Development and optimization of a qPCR assay to detect three common fish parasites, *Cryptocaryon irritans*, *Uronema marinum*, and *Neobenedenia* spp., in aquarium systems

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Cryptocaryon irritans, *Uronema marinum*, and *Neobenedenia* spp. are parasites responsible for common illnesses in aquarium fish. Marine "ich", or white spot disease, is caused by *C. irritans* and is the cause of large mortality events in many aquaria. The protozoan has a complex life cycle that allows for fast reproduction and infection. As a result, there would be a significant amount of *C. irritans* present in the water column before major infection takes place. The skin fluke *Neobenedenia* spp. has a similar free-swimming stage in its life cycle that would make it detectable in the water column for a period of time. The free-living parasite *U. marinum* does not need a living host to be able to survive, thus the parasite can remain in the water column for the entire duration of its life cycle, making its detection in water likely. At times of disease it is expected that increases in this parasite would occur in the water column. The ability to detect these parasites in the water column would allow aquarists to identify and quantify the abundance before major infection and subsequent mortality event occurs.

Archived 1L water samples were collected from the New England Aquarium and filtered through 0.22 μm filters. DNA was then extracted using the Qiagen PowerWater DNA Extraction kit and quantified. Primers previously designed for *C. irritans* targeting the 18S region by Taniguchi et al. 2011 were used. Primers were newly designed for *U. marinum* targeting the COX1 gene. Primers were confirmed via melt curve analysis in a SYBR Green qPCR assay, and linear plasmids were then developed. Probes were newly designed for both *C. irritans* (FAM) and *U. marinum* (Cy5). Singleplex TaqMan qPCR assays for *C. irritans* and *U. marinum* have been developed and verified. Primers have been newly designed for *Neobenedenia* spp., and development of the singleplex SYBR Green assay is ongoing. The goal of this research is to develop a multiplex TaqMan® qPCR assay with pathogen specific primers and probes to target *C. irritans*, *U. marinum*, and *Neobenedenia* spp. in the water column to monitor and predict parasite outbreaks. From these results, threshold levels can be developed and applied to aquarium systems worldwide. Future work includes filtering larger volumes (>100L) of water through Waterra eDNA capsules in order to obtain a more accurate sample size for tanks of larger volumes.

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