

Preliminary assessment of stream bacterial community structure and function as related to trout farming in the Ecuadorian Andes Katherine L. Krynak^{1*}, Edward M. Krynak², Dana G. Wessels³, Jane A. Lyons⁴, and Eric B. Snyder³

Introduction

ASGRALARIAS



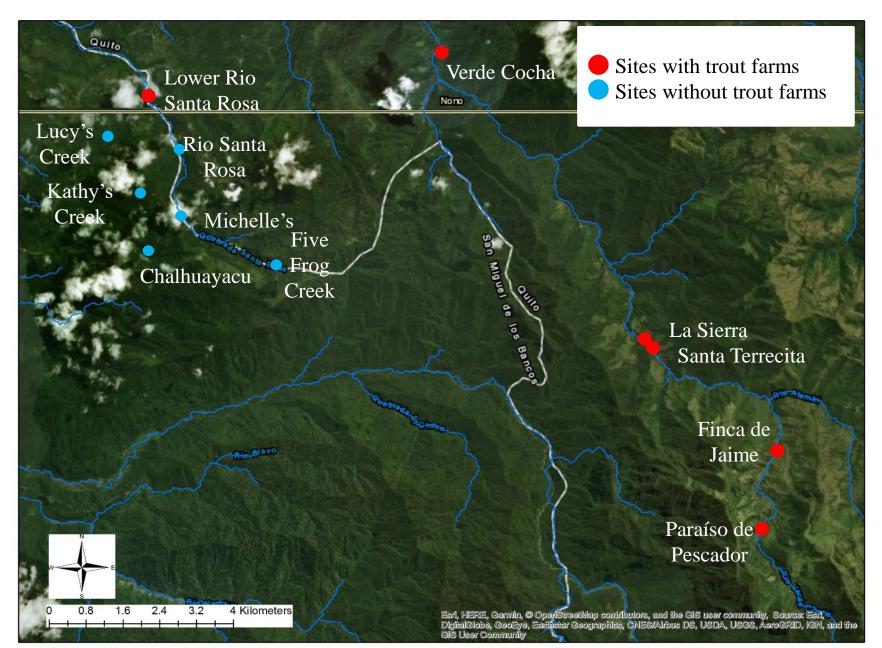
Rainbow trout (Oncorhynchus mykiss) have been repeatedly introduced into Ecuadorian streams since the 1920's.¹ The introduction of nonnative trout can have profound effects on stream

communities by means of nutrient enrichment, predation, and by competing with native taxa.^{2,3}

- How non-native trout farming affects stream microbial communities and their associated function is largely unknown.
- We examined the association of stream bacterial community structure, organic matter decay, and environmental variables among stream sites with and without trout farms.

Methods

- Sites were chosen based upon accessibility and land-owner permissions (Figure 1).
- We deployed cotton strips at 6 trout stream sites and 6 sites without trout farms (20-29 day incubation).⁴
- Bacterial communities were collected from cotton strips via standardized swabbing upon strip retrieval (n=3 per sample site; N=30 swabs in total).
- We assessed bacterial community composition with IlluminaTM (MiSeq) sequencing of the 16S rRNA gene



region (V4) of bacterial DNA (Microbial Systems Molecular Biology Laboratory, University of Michigan). • Sequence data was processed in MOTHUR⁵

Figure 1. Sampling sites. Chalhuayacu and Santa Terrecita were not included in the analysis because the cotton strips were not recovered. Lower Rio Santa Rosa was sampled immediately upstream of the trout farm

¹Ohio Northern University, Department of Biology, ²University of Western Ontario, Department Geography, ³Grand Valley State University, Department of Biology, ⁴Reserva Las Gralarias, Ecuador k-krynak@onu.edu*, ekrynak@unr.edu, wesselsd@mail.gvsu.edu, jalyons593@gmail.com, snydeeri@gvsu.edu

Methods continued

- Organic matter decay was assessed as percent loss of cotton strip tensile strength per degree day and utilized as a measure of stream bacterial community function.
- We compared bacterial community α diversity (richness and inverse Simpson index) and β diversity between trout stream sites and stream sites without trout using analysis of variance (ANOVA).
- We used non-metric multidimensional scaling (NMDS) to explore bacterial community structure as related to environmental variables and organic matter decay.
- We used permutation-based analysis of variance (PERMANOVA) to quantify the relationship between trout farming and bacterial community composition while accounting for stream order.
- All statistical analyses were conducted in R (version 3.4.3).

Results

Bacterial taxonomic α diversity measures did not differ between sites with and without trout farms.

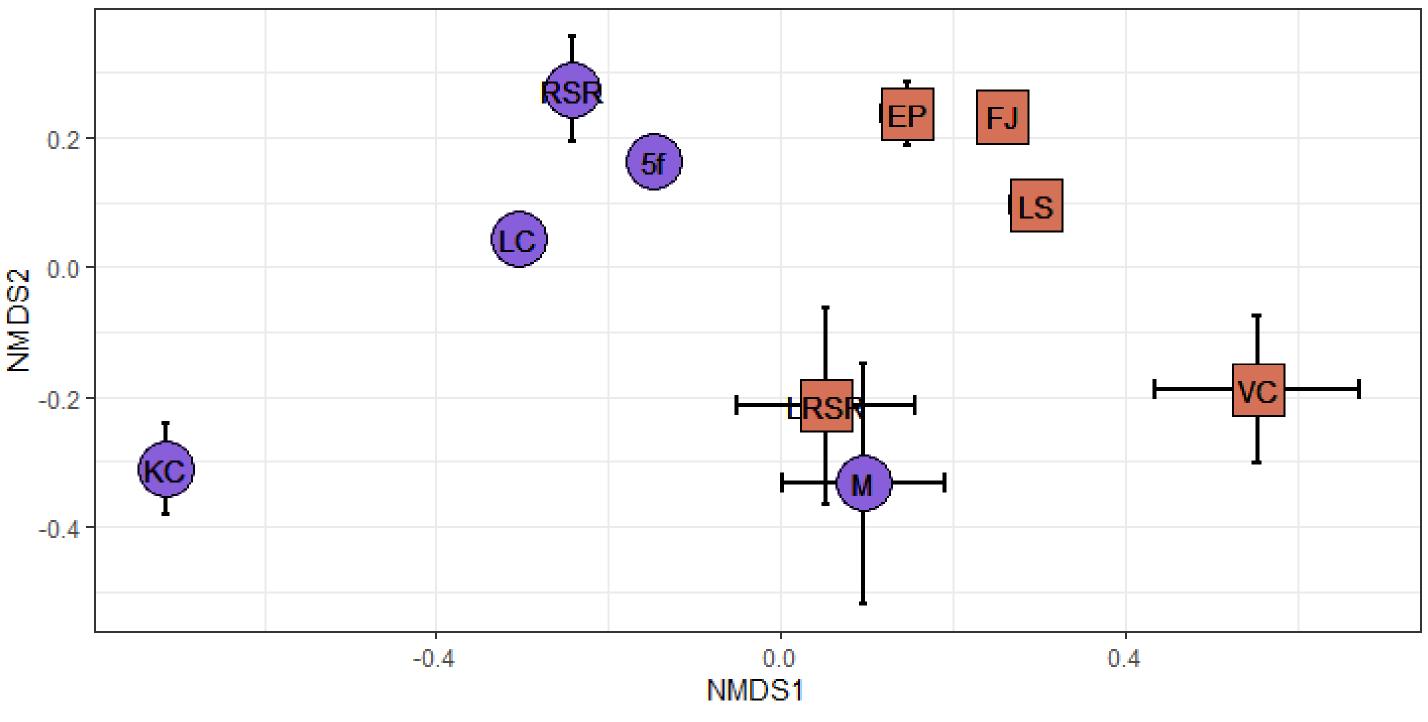


Figure 2. NMDS ordination. Stress = 10.8%. Red squares = trout farm sites, Blue circles = stream sites without trout. *note LRSR was sampled immediately above the trout farm

- NMDS ordination displayed a pattern along NMDS axis 1 indicating a potential difference in bacterial community
- The pattern observed in the NMDS ordination was not supported by permutation-based analysis of variance which indicated no significant effect of trout farming on bacterial community structure.

composition related to trout farming between sites (Figure 2).

Results continued



We thank the Fulbright Commission of Ecuador (Fulbright Early Career Fellowship issued to KLK), Grand Valley State University (Presidential Grant to DGW), and the Michigan Space grant (issued to DGW), and experiment.com for funding this research. We thank Dr. Adam Yates and the StrEAMS lab at UWO for supplying the cotton strips and use of the tensiometer. We thank the staff of Reserva Las Gralarias for their hospitality and unwavering commitment to habitat protection. All surveys were conducted under permit MAE-DNB-CM-2015-0017 issued by Ecuador's Ministry of the Environment and with permission of land owners.



Stream characteristics often altered by trout farming were correlated with NMDS axis 1 (pH: r = 0.69, p <0.001; total dissolved solids: r = 0.59, p < 0.001; dissolved oxygen: r = 0.48, p = 0.004; and percent canopy cover: r = 0.83, p < 0.001).

Rate of organic matter decay was marginally correlated with the NMDS axis 1 (r=0.30, p=0.05).

Figure 3. General flow-through design of trout farms in the region.

Discussion

• Further investigation is warranted, increasing sites sampled and sampling across additional stream systems. Potential changes in bacterial community structure and function as a result of trout farming practices could have long-term effects on the ecosystem.⁶

Bacterial community alterations of the stream could cause dysbioses between native hosts and their bacterial symbionts ultimately affecting host health.^{7,8}

Acknowledgments

References

¹. Vimos D.J., Encalada A.C., Rios-Touma B., Suarez E., and Prat, N. (2015) Effects of exotic trout on benthic communities in high-Andean tropical streams. Freshwater Science 34:770-783.

² Boaventura R., A.M. Pedro, J. Coimbra, & Lencastre E. (1997) Trout farm effluents: Characterization and impact on

^{3.} Gall, B.G., & Mathis A. 2010. Innate Predator Recognition and the Problem of Introduced Trout. *Ethology* 116:47-58. ⁴ Tiegs, S.D., Clapcott, J.E., Griffiths, N.A., & Boulton, A.J. (2013) A standardized cotton-strip assay for measuring organic-matter decomposition in streams. *Ecological Indicators 32*: 131-139.

⁵ Schloss, P.D., et al. (2009) Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl Environ Microbiol 75(23):7537-41.

⁶ Ylla, I., Peter, H., Romani, A.M., & Tranvik, L.J. (2013) Different diversity-functioning relationship in lake and stream

⁷De Palma, G., Collins, S.M., Bercik, P., & Verdu E.F. (2014) The microbiota–gut–brain axis in gastrointestinal disorders: stressed bugs, stressed brain or both? The Journal of Physiology 592(14): 2989-2997.

⁸. Woodhams et al., (2007) Symbiotic bacteria contribute to innate immune defenses of the threatened mountain yellowlegged frog, Rana muscosa. Biological Conservation 138(3):390-398

the receiving streams. Environmental Pollution 95:379-387. bacterial communities. FEMS Microbiology Ecology 85(1):95-103.