Turtle Guardians

What you leave behind matters… even if you are a turtle!

Environmental DNA: using poop to detect and protect disappearing and mysterious species

In order to protect disappearing wildlife and their habitat, we need to know where the species are. This might seem obvious, and simple to do, but that's not always the case: When a species is rare their populations are very small and scattered. If they are a territorial species, individuals may be spread out across the landscape with only one or two in an area at a time. How can we find them easily if there are so few at any given site? What if the species is really small-bodied and hides in the mud or under vegetation? What if the species lives underwater and does not come to the surface or shore regularly? There are so many factors that can make finding species in the wild difficult.

Traditional methods of surveying areas to determine which species are present and also how many are there, usually involves some kind of capturing, or less intrusively - at least seeing or hearing them. To catch, hear, or see a species in the wild (particularly a rare species), expert knowledge, time, money, and other equipment may be needed. And when it comes to rare and declining species, traditional surveying methods can be ineffective, preventing us from being able to manage or protect them and their habitat accordingly.

An emerging tool in molecular biology is making things easier for researchers to determine when those disappearing species are present in freshwater ecosystems, without having to actually locate them (Kelly et al. 2014). This tool is called environmental DNA (eDNA).

Environmental DNA is genetic material that an organism leaves through cells from urine, feces, sweat, skin, hair etc.

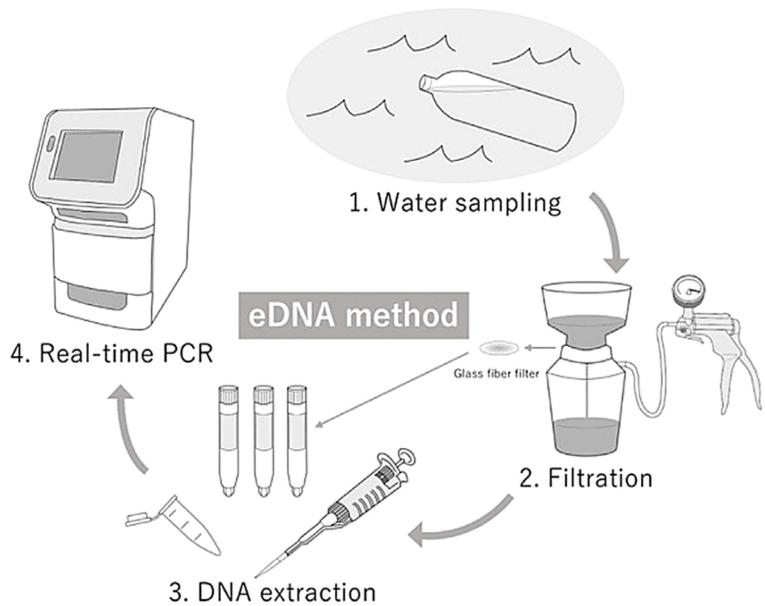
Depending on conditions like temperature, or natural forces like water flow, eDNA can remain fresh and near the location it was released for a couple weeks (Thomsen and Willerslev 2015). This means, in many areas, researchers may be able to collect samples of water, sediment or moist soil and also some cells in their sample. Then, with the right procedure, they can identify which species' DNA is contained in each sample, effectively showing them who was recently (and in many cases still is) present in the area!

The amazing way DNA is analyzed

The first step of processing a sample of eDNA is to filter the sample to separate any cells and organic matter from the medium it is in (water, sediment, soil). The DNA is then extracted and isolated, and then it is time to target the species' gene of interest. To identify a target gene, scientists choose one that is distinct for the particular species they are searching for; a gene that can distinguish the focus species from others closely related to it, and that may occur in the same sampling area. For instance, there are genes that can distinguish a Blanding's turtle from a Painted turtle. It also has to be a gene that has many copies in each cell (such as genes found in *mitochondria,* where sugar is broken down to make energy, or in *chloroplast*, where photosynthesis occurs in plant cells) (Thomsen and Willerslev 2015).

Once the gene is "targeted", it is time to "amplify it" - to make many copies of it so there is more genetic material to work with. This is done through a process called a Polymerase Chain reaction (PCR) which takes a few hours.

When genetic material is successfully amplified within a sample, it can be detected by researchers, and once detected they know that the target species is present (or was very recently present) at the location that was sampled.



Four steps required for collecting and processing eDNA. (Hashizume et al. 2017).

eDNA can identify species and confirm presence when other avenues may not work. Field surveys require expertise to see, catch, or hear a species - and if the target species resembles other species, any data collected by non-experts may be inaccurate. Also traditional survey methods might also miss cryptic species and conclude that they are not present in sites where they actually do occur. eDNA may also reduce costs, effort and the level of intrusion when searching for rare and secretive species (Davy et al. 2015).

eDNA in action

In freshwater systems, eDNA has largely been used to study fish, amphibians and molluscs (for fish see: Jane et al. 2014, Takhara et al. 2013, amphibians: Goldberg et al. 2011, Pilloid et al. 2013, molluscs: Egan et al., 2015, Diener et al. 2015). Environmental DNA has successfully confirmed suspected presence of invasive fish and frog species before they were physically found by researchers (Dejean et al. 2012, Takahara et al. 2013). Secretive species of frogs and giant salamanders that are declining in the United States have also been successfully detected using eDNA (Olson et al. 2014, Goldberg et al. 2011). These instances of effective eDNA sampling were important for conserving species and ecosystems because early detection of invasive species allowed for quick implementation of mitigation strategies, and detection of secretive declining species enabled protection of occupied habitat.

How about turtles?

Recently turtle researchers have begun to employ eDNA techniques, and they have even begun to use eDNA onsite and in the field with faster results. In 2018 in Vietnam, eDNA allowed researchers to locate one of the world's rarest turtle species, the Swinhoe's Softshell Turtle. This turtle was previously unknown to occur in that lake.

A Swinhoe's Softshell Turtle, photo by Tim McCormack.

To learn more about this story and the newest technology using eDNA watch this informative video.

https://www.youtube.com/watch?time_continue=8&v=YycZOCuDjBE&feature=emb_logo

Turtles- Closer to home

Researchers from Peterborough and Guelph, Ontario have successfully developed and tested the DNA primers necessary to perform PCR on target genes for eDNA from the eight Ontario turtle species, as well as one invasive turtle species (Davy et al. 2015). Preliminary tests using their DNA primers sampled eDNA from aquariums of captive turtles, and one outdoor pond housing invasive turtle species in Orillia at Scales Nature Park (Davy et al. 2015). The tests were successful at identifying all target species of turtles, allowing the primers to be used across Ontario.

Since then, during the winters of 2017 and 2018, eDNA has been used to successfully detect the presence of overwintering (hibernacula) Northern Map Turtles in an 8 km^2 lake in southeastern Ontario (Feng et al. 2019). A remotely powered underwater drone was then put under the ice of the lake, which confirmed that Northern Map Turtles were hibernating in the areas of the lake where high concentrations of their eDNA were found (Feng et al. 2019).

This find is significant because Northern Map Turtle populations are declining. In Southcentral Ontario and Southwestern Quebec, they are at the northernmost part of their range, and finding suitable places to overwinter where water temperatures stay above freezing is crucial for their survival. Northern Map Turtles do not tolerate low oxygen conditions well, and overwintering sites also need to have high dissolved oxygen levels (Maginniss et al. 2004). Since this Northern Map Turtles require such specific conditions for overwintering, conservation efforts need to ensure that suitable overwintering habitat is protected for them. This study in southeastern Ontario is an excellent example of how the use of eDNA accomplished something that traditional surveying methods would have struggled to do (Feng et al. 2019). Without the extensive eDNA sampling (which is relatively low cost and does not take much time), running the drone under the ice to search for overwintering turtles in such a large lake would have been ineffective, and costly.

A Northern Map Turtle, photo by Joe Crowley.

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