Measuring biodiversity with a bottle of water: development and field validation of an eDNA approach with plaice

Grosemans Tara, Jana Asselman, Pieter Boets, Karel De Schamphelaere and Colin R. Janssen

Laboratory of Environmental Toxicology and Aquatic Ecology, Environmental Toxicology Unit - GhEnToxLab, Ghent University, Jozef Plateaustraat 22, 9000 Gent, Belgium
E-mail: Tara.Grosemans@UGent.be

Marine biodiversity is crucial for ecosystem functioning and its resulting services, and is thus essential for human health and the economy. Monitoring of biodiversity is required under the EU Marine Strategy Framework Directive (2008/56/EC). However, conventional monitoring relies on taxonomic expertise and often uses destructive methods. Rapid advances in DNA analysis allow the study of biodiversity from environmental samples (e.g. water) and can significantly improve monitoring methods. With the environmental DNA (eDNA) approach routinely monitored species but also rare and cryptic species can be quickly detected without the use of selective and invasive methods. This approach has already been proven successful in freshwater, but considerably fewer studies have been performed in marine ecosystems.

In this study, a series of 24h experiments were conducted to investigate the eDNA degradation rate and detection limit for plaice (*Pleuronectes platessa*) under controlled conditions. Five aquaria with a volume of 30 L were designated as follows: two contained fish, two that had contained fish and one was a control aquarium. After one week fish were transferred from their aquarium to another aquarium to measure eDNA detection. The aquaria from which the fish were removed was sampled to measure eDNA degradation. From each aquarium 500 mL of water was taken at 0, 8, 16 and 24h. Next, samples were filtered and eDNA was extracted from the filter. Results were obtained by amplifying the eDNA on a qPCR machine with species-specific primers (117 bp) for plaice. These primers were both tested in the laboratory (on water and tissue samples) and in the field (water samples). Field samples were collected monthly for a whole year at 9 LifeWatch sampling stations located in Belgian coastal waters. To obtain positive field water samples for plaice, water was collected before starting a beam trawl at two different locations. To determine the minimal concentration of eDNA in the water for a positive detection, a technical (instrument) and biological (number of fish per volume needed to detect eDNA) limit of detection (LOD) was derived from the qPCR for plaice.

Eight hours after three juvenile fish with a total biomass of 32g were introduced in the aquaria, eDNA of plaice was detectable in the water column with no difference in eDNA quantity between 8, 16 and 24h. In the degradation experiment, there was no difference in eDNA concentration between 8 and 16h, but over the entire 24h timespan of the experiment a clear degradation pattern was observed. The technical LOD was determined at $2*10^{-10}$ ng of DNA amplicons needed to detect a signal. For the biological LOD a final quantity of $2.88E^{-13}$ µg of DNA was needed for a positive signal, which corresponds to a minimal fish density of 1 individual in 468,750 liter of seawater. At both control stations, plaice presence was confirmed with beam trawl sampling and could be detected in the water samples using the eDNA approach.

In conclusion, after 8h presence plaice could be detected via eDNA in the water and eDNA followed a clear degradation pattern once fish were removed from the water. With a minimal fish density of 1 adult per 468,750L, plaice can be detected in the water via eDNA. This is the equivalent to finding five fish in an Olympic swimming pool. Our method has proven to be successful in the field and can be used to monitor plaice in the North Sea. The eDNA approach will now be extended to other vertebrate and invertebrate species.

Keywords: environmental DNA; eDNA; degradation; detection; technical and biological detection limits; monitoring