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Lepeophtheirus salmonas Parasitism and Microbial Ecology of Atlantic Salmon, *Salmo salar*.

Leadbeater, S.*[1], Llewellyn, M.S. [2], Garcia, C.[3] Custodio, M.[4] Ang, K. P. [5] Powell, F,[5] Carvahlo, G. R,[6] Creer, S.[6] Elliot, J.[5] Derome, N[3]

[1] Saint Andrews Biological Station, Fisheries and Oceans Canada, New Brunswick, Canada , [2]School of Life Sciences, University of Glasgow, Glagsow UK,, [3] Universite Laval, Quebec, Canada, [4] Universidade Federale do Rondonia, Porto Vehlo, Brazil, [5] *Cooke Aquaculture Inc., Blacks Harbour, New Brunswick, Canada*, [6] Marine and Fisheries Genetics Laboratory, University of Wales, Bangor

Introduction

Due to the relatively small number of treatment options, a need exists to develop new and novel approaches to *Lepeophtheirus salmonis* management. Future best practices will most likely rely on multiple management strategies to balance the impacts on non-target organisms with efficient control of the parasite. As one option, the microbial ecology of a host can offer benefits via the direct or indirect modulation of its microbiota, providing the host with services such as production of inhibitory compounds, competition with potential pathogens, inhibition of virulence gene expression enhancing of the immune response. Studies on a bacterial pathogen show that the modulation did not impact the taxonomic structure, meaning the modulation could be innocuous for both the host and the surrounding bacterial communities (Boutin *et al*. 2013). Using high throughput 16S tagged amplicon sequencing, the primary goal of this work was to characterize potential differences of skin endogenous bacterial strains, for both taxonomy and functional aspects, on fish with high and low lice prevalence, with the hope of identifying bacterial strains that are correlated with low sea lice prevalence.

Methods

Infection and Housing

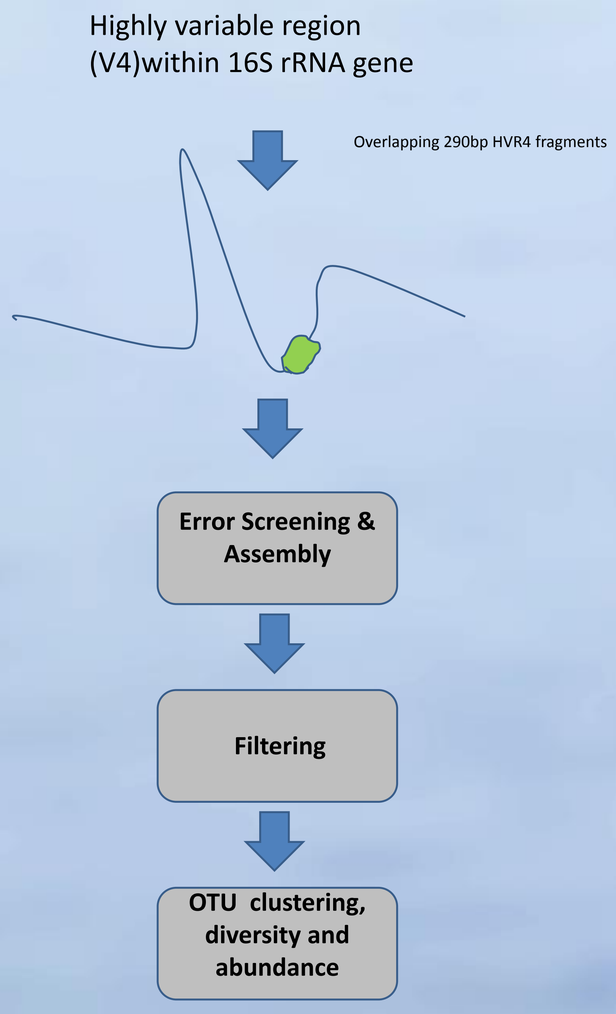
- 800 PIT (Passive Integrated Transponder) tagged salmon smolt
- Sorted to 6, 1000l UV treated flow through seawater tanks
- 12± 2°C, Salinity 30-33gL^{-1}. Density under 40kg m^{-3}, O2 maintained above 8mg L^{-1}, fish fed at a maintenance diet of 1-2%body weight day^{-1}
- Acclimation 3 weeks prior to challenge.
- Copepodid lice added to achieve an average lice load of 20 per fish

Bacteriological Sampling

- 48hours prior, 48hours, 14days, 22days and 35 days following infection
  - 25 fish from each tank
  - Tank sedated, individual netted with a clean, soft mesh net
  - Mucus, biofilm, water, collected at each time point
  - Lice samples collected at time 0 and day 35
  - Lice counts performed at day 14 and 35

Sample Analysis

- Lice counts standardized as Density on surface area (lice count * body wieght$^{-2/3}$)
- Bioinformatics analysis by 16S rRNA V4 sequencing method



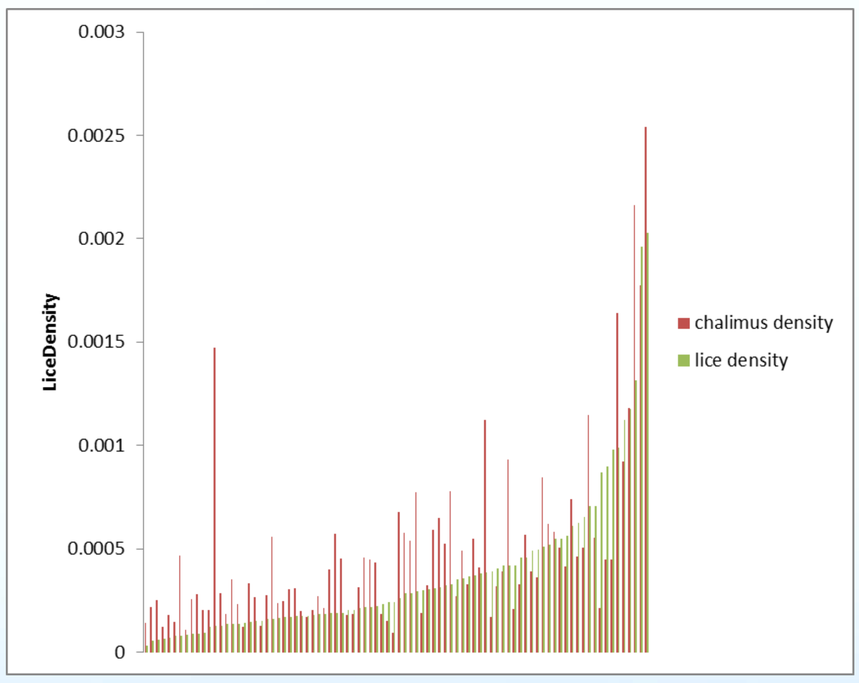


Figure 1. – Chalimus and Adult lice loads at day 14 and 30.

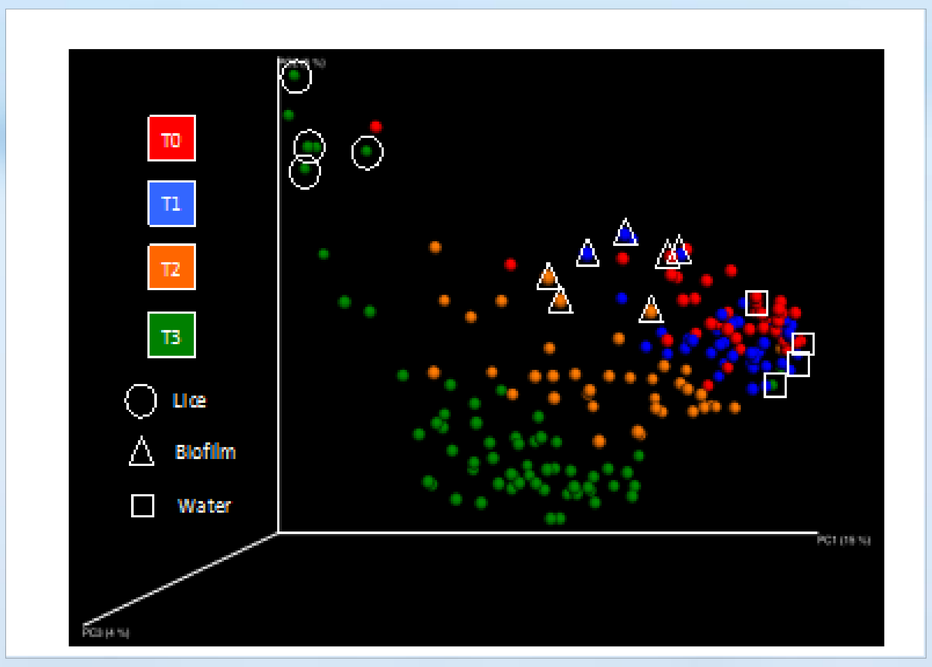


Figure 2 – Principal coordinates plot showing pairwise clustering of samples based on microbiome identity (Pairwise Unifrac)

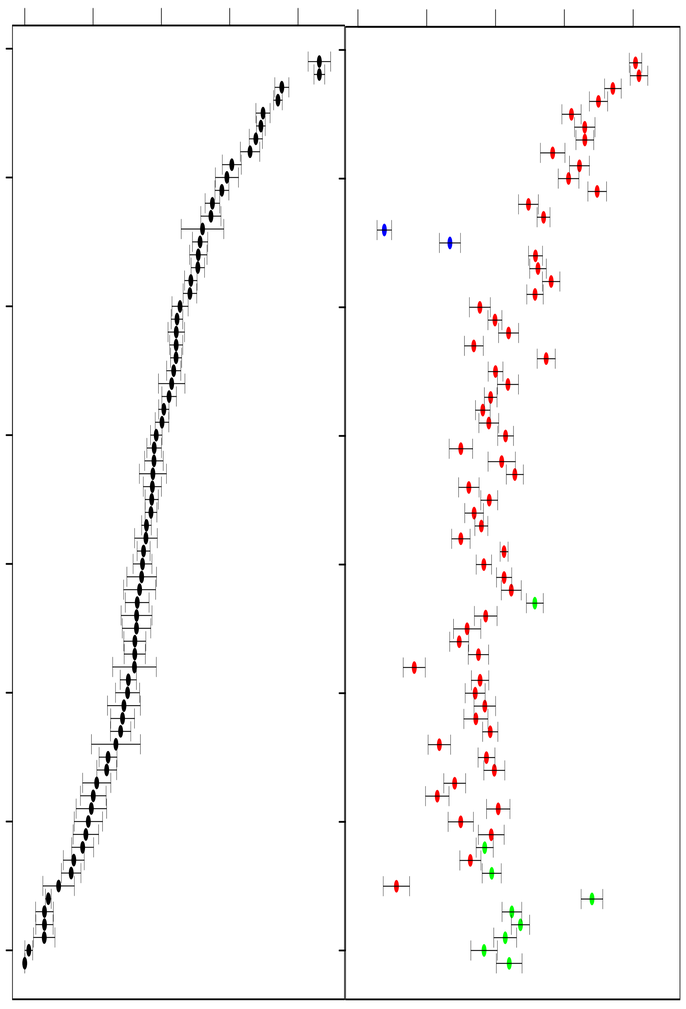


Figure 3 – Frequency distributions (log10 abundance) of abundant (top 95%) OTUs between control (left pane) and test (right pane) tanks at day 35. Blue points are taxa significantly less abundant in the test sample. Green points taxa significantly more abundant in the test samples (Kruskal-Wallis test)

Results and Discussion

Lice density and fish weight (Fig 1.)

- No significant difference in Chalimus (p=0.33) or Adult (p=0.16) lice load between treatment tanks
- High variation between individuals
- No significant difference in final fish weight, however feeding intensity was reduced in treatment tanks
- Chalimus density (Fig 1.) was not a good predictor of Adult lice density
- Perhaps Chalimus fail to reach Adult stage, jump to other fish or are otherwise lost

Bacterial Diversity

- General decline in diversity over time, however no significant (p=0.12) difference between control and treatment groups
- Instability of bacterial composition increased in infected tanks relative to controls between days 22 and 35 ($p<10^{-15}$ on day 22, $p<0.0004$ on day 35

Microbiome identity between samples (Fig 2.)

- Community identity changes over time
- Water and Biofilm tend to align with T0 and T1, while Mucus identity changes over time
- Lice community identity clusters away from that of fish
- Differences between treatment and control groups become increase over time

Frequency distributions (Fig 3.)

- Clear impact of infection on abundance
- Taxa of increased abundance (green) in treatment groups do not contain known pathogens
- Microbial imbalance can have an effect on fish health – and may increase susceptibility to colonization
- All incoming water was filtered and UV treated, however in a more open system more pathogenic colonizers may be successful

Conclusion & Future Steps

- Infestation drives imbalance in mucus microbiota
- Future directions could be:
  - Associate louse load with bacterial taxa
  - Association of louse load and pathogen susceptibility

References

Boutin, S, Audet, C and N Derome. 2013. Probiotic treatment by indigenous bacteria decreased mortality rate without disturbing the natural microbiota of Salvelinus fontinalis." Can. J Microbiol. 10.1139/cjm-2013-0443

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