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Astrid Blom, Laura M. Stancanelli, Jelle A. Dercksen, Clàudia Ylla Arbós, M. Kifayath Chowdhury, Shelby M. Ahrendt, Carolina Piccoli, Ralph M.J. Schielen, Kees Sloff & Jill H. Slinger (eds.)

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Exploring the relationship between eDNA and eRNA to advance biomonitoring techniques in rivers

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Introduction

Freshwater ecosystems around the world are vulnerable to a number of increasingly present stressors: climate change, overexploitation, invasive species, and pollution. As such, restoration campaigns will be launched to meet targets proposed by the '2030 EU Biodiversity Strategy' in the near future (Cortina-Segarra et al., 2021). This emphasizes the need for new rapid biomonitoring techniques to register the impact of those campaigns. A particularly promising novel biomonitoring technique makes use of environmental DNA (eDNA): genetic material released by organisms in various forms (e.g. faeces, shed tissue, mucous) into their environment. Due to the persistence of DNA in aquatic environments, it can be captured by collecting water samples (in volumes of 250-1000 ml) and subsequently filtering them (through filter pores of sizes typically ranging between 0.2-1.0 µm). The application of eDNA surveys have proven to be more rapid and less dependent on taxonomic expertise than traditional monitoring methods (Ji et al., 2013), while also broadening the scope of biodiversity surveys (both taxonomically and spatio-temporally). More specifically by sampling eDNA, microscopic taxa that are indicative for the state of ecosystems, i.e. bacteria and phytoplankton, can now be assessed alongside influential macroscopic taxa, i.e. fish and invertebrates. However, a significant concern when utilizing eDNA is the detection of absent species (i.e. false-positive detection) caused by the detection of older (potentially resuspended) DNA. This study aims to reduce false-positive detection rates by quantifying both DNA and RNA.

DNA versus RNA

DNA and RNA are both polymers found in organismal cells. Their structural differences (e.g. DNA consists of two complementary genetic strands whereas RNA consists of one strand) and functional differences (e.g. the storage of information by DNA, and the control on gene expression by RNA), however, allow for the fulfilment of various key cellular functions. As of yet, most genetic biomonitoring studies have focussed on the analysis of DNA while neglecting the analysis of its derivative RNA. Comparatively, shed RNA in the environment is detectable for significantly shorter periods of time compared to DNA (Fig. 1). However, the isolated analysis of DNA introduces a number of challenges, for instance: the observed variable persistence of eDNA in aquatic ecosystems is ill-defined and the capture of legacy eDNA may lead to the false-positive detection of species (Laroche et al., 2017). To address this issue, Marshall et al. (2021) proposed the quantification of both DNA and RNA. They demonstrated that the ratio of RNA to DNA decreased significantly throughout the degradation process. This is important as the supplementary quantification of eRNA may provide estimates for the age of genetic material, thereby strengthening the application of eDNA methods. In their experiment, Marshall et al. (2021) used a static volume of water whereof only surface water was sampled. Although their results seem promising, questions remain on the relationship between RNA and DNA. For

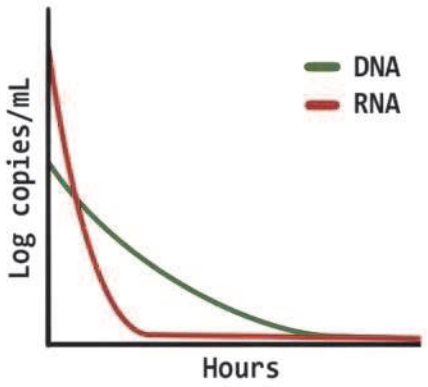

Figure 1. Schematic illustration of (e)DNA and (e)RNA concentrations (in log copies of DNA or RNA sequences per ml) degrading variably through time after release by its source organism.

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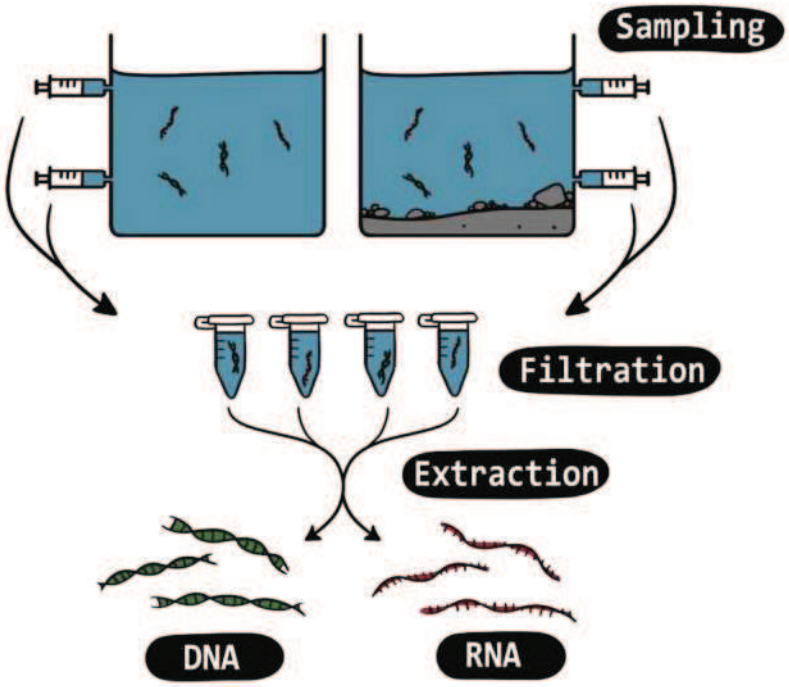

Figure 2. Schematic illustration of an experimental setup and workflow, wherein (1) water samples are taken at various depths in experimental water tanks, (2) samples are filtered and (3) extractions are performed to separately target DNA and RNA.

instance: is the ratio of RNA to DNA comparable at variable depths? How does the presence of sediment influence the ratio of RNA to DNA?

Advancing eDNA

We propose to quantitatively monitor the relationship between RNA and DNA at various depths and intervals in an experimental water volume (Fig. 2), to assess whether this relationship is reliably observable under variable circumstances. If so, false positive detection rates may be reduced, thereby advancing the application of eDNA-based methods. Since the presence of a sediment layer demonstrably changes the detectability of eDNA (Stoeckle et al., 2017), it will be accounted for in our proposed experimental setup. After sampling, filtration of genetic material is swiftly performed on-site to reduce degradation of eDNA and eRNA. Filtered samples are split in two, after which DNA is extracted from one half and RNA is extracted from the other half (Fig. 2). Depending on the targeted species (e.g., a species of fish), relevant genetic markers are selected. Subsequently, DNA and RNA concentrations are measured using quantitative polymerase chain reaction (i.e. qPCR) methods as described by Marshall et al. (2021). Challenges for this experiment arise in the design of an experimental facility that eliminates disturbances (e.g. during sampling) to the volume of water, while meeting microbiological requirements (sterility and reduced odds of contamination).

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