

## Using eDNA to reconstruct logbook information and improve estimates of by-catch.

Green, M. E., Craw, P., Hardesty, B. D., Deagle, B., Wilcox C.

### Introduction

Fish and seafood products are heavily relied on as a key source of protein for millions of people globally (FAO 2020). For some nations, fisheries are the main source of gross domestic product and support the livelihoods of local coastal communities (Vince et al 2020). Wild capture fisheries are the largest contributor to global fisheries production, estimated to have reached 96.4 million tonnes in 2018 (FAO 2020). Appropriate management of fish stocks to prevent overfishing and promote long-term use of a resource is often challenging given the scale and diversity of fishing activity. Overfishing is the primary cause of population decline for marine species and if not intervened can lead to localised or functional extinctions and detrimental ecosystem shifts. It is reported 34.2% of all fished species are currently overfished (FAO 2020). Effective fisheries management and stock structure analyses requires good records of landings including species level information about both catch and by-catch.

Recent discussions during the Indian Ocean Tuna Commission (IOTC) working party on ecosystems and bycatch (WPEB) found that despite improvements in the data reporting for sharks and rays, the overall data remains low quality and highly incomplete (WPEB17, 2021). It is estimated 50 per cent of all shark catches are aggregated and species level information for landings is unavailable. The WPEB have formally acknowledged this is a long-standing issue in reporting for sharks and rays (WPEB17, 2021). Artisanal fisheries operating in the IOTC contribute to the majority of reported nominal catches of shark and ray species, however only 5 per cent of catches are reported. Low reporting in commercial and artisanal fleets can be due to a range of reasons and any efforts to increase coverage are helpful for fisheries management.

eDNA is a promising tool for cost-effective, rapid and non-invasive fisheries monitoring. In recent years, the literature demonstrates a growing interest in the use of eDNA to monitor aquatic ecosystems for species that are cryptic or no longer visibly present in the sampled environment (Evans & Lamberti 2018). Two key features of eDNA are of particular interest. First, in natural systems eDNA has a limited residency time, in the order of a few weeks (Bista et al 2017). eDNA is sensitive to physical and environmental conditions including temperature, pH, UV and microbial activity which can degrade short fragments of DNA (Harrison et al 2019). This means that samples relate to a known and relatively short period of time, and thus are informative about the status of a system, instead of integrating over a long and unknown period. Second, the concentration of sequences extracted from eDNA have been shown to be proportional to the biomass or density of a detected species (Yates et al 2019). While this field of research is still in its infancy it does mean eDNA monitoring could be used to not only to confirm the presence of a species but also provide an estimate of its abundance in closed systems.

eDNA is an emerging tool in fisheries science serving to better understand seafood supply chains at stages where regular monitoring and surveillance is limited or unavailable. Recent studies have started to collect eDNA from trawl nets and brine tanks in hope to reconstruct the landings from that vessel. Researchers have tried collecting water from the cod-end of a trawl

net immediately after it was hauled onboard (Russo et al 2020). The trawl net experiments had some success with DNA from 30 species detected in samples. Not all 30 species were landed in trawls and more work needs to be done to understand the contamination from surrounding sea water. Another recent pilot project conducted in Ecuador sampled water from the brine tanks of commercial and artisanal fishing vessels (Willette et al 2021). The study compared species identified from eDNA collected in tanks and reports from captain and crew about which species were being targeted. Unfortunately, the pilot project was only able to identify tuna to genus level due to their conserved genomes and the study did not know the true fish species retained in the tanks to confirm findings. Nonetheless, the results from the Willette et al (2021) study is a positive sign that samples collected from fish holds can be used to better understand vessel landings. Additionally, eDNA sampling could also help detect transshipment activities in some cases. The application of eDNA as a forensic tool to improve traceability in fisheries and seafood supply chains is a growing area of research. It is important we understand how to best design the tool in order to get the most reliable estimates of species catch.

Current investigations being undertaken at CSIRO in Australia are developing a standardised method to sample brine tanks on-board fishing vessels and reconstruct landed species using a forensic eDNA approach. Our project currently collects samples from the brine and slurry tanks of tuna longline vessels operating in the Eastern Tuna and Billfish Fishery, Australia. Samples are collected from tanks with known quantities of fish species in order to validate eDNA results. The eDNA samples are currently being sequenced and results from the first trial will be available soon. The below section introduces our collection method developed for fishing vessels.

## METHOD

Water (500ml-1000ml) is collected using a Nalgene wide mouth bottle from each of the holds on the vessel. Samples (30ml) are then decanted into 50ml falcon tubes with 10ml of Longmires buffer added (Figure 1). Samples are then inverted to mix and stored in cool dark location. Longmire's buffer preserves the eDNA and can allow the sample to be stored for 6-12 months in temperatures up to 51°C. Samples are then sent to CSIRO laboratories for DNA extraction and processing.



Figure 1. Process of decanting hold samples into preservation medium. Left- Sample collected from hold onboard fishing vessel. Middle- 30ml of sample poured into collection tube. Right- 10ml of Longmire Buffer added to tube for DNA preservation.

Once arrived at the laboratory eDNA is extracted from samples using a precipitate method as described in Edmunds et al (2020). In order to identify the fish and shark species present, universal metabarcoding primers are used. Currently a number of primers are being trailed in order to discriminate between tuna and tuna like species as well as sharks and rays. Samples undergo PCR in order to amplify specific barcode sections of DNA used to identify fish and sharks to species level. Samples are then sent to Ramaciotti Centre for Genomics (UNSW), Sydney Australia for Illumina sequencing. Returned metadata is processed through bioinformatic pipelines developed at CSIRO and unique sequences are compiled per sample, per hold to reconstruct landings on the vessel.

The current CSIRO project has 4 main aims:

1. Establish a simple sample collection method that can be used onboard a variety of vessels with fish holds
2. Optimise genetic markers for robust species identification
3. Test the accuracy of reconstructing logbooks or observer records including relative abundance of catch accounting for base level contamination
4. Evaluate the effectiveness of eDNA as a tool for monitoring catch or identifying unauthorized/ unreported catches

This project is developing a cost-effective, reliable and quantitative method for monitoring and reconstructing fisheries catches. The universality of DNA means this method can be applied to a variety of fisheries at a global scale for routine monitoring and targeted inspection.