## Environmental RNA degrades more rapidly than environmental DNA across a broad range of pH conditions

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## Abstract

Although the use and development of molecular biomonitoring tools based on eNAs (environmental nucleic acids; eDNA and eRNA) have gained broad interest for the quantification of biodiversity in natural ecosystems, studies investigating the impact of site-specific physicochemical parameters on eNA-based detection methods (particularly eRNA) remain scarce. Here, we used a controlled laboratory microcosm experiment to comparatively assess the environmental degradation of eDNA and eRNA across an acid-base gradient following complete removal of the progenitor organism (*Daphnia pulex*). Using water samples collected over a 30-day period, eDNA and eRNA copy numbers were quantified using a droplet digital PCR (ddPCR) assay targeting the mitochondrial *cytochrome c oxidase* subunit I (COI) gene of *D. pulex*. We found that eRNA decayed more rapidly than eDNA at all pH conditions tested, with detectability—predicted by an exponential decay model—for up to 57 hours (eRNA; neutral pH) and 143 days (eDNA; acidic pH) post organismal removal. Decay rates for eDNA were significantly higher in neutral and alkaline conditions than in acidic conditions, while decay rates for eRNA did not differ significantly among pH levels. Collectively, our findings provide the basis for a predictive framework assessing the persistence and degradation dynamics of eRNA and eDNA across a range of ecologically relevant pH conditions, establish the potential for eRNA to be used in spatially and temporally sensitive biomonitoring studies (as it is detectable across a range of pH levels), and may be used to inform future sampling strategies in aquatic habitats.

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