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Improving Wild and Hatchery-Reared Lake Trout Health via Development of Novel Epizootic Epitheliotropic Disease Prevention Methods

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ABSTRACT:

Great Lakes fishery managers have grappled with a virulent virus of lake trout (*Salvelinus namaycush*) since the 1980's, when Epizootic Epitheliotropic Disease Virus (EEDV; syn. *Salmovirus salmonidallo3*; Family *Alloherpesviridae*) emerged in Great Lakes hatcheries across multiple states, leading to the death of millions of hatchery-reared lake trout that negatively affected lake trout restoration efforts at that time. Decades later, EED continues to be a problem, causing 20 - 30% losses in affected hatcheries, derailing lake trout stocking plans, and having very few contemporarily available disease prevention and control options. Thus, the overarching goal of this GLFC-funded study was to improve Great Lakes lake trout health by more effectively preventing and controlling EEDV infections in hatchery-reared stocks through the development of novel technologies & modifying currently employed hatchery practices. In this vein, our team generated two novel vaccine preparations against EEDV, verified the virus inactivation process via *in vivo* laboratory experiments, and tested the ability of each vaccine to protect laboratory-raised lake trout that were experimentally immunized and subsequently exposed to infectious EEDV ~ 1 month later. Should these new vaccines provide adequate protection, the newly generated protocols and preparations provide a means to produce similar vaccines from other originating lake trout hatcheries that can be deployed throughout the Great Lakes basin. To enhance the detection and diagnosis of EEDV and subsequently guide management decisions that reduce the risk of virus transmission during gamete collection and the subsequent hatchery rearing cycle, our team modified and optimized a loop-mediated isothermal amplification (LAMP) assay and a quantitative polymerase chain reaction (qPCR) assay to enhance the detection of EEDV in the reproductive fluids (e.g., ovarian fluid, seminal fluid) of lake trout. Notably, the new LAMP assay yields results more rapidly (< 30 min), offers improved visual differentiation via the naked eye, is ideally suited for hatchery/field settings, and consistently detects EEDV at loads of ~103 copies per μl of spiked ovarian and seminal fluids. The optimized qPCR assay was even more sensitive, consistently detecting the virus in spiked lake trout ovarian and seminal fluids at estimated loads of ~101 and ~102 copies per μl , respectively. When deployed on reproductive fluids collected from four year classes of hatchery-reared lake trout broodstock with a history of EEDV, both assays successfully detected the virus in a subset of the analyzed fish, thereby representing a valuable tool for fishery managers interested in rapidly screening lake trout reproductive fluids and making management decisions that minimize virus transmission risk. Also in the vein of enhanced EEDV detection methods and using our team's newly cloned and expressed EEDV glycoprotein, we developed an experimental indirect enzyme-linked immunosorbent assay (ELISA) that can be used to determine if lake trout have been previously exposed to this virus. Within this GLFC-funded project and via a series of additional *in vivo*

laboratory experiments, our team provided evidence that lake trout can remain infected with EEDV for nearly a decade and intermittently shed the virus, and that the virus can be readily transmitted on contaminated fomites during fin clipping, a practice commonly used in hatcheries raising lake trout for conservation and stock enhancement purposes. Importantly, results from experiments conducted herein revealed that a brief dip of virus contaminated equipment in Virkon Aquatic® reduced transmission risk, and a 20-minute soak in Virkon Aquatic® eliminated detectable virus transmission, thereby equipping fishery managers with a potential tool to break EEDV transmission. Finally, two additional series of *in vivo* laboratory experiments evaluated the ability of three compounds (e.g., Sodium Chloride, Hydrogen Peroxide, and Chloramine-T), widely used in hatcheries to control other diseases, to reduce EEDV transmission and associated mortality. Although further investigation is needed, results suggest that hydrogen peroxide may be capable of reducing/delaying virus transmission and mortality during EED outbreaks. Collectively, this GLFC-funded study led to the development and testing of several novel tools and strategies that will enhance the ability of fishery managers to detect and prevent EED outbreaks in lake trout while simultaneously reducing the risk of EEDV transmission more efficaciously.