Title - Characterizing the Cutaneous Microbiome of the Peaks of Otter Salamander (*Plethodon hubrichti*) in the Context of Infectious Disease

Program of Study – Department of Biology and Chemistry

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Abstract: In recent years, massive extinctions and declines in amphibian populations around the world have been implicated to the emerging infectious disease chytridiomycosis, caused by the fungus Batrachochytrium dendrobatidis (Bd). Although some amphibian populations have been observed to be resistant to chytridiomycosis, others are highly susceptible to the disease and have undergone significant losses as a result of the infection. Scientists have discovered that the microbiome of amphibian skin can significantly influence the severity and lethality of this disease. For example, the bacterial species Janthinobacterium lividum has been found to colonize the skin of the red-backed salamander, producing an antifungal biomolecule which powerfully inhibits Bd and effectively protects the animal from morbidity. In view of the chytridiomycosis pandemic, we studied the microbial symbionts of the Peaks of Otter salamander (Plethodon hubrichti) to understand how they might modulate disease risk in this species. At a field site in the Blue Ridge Mountains, we captured P. hubrichti individuals (n=13) and swabbed their skin to collect microbial samples. We then cultured these samples, isolated distinct species, extracted their DNA, and analyzed their 16S rRNA gene sequence for the purpose of taxonomic identification. We found that the salamander microbiome harbors a robust diversity of microbial

symbionts, including those of the genera *Arthrobacter*, *Bacillus*, and *Pseudomonas*, which are bacteria that have previously been shown to inhibit *Bd*. Based on our microbiome data we made predictions about which of these microorganisms may have the potential to protect their amphibious host against the deadly infection. Our future work will consist of *in vitro* assays that measure the ability of microbial isolates to produce biomolecules that inhibit *Bd*. With our understanding of the microbiome of this species, we consider bioaugmentation strategies for the purpose of conservation and suggest a method for selecting a probiotic that may mitigate disease risk.

Christian Worldview Integration: According to the Judeo-Christian worldview, God created the cosmos and all living things, with the human being as preeminent among earthly creatures. Within the creation account, the Creator grants his human image-bearers the authority to rule over the other creatures: "Then God said, 'Let us make man in our image, after our likeness. And let them have dominion over the fish of the sea and over the birds of the heavens and over the livestock and over all the earth and over every creeping thing that creeps on the earth" (Genesis 1:26, ESV). One of the first mandates given to humanity is to oversee the organisms of the earth, a responsibility demonstrated by Adam's naming the animals. The modern Christian who seeks to follow the divine mandate of environmental stewardship will value such goals as habitat preservation, the minimization of pollution, and protection for certain wildlife. Our research contributes to these efforts by seeking greater knowledge of the Peaks of Otter salamander, a species endemic to the Blue Ridge Mountains. This species may be susceptible to certain infectious diseases, and our intention is to gain information about the natural protection that may be afforded by symbiotic bacteria, a relationship that has been demonstrated in other amphibian species. Our research will increase our understanding of this species and may lead to

bioaugmentation attempts to help protect and conserve this unique species. Pursuing knowledge about creation and using this information to develop strategies to protect wildlife from disease is one way that we as Christian scientists can demonstrate our love for God and His creation.