How long has each particular species' eDNA been outside the organism from which it came? Some thoughts on the possibility to obtain more information from eDNA analysis of water samples

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31 January 2022
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eDNA already allows learning an enormous amount from a sample. The approach discussed here might allow even more information to be extracted: to tell if eDNA comes from animals immediately in the area or if they were either there in the recent past (ca 2 days by current estimates) and/or if currents flow from a distance where the species reside.

The presence and relative numbers of each species' sequence reads taken at different points that have physical continuity can be interpreted as a "phylogeny of space" related to the direction currents flow (Mark Stoeckle). When a species is present, that location acts as a source for species-specific eDNA. The quantity of species-specific DNA decreases with distance from its source.

Two distinct mechanisms decrease species-specific DNA as a function of distance from the source: dilution or degradation. Dilution by simple diffusion in 3D space decreases concentration as the third power of distance from the source. Currents, convection, and waves modulate the dilution effect expected by simple diffusion.

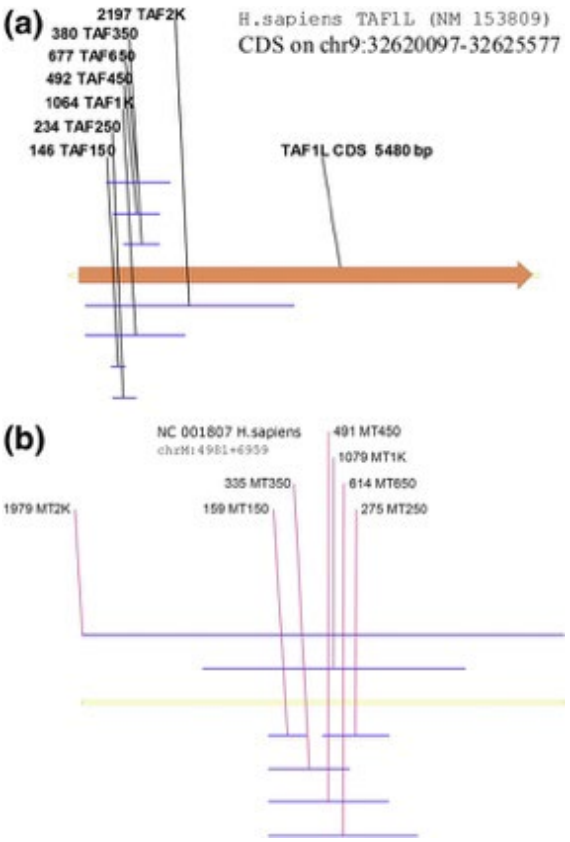
Degradation is the consequence of physical, chemical, and enzymatic activities that break covalent bonds in the phosphodiester backbone of DNA. Most degradation in the aquatic environment is due to DNase from either intracellular sources and/or secreted by ravenous bacteria. Other things being equal, degradation is a function of time.

Although both mechanisms lead to a decrease in signal, by hypothesis, it is possible to distinguish between dilution and degradation via assay of DNA fragment sizes. If eDNA is simply diluted, then all fragment sizes should diminish but the ratio of one size to another should remain the same.

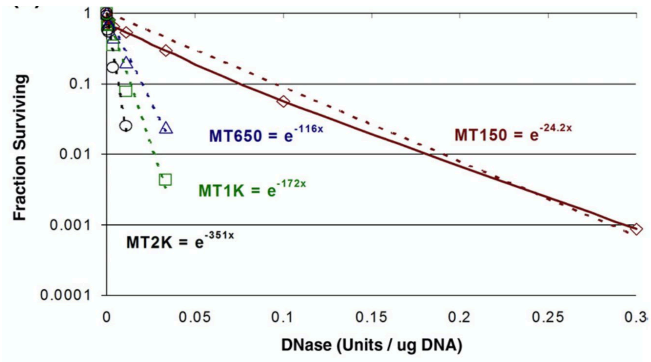
On the other hand, when DNA is degraded, large amplicons decrease faster than small ones. Here is a good way to think about it: Breaking the DNA anywhere between two primer sites prevents exponential amplification. It is more likely that damage will occur between primer sites that are further apart.

For other purposes several years ago, we tested this model *in vitro* by qPCR for three types of damaging agents: DNase, UV, and gamma radiation [1]. We nested primers with amplicons of sizes from 150 to about 2,000 base pairs.

A figure from the paper (which has more details) shows how these amplicons cover the same sequences, so effects are based on length rather than sequence or accessibility effects.



The assay works in a quantitatively straightforward way on human mitochondrial DNA treated with DNase. As the model predicts, large targets are lost faster than small ones: About a 1,000-fold decrease in the 2kb target occurs with the same DNase treatment that yields a ca 50% decrease in a 150bp target.



We believe, but have not tested, that this thinking applies to eDNA. By hypothesis, the ratio of small to large amplicons will be a measure of how long the DNA has been outside the organism and subject to environmental degradation. When first coming from the aquatic organism (e.g., when a fish poops), the DNA is expected to have an equal number of long and short amplicons but as it degrades targets will be lost at a rate proportional to their length.

The loss of different target sizes could be calibrated against chronological time and/or distance from the source. Once calibrated, a single standard curve might suffice to make estimates from single-site samples.

By hypothesis, it may become possible from a single assay and analysis to tell not only if a particular species is in a water but how long ago the DNA came out of the animal. If fish are proximal, then a lot of their eDNA will be "young eDNA" and contain long targets. If the fish have left the area or eDNA has been carried from somewhere else, then long targets will be differentially rare.

This approach was developed and tested with qPCR and human-specific primers. It could be adapted and extended to more general primers so that many species can be assayed in the same experiment by NGS.

We hope to discuss with fisheries labs and/or others interested how to go further with this idea and proposed project. Progress could amplify the value of eDNA studies by providing reliable information on how long has each particular species' eDNA been outside the organism from which it came.

1. Spangler R, Goddard NL, Spangler DN, Thaler DS: **Tests of the single-hit DNA damage model**. *Journal of molecular biology* 2009, **392**(2):283-300.