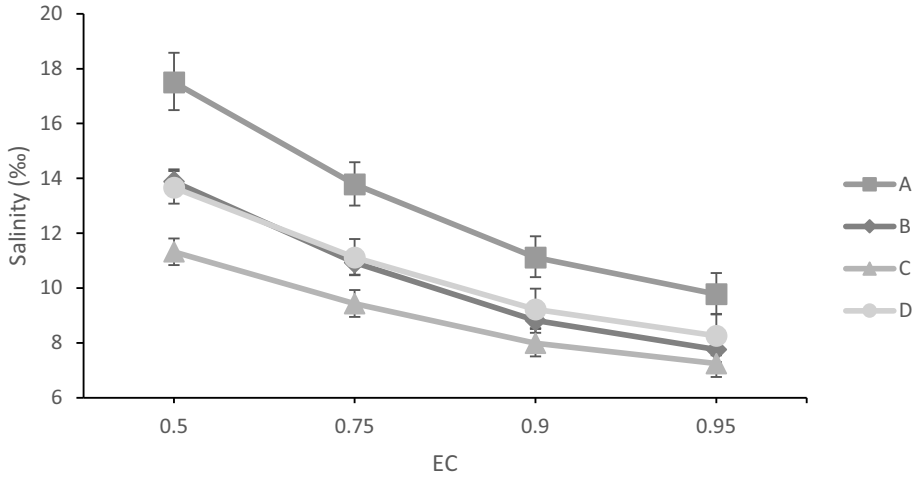

Fig. 3. Dose-response curve for copepodids from four *Lepeophtheirus salmonis* populations exposed to different salinities (‰) for 24 h to determine the median survival salinity, provided as a range from EC_{50} to EC_{95} (with upper and lower 95% CI).

variability in salinity tolerance across the life stages, lowest for copepodids, higher for pre adults and highest for adults. Observations of the surviving copepodids showed that they remained unaffected until the salinity reached 9‰; above this level, the active copepodids exhibited normal swimming behaviour and normal appendage movement, as observed in the control group. As the aim of the study was bioassay development with a clear endpoint, fish were not infested with copepodids exposed to low salinity. Thus, no conclusion regarding possible effects on copepodids' ability to infest a host could be made. Bricknell et al. (2006) observed that exposure to low salinity levels seemed to compromise this ability.

The bioassays that we conducted using the copepodid stage indicate that copepodids in the water column may be able to survive for sufficiently long periods, allowing them to relocate to more saline areas or depths. This may reduce the overall efficacy of the freshwater layer in coastal/estuarine areas which is generally seen as a natural salmon louse deterrent. In addition, the differences between our results and previously published results may be due to variation among *L. salmonis* populations. The results of the current study support this possibility; for example, population A had a mean EC_{50} of 17.5‰, whereas population C had a mean EC_{50} of 11.3‰. The EC_{50} values of the remaining populations fell between these two values.

Several studies have studied the efficacy of freshwater (< 5‰) in controlling attached parasites, and the reported durations required for eradication are very diverse, including 4 h (Reynolds, 2013), 4.5 days (Connors et al., 2008), > 8 days (Wright et al., 2016), and 13 days (Finstad et al., 1995). However, prior to the current study, no work had been published on detached PA IIs. Although detached from fish, the *L. salmonis* in these bioassays were able to attach to the treatment container throughout the treatment period (Aaen and Horsberg, 2016; Helgesen and Horsberg, 2013; Sevatdal and Horsberg, 2003). Even though the parasites did not receive salts and nutrients, which assist with osmoregulation, from a host when attached to the container, this attachment may have provided some protection from low salinity. This study found that after 24 h of exposure, PA II (males and females combined) exhibited survival rates above 80% at water salinities above

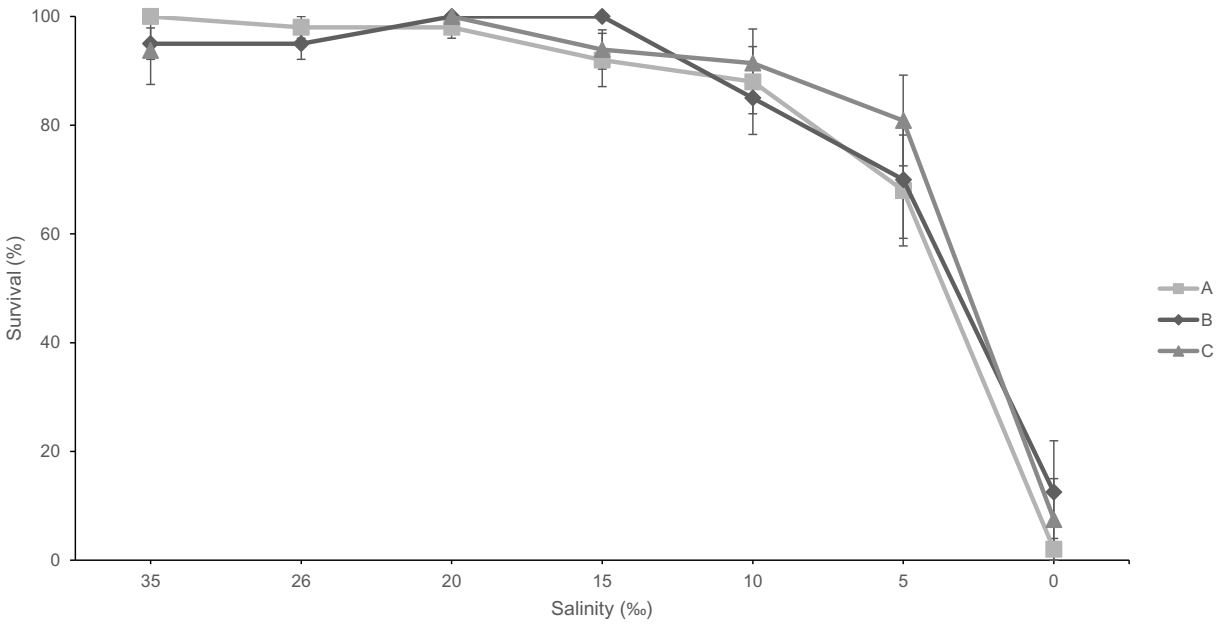

Fig. 4. Survival (%) for the pre-adult II stage from three *Lepeophtheirus salmonis* populations following 24 h exposure to a range of salinities (‰), standard error bars (SE) are also shown.

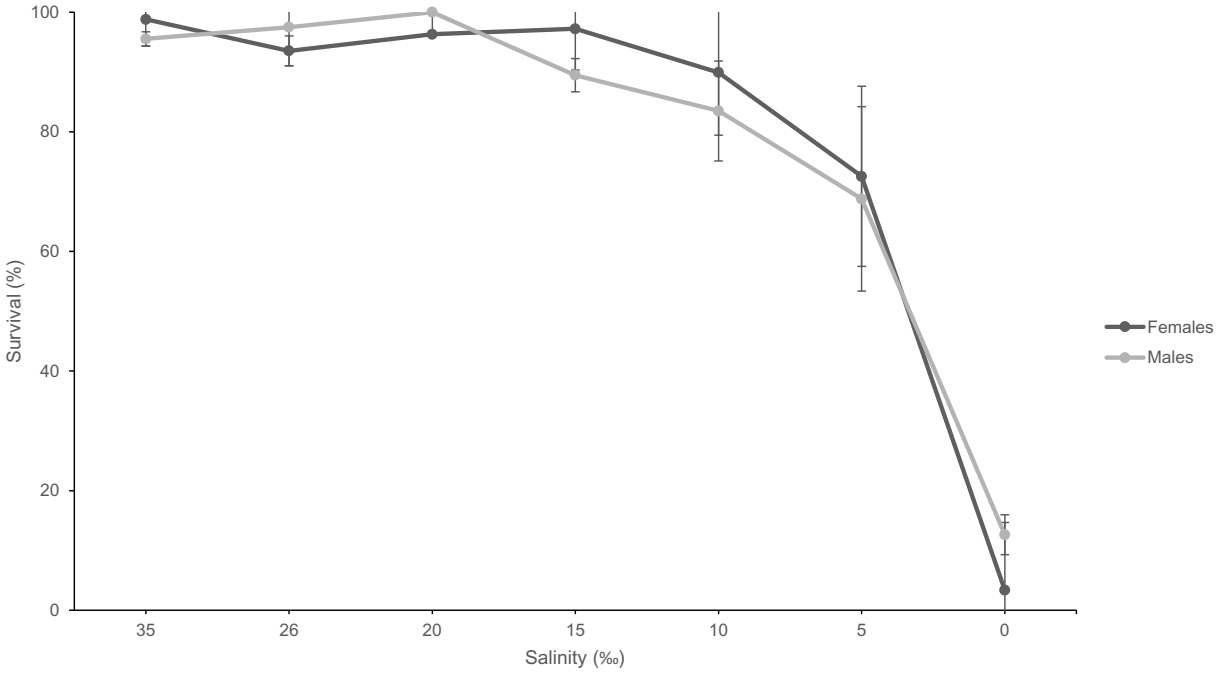

Fig. 5. Survival (%) for the male and female pre-adult II stage from three *Lepeophtheirus salmonis* populations following 24 h exposure to a range of salinities (‰), standard error bars (SE) are also shown.

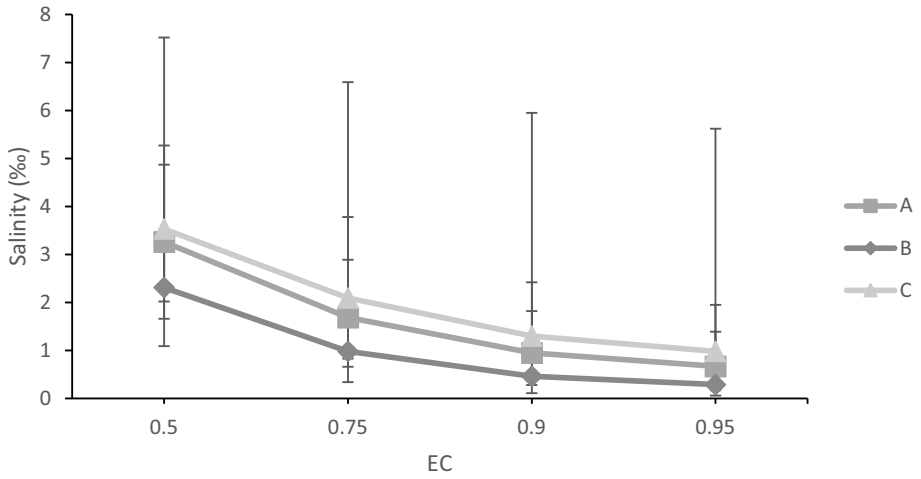

Fig. 6. Dose-response curve for the pre-adult II stage from three *Lepeophtheirus salmonis* populations exposed to different salinities (‰) for 24 h to determine the median survival salinity, provided as a range from EC_{50} to EC_{95} (with upper and lower 95% CI).

10‰. Survival decreased to 72% at 5‰, with an eventual EC_{50} of 2.8‰. The EC_{50} were similar when comparing the sexes of PA II (females: 2.4‰; males: 2.6‰). All surviving parasites exhibited strong attachment capabilities and swimming behaviour. No significant differences in survival rates were observed among the three salmon louse populations tested, however a larger cohort of populations might have demonstrated such differences.

Previous studies have shown that adult *L. salmonis* can tolerate long periods in freshwater, such as 4.5 days (Connors et al., 2008), > 7 days (Bricknell et al., 2006) and > 21 days (Arriagada et al., 2016), when attached to a host fish. However, only one study, conducted by Hahnenkamp and Fyhn (1985), investigated detached adult *L. salmonis*; they reported survival durations > 8 h in freshwater. Our adult *L. salmonis* bioassays support these findings, with high survival after 24 h exposure across the salinity range down to 5‰. The fact that adult *L. salmonis* are more tolerant to lower salinities than pre-adults may be overlooked by aiming the freshwater treatments at the PA II stage. This is relevant for treatment success because it will ensure that fewer parasites reach maturity.

The efficacy of freshwater treatment may be associated with geographic origin of populations, as water chemistry and environmental conditions vary greatly among regions. Water chemistry, water temperature and currents could all contribute to differences in salinity tolerance, which would be beneficial for the infectious stage that relies on distribution *via* water currents as has been suggested by Mordue (Luntz and Birkett (2009)). Differences in baseline salinity sensitivity were demonstrated for another ectoparasitic copepod, *Caligus rogercresseyi,* collected from four different areas with different salinities in southern Chile. The survival rate at low salinity was highest for parasites collected from Hornopirèn, an area with brackish water (Bravo et al., 2008). The authors concluded that parasites from the sites with low salinity levels have adapted to these conditions, resulting in better

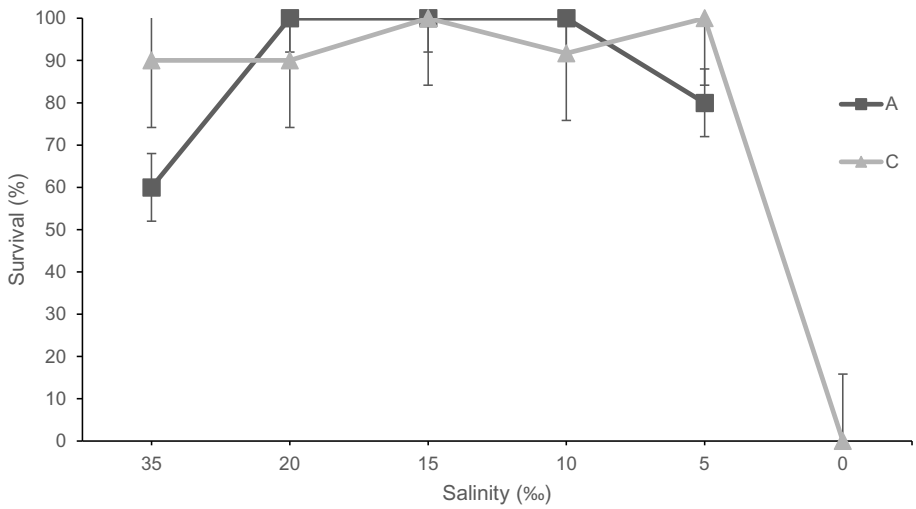

Fig. 7. Survival (%) for the adult stage from two *Lepeophtheirus salmonis* populations following 24 h exposure to a range of salinities (‰), standard error bars (SE) are also shown.

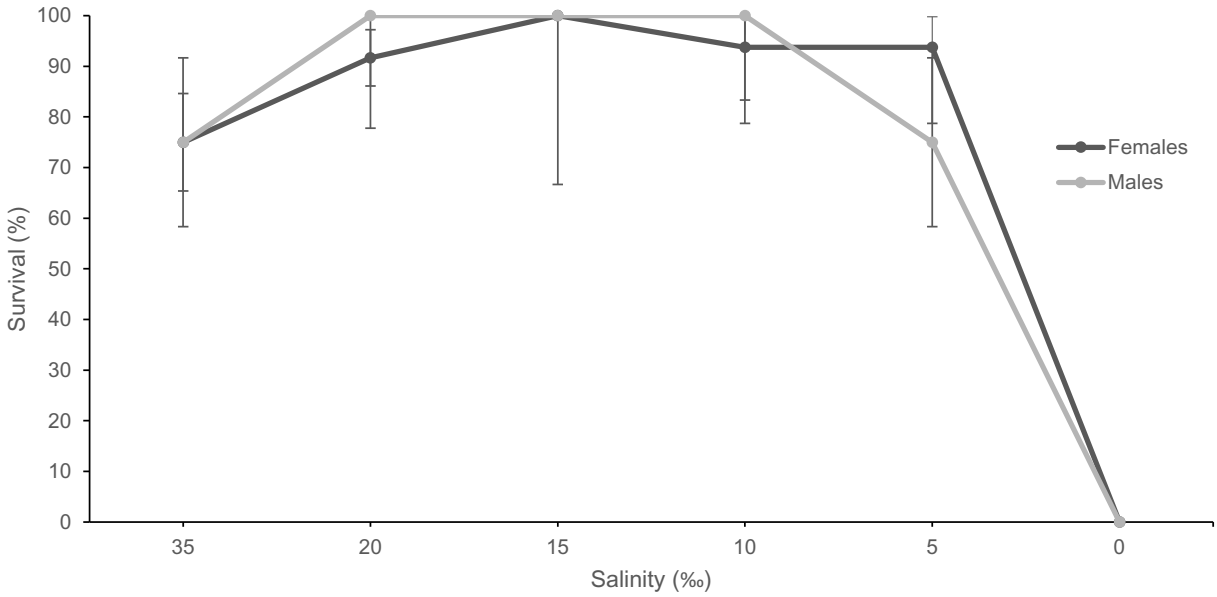

Fig. 8. Survival (%) for the male and female adult stage from two *Lepeophtheirus salmonis* populations following 24 h exposure to a range of salinities (‰), standard error bars (SE) are also shown.

survival rates as opposed to parasites in higher salinity levels. They also found that female parasites were more tolerant to lower salinity than males, which would assist in the spread of more tolerant nauplii to areas of low salinity. This points to a possible genetic aspect of salinity tolerance, with parasites illustrating greater tolerance in regions that are more often subjected to lower salinities. An in depth genetic study should be conducted to determine if there are genetic differences between geographically distinct populations.

The effects of freshwater on *L. salmonis* vary among developmental stages. Wright et al. (2016) found that adult stage can survive long periods (> 7 days) in freshwater when attached to a fish host in the wild. Our study found that detached PA II survived 24 h of exposure to < 5‰ water. However, we found that the treatment was effective at the copepodid stage. Powell et al. (2015) presented efficacy numbers from a freshwater treatment in a review paper. They did not list the original reference, nor the holding time, thus an in-depth interpretation is difficult. The freshwater treatment removed approximately 80–90% of mobile stages, whereas pumping alone removed approximately 25–30% of these stages. This is in line with Reynolds (2013) and could point to a reduced ability of the parasites to stay attached when influenced by fresh water.

In conclusion, this study demonstrated that a 24 h bioassay with stepwise exposure of PA II *L. salmonis* is a simple and effective method for determining the salinity at which a local louse population should experience a decrease in survival. In addition to presenting an on-site bioassay method, this study demonstrated that the stages tested tolerated low salinity levels well for 24 h, with differences in sensitivity between strains. In addition, we found that the infective copepodid stage is capable of withstanding relatively low salinities for long time periods. This raises the concern that more tolerant copepodids would be capable of moving between regions, increasing the spread of more tolerant *L. salmonis* populations. Based on experiences with resistance development against conventional chemical treatments, this suggests a possibility for selecting the most salinity-tolerant parasites during freshwater treatment. Thus, salinity tolerance is recommended to be monitored routinely to stay ahead of such a development.

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