50. What were the outcomes of the award?

i. Executive Summary

Our grant focused on investigating if the presence of co-stressors (here a pathogen) altered the toxicity of microplastics to exposed wildlife. Specifically, trout (*O. mykiss*) were exposed to a series of concentrations of polystyrene microplastics, nylon fibers and particulates (generated from senescent/dead shoots of *Spartina alterniflora*; a common saltmarsh plant, also known as smooth cordgrass). Salmonid fishes, such *O. mykiss*, are important components of ecosystems, as well as sought-after for human consumption via the wild-caught and aquaculture fisheries. Salmonids are also dietarily and culturally iconic to Northwest indigenous peoples. The polymeric materials chosen for initial study were polystyrene (widely used in aquaculture and fishing floats, docks, vessels, single-use containers and structural insulation - common coastal tsunami debris); nylon fibers (common in fishing lines, nets and abundant in textiles) and spartina detritus (abundant in U.S. East and Gulf coastal habitats and has invaded other habitats, including the West Coast). Few studies to date have compared the toxicological consequences of naturally derived polymeric materials, such as vegetative detritus. Such material is far more abundant than synthetic polymers in the environment. In addition, there are efforts to replace fossil-fuel derived polymers with those derived from natural biomaterials. Hence, it is critical to ascertain if vegetative detritus influences toxicological outcomes, i.e., do observed effects relate to the chemical composition or the physical morphology of the particle.

Our initial findings were that microplastics, or microparticles derived from spartina, alone were not lethal to trout exposed for 56 days (daily renewal) in the lab. Mortalities were induced upon one-time exposure to the IHNV alone (Day 28), but increased significantly when fish were co-exposed to virus and microplastics, particularly microfibers. This indicates that microplastics can have a significant impact on population health when presented with a co-stressor. Further, we observed that mortalities correlated with host viral load, mild gill inflammation, immune responses, and transmission potential. We hypothesize that microplastics can physically compromise host tissues, allowing pathogens to bypass defenses. These findings are described in a recent paper published in *Science of the Total Environment.*

To further elucidate possible microplastic mode of action, we investigated differences in mortality when fish were exposed to nylon fibers continuously (56 days), before (up to Day 28), or only (post Day 28) after pathogen introduction. Additionally, we investigated if nylon elicited a greater effect due to physical conformation (challenged with either fiber or powder). Virulence significantly increased when fish were co-exposed to nylon microfibers chronically, but not when introduced after the virus exposure or when nylon powder alone was chronically dosed. This strengthens our hypothesis that fibers can damage delicate organismal surfaces and facilitate virus penetration (especially via the gills), resulting in heighted virulence. The latter was supported by pathology results. Future work should consider the impact of synthetic microfibers compared to semi-synthetic (e.g., rayon) and natural polymer microfibers (e.g., cellulose), as these fibers are highly abundant in the natural environment from textile pollution and natural sources. We performed some preliminary experiments on this premise but encountered issues with fiber clumping and hence effective dose.

We also addressed if UV-weathering affected the propensities of different microplastics and a naturally derived microparticle (from spartina) to alter virulence of IHNV in trout populations. We originally hypothesized that UV-weathering might increase the hydrophilicity and surface complexity of microparticles. This might enhance their likelihood to interact with and disrupt sensitive gill and mucosal epithelia in fish, increasing virulence. However, in general, the observed magnitude of virulence was

lower for all UV-weathered particles than for unweathered particles (based on mortality rates observed in our previous work). Interestingly, the type and doses of UV-weathered particles eliciting the most notable effects differed from those for the unweathered particles. For example, the highest concentration of UV-weathered spartina was more influential on virulence than expected. We did include an unweathered nylon microfiber treatment (previously observed to be most impactful on virulence) and results from this suggested that some of the observed response differences might be attributable to differences in susceptibility of the fish cohort used in this follow-up experiment. The fish here were smaller and a different line from the previous work. As such, our ability to compare between studies was confounded. Future work should address the effect of unweathered and UV-weathered microplastics in identical systems. However, this is logistically difficult due to extensive test organism husbandry requirements. Further, microplastics in the environment may be weathered for periods of time far longer than the exposure we simulated here. Biofilm formation on microplastics may also be controlling, but this was beyond the scope of the current study.

We also investigated the effects of small (0.8–20 μm) polystyrene microplastics on the abundance of B lineage cells in primary cultures of developing immune cells from the anterior kidney of rainbow trout. We observed that trout phagocytic B cells efficiently took up small (0.83–3.1 μm) microbeads within hours of exposure. In addition, our data (using flow cytometry and RT-qPCR) revealed that microplastic exposure significantly decreased the abundance of a population of non-phagocytic developing B cells. Polystyrene microplastics-induced loss of developing B cells further correlated with reduced gene expression of RAG1 and the membrane form of immunoglobulin heavy chains mu and tau. Based on the induced loss of developing B cells observed, we speculated that in vivo, chronic polystyrene microplastic-exposure may lead to suboptimal IgM/IgT levels in response to pathogens in teleost species. Considering the highly conserved nature of vertebrate B lymphopoiesis it is likely that such microplastics will similarly reduce antibody responses in higher vertebrate species, including humans. Further, RAG1 provides an effective biomarker to determine effects of PS microplastics on B cell development in teleost species.