CERMAQ

Mr. Andrew Thomson,
Regional Director, Science
Fisheries and Oceans Canada
Pacific Biological Station
3190 Hammond Bay Road, Nanaimo, BC, V9T 6N7

May 14, 2021

Dear Mr. Thomson,

I am the Senior Veterinarian with Cermaq Canada, and through my clinical experience in the BC aquaculture industry for the last 10 years, I have accumulated considerable knowledge of mouthrot (also known as tenacibaculosis or yellow mouth). During this time, I have completed a PhD entitled "Mouthrot in farmed Atlantic Salmon", which makes me an expert in the field of *Tenacibaculum maritimum* infections in farmed Atlantic salmon.

Dr. Miller-Saunders was a coauthor on a recent study, Bateman *et al*. "Descriptive multi-agent epidemiology via molecular screening on Atlantic salmon farms in the northeast Pacific Ocean" in Scientific Reports[1]. There are several scientific issues with this article. One significant concern that is not appropriately addressed by the authors is the detection of *T. maritimum* in fish sampled in freshwater facilities. Dr. Miller-Saunders' laboratory was responsible for running the samples analyzed in this study, and therefore responsible for these results.

The discussion section of the paper states*: "The sampling environments (freshwater or marine) of several detections were unexpected. In particular, we detected K. thyrsites and T. maritimum in freshwater hatcheries, although these agents are considered marine species."*

The authors reference Suzuki et al., 2001[2], which first describes the *Tenacibaculum* genus and the phenotypic description of *T. maritimum* which is a strictly marine organism. There are dozens of other articles that indicate that seawater is required for the growth of *T. maritimum*. To date, there are 36 described species of *Tenacibaculum*[3] and all are from the marine environment. There has never been a case of describing the isolation of *Tenacibaculum* from freshwater, nor a case of tenacibaculosis or similar disease in freshwater in which a *Tenacibaculum* species has been described as the disease giving and/or associated agent.

Bateman et al. explained their finding by saying: "*It is possible that these hatcheries introduced saltwater in the weeks before ocean transfer, to prepare smolts for release*." However, this is not the case. For example, Cohort 2 which was sampled in freshwater prior to transfer were sampled from Little Bear Bay Hatchery. This hatchery is a flow-through hatchery that only uses freshwater and does not have the ability to pump in seawater.

The emphasis of the authors should have been that the likely source of the positive molecular detection of *T. maritimum* was the lab where the tests were conducted – rather than the freshwater from which no *Tenacibaculum* species has ever been isolated. However, no other possible explanation was given by the authors which is concerning in terms of the methods used in the study.

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[1] Bateman, A.W., Schulze, A.D., Kaukinen, K.H. *et al.* Descriptive multi-agent epidemiology via molecular screening on Atlantic salmon farms in the northeast Pacific Ocean. *Sci Rep* **11,** 3466 (2021). https://doi.org/10.1038/s41598-020-78978-9

[2] Suzuki, M., Nakagawa, Y., Harayama, S. & Yamamoto, S. Phylogenetic analysis and taxonomic study of marine Cytophaga-like bacteria: proposal for Tenacibaculum gen. nov. with *Tenacibaculum maritimum* comb. nov. and *Tenacibaculum ovolyticum* comb. nov., and description of *Tenacibaculum mesophilum* sp. nov. and *Tenacibaculum amylolyticum* sp. nov.. *Int. J. Syst. Evol. Microbiol.* **51**, 1639–1652 (2001).

[3] Search result on dsmz.de: https://lpsn.dsmz.de/search?word=TENACIBACULUM

Given that no seawater was introduced into the hatchery, the most likely explanations of how the authors could have detected *T. maritimum* through molecular methods in the fish samples from freshwater are: either there was contamination with genetic material from *T. maritimum* infected tissue in the lab at some point during the process or the assay used for detecting *T. maritimum* is not specific to the bacterium and is detecting a closely related bacterium that is present in freshwater.

Tissues infected with *T. maritimum* result in a very high load of bacteria[4] making contamination from infected tissues highly likely. On top of this, *T. maritimum* has a high affinity to plastic[5] furthering the risk of cross-contamination in a laboratory environment. Quality controls are required at every step to ensure that contamination has not occurred. The method used by Dr. Miller-Saunders's laboratory includes a pre-amplification step where multiple sets of primers are mixed. This step introduces contamination risks. Despite the dilution step, there is the potential for this pre-amplification to create large numbers of amplicons from positive samples that can easily be transferred to negative ones especially when no Ct cutoff is used.

In terms of specificity of the primers used for *T. maritimum*, this bacterium was not part of the original list of organisms when the Fluidigm BioMark Platform was evaluated through a CSAS review[6]. It is not entirely clear what set of primers was used for *T. maritimum* in the Bateman *et al*. paper; however, it is likely based on the 16S gene which is a highly conserved gene in bacteria. This can be problematic for two reasons: one, *T. maritimum* has several copies of this gene which greatly increases the risk of contamination and two, there is a high risk for false positives by detecting closely related species.

It is common practice in scientific publications to confirm results such as the ones discussed here from the Bateman *et al.* paper; however, this was not performed. Ideally this would be through sequencing of the organism in question and minimally by confirming using another method such as classical qPCR or digital PCR.

Given the importance of this finding, and its contradiction with a significant number of peer-reviewed science papers, concerns are raised around the validity of the work performed. We are deeply concerned about this and believe that the evidence above warrants a review of the quality assurance and quality control practices of Dr. Miller-Saunders' laboratory. This request is not being made lightly, but the fact is Dr. Miller-Saunders is consistently linking our industry to declines in wild salmon and challenging the legitimacy of operations in BC and we feel we have no choice but to bring this matter to your attention. I look forward to receiving your response to these concerns.

This letter will also be forwarded to the Fisheries and Oceans Parliamentary Standing Committee, currently studying the *State of Pacific Salmon*.

Regards,

Dr. Kathleen Frisch, BVSc, MSc, PhD
Senior Veterinarian, Cermaq Canada

[4] Frisch, K., Småge, S.B., Johansen, R., Duesund, H., Brevik, Ø.J. & Nylund, A. Pathology of experimentally induced mouthrot caused by Tenacibaculum maritimum in Atlantic salmon smolts. *PLoS ONE* (2018). https://doi.org/10.1371/journal.pone.0206951
[5] Avendaño-Herrera, R., Toranzo, A. & Magariños, B. Tenacibaculosis infection in marine fish caused by *Tenacibaculum maritimum*: A review. *Dis Aquat Org* **71**, 255-266 (2006).
[6] Miller, K.M., Gardner, I.A, Vanderstichel, R. *et al*. Report on the Performance Evaluation of the Fluidigm BioMark Platform for High-Throughput Microbe Monitoring in Salmon. CSAS Research Document 2016/038 (2016).