This is all quick and dirty exploration. Any conclusions are entirely conditional on these results persisting to selection by more rigorous protocols.

Graphical user interface, chart

Description automatically generated

Decay slopes estimated for ranges from 0-6h to 0-96h, showing dependence of estimated decay rate on range of data considered. This is for data averaged across qPCR replicates within a water sample. Based on this analysis, there is, I think, pretty compelling indication of a systematic flattening of the decay curve as more time steps are included. Most rapid decay is observed in early time steps – rates on the order of -0.2 to -0.4, which would come into line with literature values and yield shedding rates in the correct range. Note variation in range of y-axis, retained to assist visualization (see below for scaled comparison).

A couple of interesting patterns also catch my eye. In 25:1 and 5:1 (and to a lesser degree 5:2), there are a set of water samples that remain eDNA rich even as concurrent samples become quite depleted. This suggests to me that these samples were seeded with really different concentrations of eDNA or are experiencing very different decay rates, perhaps due to differences in how the eDNA is packaged (e.g., particle size, characteristics, etc.). In this regard, the 25:2 might be the most coherent resolution of decay processes observed in this experiment – samples within time step are all in agreement & do not suggest divergent trajectories.

Chart, line chart

Description automatically generated

Same as above, but on same y-axis for comparison.

Chart

Description automatically generated

Results from fitting a linear mixed effects model allowing for random variation in intercept (initial concentration) in each sample. Note, I have *not* averaged across qPCR replicates, under the assumption that each is an independent estimate of the concentration of eDNA in the sample, and recognizing that the random intercept for each water sample captures this correlation between qPCR replicates.

mod.a <- lme4::lmer(log.copies.liter ~ Time + (1|individualWaterSample),

data = data)

This model estimates fixed effects for intercept and slope of a linear model through the entire data set, and then the distribution of random offsets from the intercept for the set of samples. An intercept (initial concentration) is estimated for each sample, conditional on all having equal decay rates.

For 25:2, the distribution of random intercepts is not symmetrical, and when plotted over time, shows a clear temporal pattern consistent with the curvature in the data. This would require highly unlikely correlation between the selection of samples for assay and the initial concentration of eDNA. For 5:1, the distribution of random intercepts almost suggests some bimodality – presumably reflecting broad variation among samples at intermediate time steps.

Chart

Description automatically generated

NOTE: I do not think the following model makes much sense, but have included to illustrate some of the potential contortions required in initial conditions and decay rates to achieve some observed patterns assuming that some common fixed effect exists, and that each water sample is a different realization of potential dynamics.

Results from fitting a linear mixed effects model allowing for random variation in intercept (initial concentration) and slope (decay rate) for each sample. Note, I have *not* averaged across qPCR replicates, under the assumption that each is an independent estimate of the concentration of eDNA in the sample, and recognizing that the random intercept for each water sample captures this correlation between qPCR replicates.

mod.a <- lme4::lmer(log.copies.liter ~ Time + (1+Time|individualWaterSample),

data = data)

Model coefficients include fixed effects for intercept and slope of a linear model through the entire data set, and then random effects (e.g., offsets for both intercept and slope) for each sample (and the distribution of these random effects).

For 5.1, the interpretation remains that bags were seeded with different amounts of eDNA and experienced similar decay rates – the random intercept model would probably be preferred here.  
  
My interpretation for e.g., 25:2 is that the persistence of eDNA at levels observed in some of the later samples is not entirely consistent with evidence for more rapid decay during the early part of the experiment.