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Environmental DNA (eDNA) survey of sawfish in Groote Eylandt

Report

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Front cover photograph: Largetooth sawfish (photo: Peter Kyne).

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Acronyms and abbreviations

ALS..... Anindilyakwa Land & Sea Rangers

eDNA..... environmental DNA

LOD ..... limit of detection

NESP..... National Environmental Science Program

qPCR..... quantitative real-time polymerase chain reaction

TropWATER ... Centre for Tropical Water and Aquatic Ecosystem Research

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Executive summary

Sawfish populations worldwide have suffered major declines and there is an urgent need to determine the extent of their geographical distributions. While fisheries-dependent data and citizen science reports have provided some data on contemporary occurrence, this is not enough to accurately confirm distributions of these species. Targetted surveys using traditional methods have provided valuable information, however the ability to conduct this research is challenged by the highly turbid and often remote habitats that sawfishes inhabit. In addition, these sampling methods are often time intensive and require highly trained staff and equipment that are not always readily available. Environmental DNA (eDNA), or the DNA shed by organisms into their environment, constitutes a non-invasive, time- and cost-effective tool to assess species detection without having to trap or sight individuals. Sample collection requires only simple equipment, which allows researchers to enagage with non-specialists and conservation stakeholders for sample collection. This also increases the monitoring capacity by orders of magnitude and builds on the capacity for widespread, comprehensive assessment. In the present study we engaged with the Anindilyakwa Land & Sea (ALS) Rangers for eDNA sample collection in Groote Eylandt using a simplified field protocol developed at TropWATER. The ALS Rangers collected samples from four sites at a saltwater lake where there is anecdotal evidence of sawfish presence. We extracted eDNA from water samples and screened it for presence of largetooth sawfish (*Pristis pristis*) and dwarf sawfish (*P. clavata*). Although no positive detections were obtained, we provide details for a field protocol that was user-friendly and easy to follow by non-specialists. We propose the method as a useful tool for future surveys and provide recommendations on strategies to maximise detection probability.

1. Introduction

Sawfishes (family Pristidae), a family of shark-like rays, are considered among the most threatened marine fishes in the world (Harrison & Dulvy, 2014). Three major factors account to their threatened status: low population growth rates, high catchability in fisheries and high commercial value of their fins (Dulvy et al., 2016). The Global Sawfish Conservation Strategy states the need for capacity building towards effective sawfish conservation and management (Harrison & Dulvy, 2014), however, information of the contemporary distribution of sawfish is limited, hindering conservation initiatives (Dulvy et al., 2016). Traditional sawfish survey methods include visual observations, fishing using gillnets or lines, traditional ecological knowledge, and public encounter data (Guttridge et al., 2015; Leeney, Mana, & Dulvy, 2018; Simpfendorfer, Wiley, & Yeiser, 2010). Of these, the most useful method for compiling important biological data has been capture with gillnets and line fishing, however, fishing becomes logistically difficult in remote and sparsely populated regions where sawfish are known to occur (Simpfendorfer et al., 2016). Traditonal ecological knowledge has been valuable for mapping trends in occurrence and abundance over time, but contemporary evidence is lacking given the rarity of sawfishes. There are four sawfish species that are known to occur in the Indo-West Pacific region (largetooth sawfish *Pristis pristis,* dwarf sawfish *P. clavata*, green sawfish *P. zijsron*, and narrow sawfish *Anoxypristis cuspidata*). The coastal waters of Northern Australia are considered one of the last safe havens for these four species (Dulvy et al., 2016).

Capture surveys and field observations indicate that, while now rare, all four Indo-West Pacific sawfish species utilise the shallow estuarine and coastal habitats of Northern Teritory (Field, Charters, Buckworth, Meekan, & Bradshaw, 2008; Salini et al., 2007). Some species also occupy tidal rivers and largetooth sawfish range extends into freshwater habitats (Devitt, Adams, & Kyne, 2015). Dwarf sawfish has the smallest historical distribution, being found only in coastal zones of India, Indonesia, Papua New Guinea and Australia (Dulvy et al., 2016). The species is listed as Endanagered on the IUCN Red List of Threatened Species (Kyne, Rigby, & Simpfendorfer, 2019). Currently, its geographic range is considered to have reduced to less than 70% of its fromer range, with the only known extant populations occurring entirely in Northern Australia (Dulvy et al., 2016). Largetooth sawfish have the largest historical distribution, including coastal waters of 75 countries, however, this species has experienced a 61% decline in geographic size range (Dulvy et al., 2016) and is listed as Critically Endanegred on the IUCN Red List of Threatened Species (Kyne, Carlson, & Smith, 2013).

Sawfishes (excluding narrow sawfish) are protected in Australia and, given their imperilled state, there has been a surge in research and collaborative conservation efforts. Sawfish and their saws also have cultural value and historical significance among many Indigenous communities. However, sawfish constitute bycatch of recreational and commercial fisheries (Field et al., 2008), though capture records are declining, and there are uncertainties of their occurrene in large parts of their range. The ability to resolve this uncertainty and implement effective safeguards for their recovery is limited by the difficulty in detecting sawfishes in the coastal and riverines habitat where they are thought to occur.

Environmental DNA (eDNA) is the DNA released into the environment via mucous, faeces, skin cells, etc., that can be isolated from water samples and screened for species of interest (Jerde, Mahon, Chadderton, & Lodge, 2011). Environmental DNA is gaining momentum as a

monitoring tool given its sensitivity to detect the presence of species that occur at low abundance (Sigsgaard, Carl, Møller, & Thomsen, 2015). It is a time and cost-efficient method of assessing species distribution and in some cases, it has shown to have higher sensitivity to rare species that net surveys (Thomsen et al., 2012). Environmental DNA analysis has successfully been applied to detection of largetooth sawfish in Australia (Simpfendorfer et al., 2016) as well as smalltooth sawfish, *P. pectinata*, in the western Atlantic (Lehman et al., 2020). Environmental DNA sample collection has the potential to build the capacity of citizen scientists and traditional owners to participate in regional sawfish conservation action. However, the existing sawfish eDNA research has involved collecting eDNA via water filtration. The downside of this eDNA capture technique is that it involves many procedurial steps, that requires specific filtration equipment and may impose higher chance of sample contamination (Huerlimann, Cooper, Edmunds, Villacorta-Rath, Le Port, et al., 2020), and this method may be especially impractical for citizen science and traditional owner groups.

Despite major advances in filtering technology that make on-site sampling easier (e.g., portable pumps) and less prone to contamination (e.g., enclosed filter units), filtering water can be time-consuming and is therefore not an attractive option to non-specialists who could otherwise be usefully involved in eDNA-based monitoring programs. In addition, when filtration is done off-site, water samples need to be kept on ice and filtered within 24 hours of collection in order to prevent further eDNA degradation and maximize eDNA yield (Hinlo, Gleeson, Lintermans, & Furlan, 2017). Remote sampling locations in northern Australia can be several hours away from laboratories where filtration is often performed and keeping samples on ice is difficult due to high ambient temperature (Huerlimann, Cooper, Edmunds, Villacorta-Rath, LePort, et al., 2020). An alternative to filtration is to mix whole water samples with buffer or preservation solutions, however, preservation of eDNA samples at ambient temperatures above 30°C in the tropics where sawfish occur requires a temperature-stable preservative solution (Huerlimann, Cooper, Edmunds, Villacorta-Rath, LePort, et al., 2020).

TropWATER has developed a simplified eDNA field method that is routinely used for collection of whole water samples and preservation at ambient tropical temperatures for extended periods (Edmunds & Burrows, *in press*). The method uses a non-toxic, non-alcohol preservation solution called Longmire's buffer to preserve eDNA in whole water samples (Villacorta-Rath & Burrows, 2019, 2020; Villacorta-Rath, Edmunds, & Burrows, 2019; Villacorta-Rath & Burrows, 2020). This method has also been successful in preserving sawfish eDNA on filters (Cooper et al., *in review*) and in unfiltered water samples for up to three months (unpublished data), allowing for samples to be shipped from remote areas to the laboratory facility in Townsville. In addition, the TropWATER field method, which involves collecting whole water samples, can be carried out with ease by non-specialists without formal training by just following an instruction manual. The protocol has been tested at different locations and for detection of other target species, including fish and amphibians (Villacorta-Rath & Burrows, 2019, 2020; Villacorta-Rath et al., 2019; Villacorta-Rath & Burrows, 2020). Whole water collection and immediate preservation represents a lower risk of contamination than conventional water filtration methods. The development of robust water sampling methods that can be used by citizen scientists and/or traditional owners will allow eDNA samples to be collected by the wider community. Community participation will not only raise awareness but will assist in comprehensive sawfish distribution data to be collected. This crucial baseline information can advise regional conservation actions for species protection and habitat conservation.

In the present study, we engaged with the Anindilyakwa Land & Sea Rangers (ALS), Groote Eylandt, to conduct sawfish eDNA sampling during December 2019. Sawfish constitute species of cultural significance for certain clans of the Anindilyakwa people (McDavitt, 2005). Environmental DNA sampling was carried out by the ALS following TropWATER's field protocol in a saltwater lake where there is anecdotal evidence of sawfish presence. Although the particular sawfish species that could inhabit the lake is unknown, it is suspected to be largetooth or dwarf sawfish. Based on their likelihood of occurence at the sampling location, eDNA samples were screened for presence of largetooth and dwarf sawfish. The aims of the present study were to: (1) trial our eDNA field sampling methods without any formal training and just following a field manual; (2) screen field samples for presence of dwarf and largetooth sawfish eDNA.

This study and report complements similar work undertaken by the researchers in Arnhem Land in the Northern Territory, which can also be found on the hub website.

2. Methodology

2.1 eDNA sampling

Water samples for eDNA analysis were collected directly from the water surface at four sites in a saltwater lake in Groote Eylandt during December 2019 (Table 2.1, Figure 2.1). At each site, five replicate 500 mL samples were collected using a new, clean bottle and then decanted into a new, clean jar containing 125 mL of Longmire's preservative solution. A field blank was included at each site to ensure that the process of sample collection did not introduce contamination. The field blank consisted of decanting 500 mL of laboratory-grade water into a jar containing 125 mL of preservative solution in the same manner as field samples. The final volume of all samples, including controls, was therefore 625 mL.

Table 2.1. Field sites in a saltwater lake, Groote Eylandt, sampled for sawfish eDNA detection.

Site name	Latitude	Longitude	Collection date
Campsite	-13.95316	136.71930	05/12/2019
Channel-Lakeside	-13.95609	136.75858	03/12/2019
Main	-13.92887	136.72371	05/12/2019
Track	-13.96258	136.76572	03/12/2019

Figure 2.1. Field sites sampled for sawfish eDNA detection in a saltwater lake, Groote Eylandt.

2.2 Environmental DNA extractions

Environmental DNA extractions were carried out at the dedicated TropWATER eDNA laboratory, James Cook University, Townsville. Prior to eDNA extraction, bench top surfaces and floor were decontaminated with 10% bleach and subsequently wiped with water and ethanol. Jars and lids containing the field samples were wiped using the same procedure to avoid cross contamination during tube handling. A volume of 300 mL of water sample plus Longmire's preservative solution of each field sample was aliquoted into 15 Falcon tubes of 50 mL capacity, each containing 20mL of water sample and preservative solution for eDNA extraction (Figure 2.2).

We followed a glycogen-aided isopropanol precipitation protocol developed at TropWATER, as described by Edmunds & Burrows (*in press*). For all extractions, 20 mL sample aliquots were mixed with 5 µL glycogen (200 mg/mL), 20 mL isopropanol and 5 mL NaCL (5M). Samples were then incubated overnight at 4°C and subsequently centrifuged at 6,750 g for 10 min to form a pellet. The supernatant was then discarded, and pellets were dissolved in 600 µL of lysis buffer (guanidinium hydrochloride and TritonX; pH 10), transferred into a 2 mL tube, and frozen overnight. Environmental DNA present in the samples was lysed at 50°C for five hours and a subsequent precipitation step was carried out by adding 1 µL glycogen and 1,800 µL polyethylene glycol (PEG) buffer to the samples. Samples were centrifuged at 20,000 g for 30 min to form a pellet that was then washed twice using 70% ethanol. After the ethanol washes, the pellet was dried and eDNA was resuspended in 100 µL MilliQ water. Finally, eDNA was purified using the Qiagen DNeasy® PowerClean® Pro Cleanup kit and eluted in 100 µL elution buffer (Figure 2.2).A negative extraction control (EC) was added to each batch of eDNA extractions to ensure that no contamination was introduced during laboratory procedures (Goldberg, Turner, Deiner, Klymus, Thomsen, Murphy, Spear, McKee, Oyler-McCance, Cornman, Laramie, Mahon, Lance, Pilliod, Strickler, Waits, Fremier, Takahara, Herder, & TABERLET, 2016).

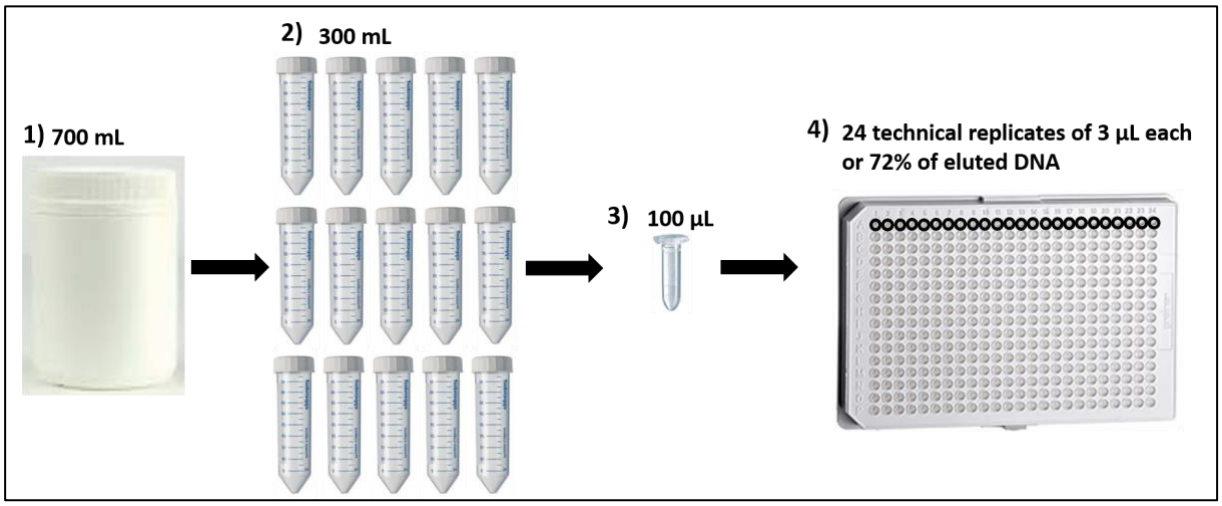

Figure 2.2. Field samples consisting of 700 mL of water plus preservative buffer, concentrated into 100 µL of DNA eluted in laboratory-grade water, out of which 72 µL, or 72% of eluted DNA in the case of samples from 'Main' and 'Channel-Lakeside' where loaded into a qPCR plate.

2.3 Inhibition tests

Presence of PCR (polymerase chain reaction) inhibitors in water samples was tested by spiking 180 copies of artificial DNA (aDNA) of *Litoria lorica*, a rainforest frog, into triplicated samples from each site sampled in Groote Eylandt. Additionally, the same number of aDNA copies was spiked into three technical replicates containing only MilliQ water, representing the control. Any shift in the quantitative PCR (qPCR) amplification threshold (Ct value) between the field samples and the control in more than three cycles would be indicative of PCR inhibition (Hartman, Coyne, & Norwood, 2005).

2.4 Detection of species-specific DNA by quantitative polymerase chain reaction (qPCR)

Detection of largetooth dwarf sawfish was performed using species-specific qPCR assays. The assays include unique primer pairs and TaqMan MGB probes that were previously developed and optimised at James Cook University (Cooper et al., *in review*). The assays were designed to detect a short segment of the *12S* rRNA mitochondrial gene of largetooth sawfish (179 bp) and dwarf sawfish (111 bp) with an LOD at 1.25 and 5 copies per reaction, respectively. The *12S* segments were chosen based on adequate interspecific sequence divergences and intraspecific sequence similarity using reference sequences in Geneious 10.2.6 software. The largetooth sawfish 12S assays uses forward primer 5'-GTGCCTCAGACCCACCTAGA-3', reverse primer 5'-CATCATACTGTTCGTTTTTTCTTAGGAG-3', and probe 5'-VIC-AAATGAACTAACCTTCAATACG-MGBNFQ-3'. The dwarf sawfish 12S assays uses forward primer 5'-GGTGCCTTAGATCCACCTAGAG-3', reverse primer 5'-CTGACGTATTGAAGGTGGGTTCT-3', and probe 5'-FAM-CATTTCTTGCTATCAACC-MGBNFQ-3'.

qPCR assays were run on a QuantStudio™ 5 Real-Time PCR System (Thermo Fisher Scientific Australia Pty Ltd) in a total of five MicroAmp™ Optical 384-well plates sealed with optical films (Applied Biosystems, ThermoFisher Scientific, Victoria, Australia). Twenty four technical replicates of each field sample, representing 72% of the eluted DNA volume, were run on qPCR (Figure 2.2).

Sites 'Campsite' and 'Main' were only screened for presence of largetooth sawfish, given that they were located downstream from the two saltwater lake tributary creeks and it is known that largetooth sawfish inhabit freshwater environments (Thorburn, Morgan, Rowland, & Gill, 2007; Thorson, 1982b, 1982a). Each technical replicate consisted of 10 µL reactions containing 3 µL of template DNA and 7 µL of master mix (5 µL TaqPath™ ProAmp™ Multiplex Master Mix; 0.3 µL *P. pristis* forward primer at 100 µM; 0.3 µL *P. pristis* reverse primer at 100 µM; 0.25 µL of *P. pristis* TaqMan™ MGB probe; 1 µL Bovine Serum Albuminum, BSA; 1 µL MilliQ® water). Thermal cycling profile was as follows: initial denaturation at 95°C for 10 min, followed by 55 cycles of 95°C for 15 sec and 60°C for 1 min. In each plate, six positive reference samples containting largetooth sawfish 12S synthetic DNA (gBlocks® Gene Fragments; Integrated DNA Technologies Pty Ltd, New South Wales, Australia) were added, as well as three non-template controls (NTC) and EC from all eDNA extractions. The NTC and EC samples did not contain the target species DNA and their lack

of amplification indicated that no contamination was introduced during plate handling or extraction, respectively.

Sites ‘Track’ and ‘Channel-Lakeside’ were additionally screened for presence of dwarf sawfish in a duplex reaction (two assays per reaction), given that they were located close to the lake outlet into the ocean. Each technical replicate consisted of 10 µL reactions containing 3 µL of template DNA and 7 µL of master mix (5 µL TaqPath™ ProAmp™ Multiplex Master Mix; 0.03 µL *P. pristis* forward primer at 100 µM; 0.03 µL *P. pristis* reverse primer at 100 µM; 0.03 µL *P. clavata* forward primer at 100 µM; 0.03 µL *P. clavata* reverse primer at 100 µM; 0.025 µL of *P. pristis* TaqMan™ MGB probe; 0.025 µL of *P. clavata* TaqMan™ MGB probe; 0.5 µL BSA; 1.33 µL MilliQ® water). Thermal cycling profile was as follows: initial denaturation at 95°C for 10 min, followed by 55 cycles of 95°C for 15 sec and 60°C for 1 min. In each plate, two triplicate samples containting gBlocks DNA from largetooth sawfish were added, as well as three NTC and EC from all eDNA extractions. The NTC and EC samples did not contain the target species DNA and their lack of amplification indicated that no contamination was introduced during plate handling or extraction, respectively.

2.5 Data analysis

All plates were analysed with a common fluorescence threshold (0.4) using QuantStudio™ Design and Analysis Software (version 1.4.2; Thermo Fisher Scientific Australia Pty Ltd) before export and subsequent analyses in Microsoft Excel. Samples were considered positive for largetooth or dwarf sawfish detection ifthere was a fluorescence signal and the association amplification curve crossed the common fluorescence threshold within 50 cycles.

3. Results

3.1 Inhibition tests

Extracted eDNA from water samples collected at ‘Campsite’ and ‘Track’ showed a Ct difference of 0.2 and 2.5 cycles, respectively, indicating that the eDNA was not inhibited. Conversely, water samples collected at ‘Main’ and ‘Channel-Lakeside’ showed a Ct difference of >9 cycles. To counteract inhibition, a 1:2 dilution to water samples from those sites was applied. After sample dilution, inhibition tests were repeated for those two sites and confirmed that inhibition was resolved (Ct ≤3 cycles).

3.2 Detection of sawfish eDNA via qPCR

Based on the assessment criteria to consider a positive detection of the target species, no presence of largetooth or dwarf sawfish at any sampling site was detected. All field and extraction control samples were verified to not contain target species eDNA by qPCR.

4. Discussion

In the present study, we enagaged with the ALS Rangers, Groote Eylandt, to collect eDNA samples from a saltwater lake where there is anecdotal evidence of sawfish occurrence. Based on the location and knowledge of sawfish habitat utilisation, we determined that the sawfish species that are likely to inhabitat the location would be largetooth sawfish or dwarf sawfish. Therefore, eDNA samples were screened for these two species. Although we did not find sawfish eDNA in the samples, our field method proved to be user-friendly and was successfully followed by the ALS Rangers.

4.1 Field collection method

Environmental DNA analysis relies on the premise that we can collect and analyse trace amounts of target DNA that is suspended in water. The method is therefore very sensitive to contamination that could lead to false positive detections. Contamination can occur during multiple stages of the analysis, in both the field and the laboratory, therefore blank controls need to be implemented in order to monitor potential contamination (Goldberg, Turner, Deiner, Klymus, Thomsen, Murphy, Spear, McKee, Oyler-McCance, Cornman, Laramie, Mahon, Lance, Pilliod, Strickler, Waits, Fremier, Takahara, Herder, & Taberlet, 2016). These include: field controls, eDNA extraction negative controls and qPCR negative controls (Goldberg, Turner, Deiner, Klymus, Thomsen, Murphy, Spear, McKee, Oyler-McCance, Cornman, Laramie, Mahon, Lance, Pilliod, Strickler, Waits, Fremier, Takahara, Herder, & Taberlet, 2016).

Laboratory contamination is easier to eliminate by conducting all eDNA extractions and pre-PCR steps in a dedicated ‘low DNA copy’ space (Taberlet, Waits, & Luikart, 1999), such as the facility used in this study. Specifically, the TropWATER eDNA lab does not contain or permit the handling of genomic DNA of any target species and utilises thorough decontamination protocols. On the other hand, field contamination of samples is typically harder to avoid. Especially when involving non-specialists who may not be immediately aware of the sensitivity of the method and the special care that is required when carrying out this work. TropWATER’s simplified field collection method involves collecting water samples and preserving them directly in Longmire’s buffer. This avoids having too many procedurial steps and equipment handling, where contamination could be introduced.

The simplified field collection method was succesfully applied by the ALS Rangers, who reported that ‘the field method was simple and the instructions were very clear, so the whole process was very straightforward’. Importantly, the fact that we did not observe positive amplification in any of the field control samples collected in the present study demonstrated that the field method was stringent enough to avoid contamination during field sampling.

4.2 Sawfish eDNA detection

The application of eDNA as a detection tool for elasmobranchs (sharks and rays) in dynamic coastal environments is still in its infancy but presents an unparalleled opportunity to survey rare shark and ray species rapidly and effectively. We did not detect the presence of dwarf or largetooth sawfish eDNA at four sampling sites within the saltwater lake, Groote Eylandt, during this survey. There are many factors that could explain the lack of positive detections and lack of sawfish eDNA detections in the present study is not always indicative of the

species absence at the sampling location. The capture and detectability of eDNA is contingent on not only the release of eDNA from the species when it is present in the habitat, but also the persistence and degradation of eDNA in the water (Thomsen et al., 2012).

Very little is known about the source of eDNA from elasmobranchs or the rate it is shed into the environment, which can depend on a number of factors including the species, size, abundance, life stage, stress, and water conditions (Harrison, Sunday, & Rogers, 2019). Lack of such information makes inference of non-detections difficult. Moreover, eDNA transport and degradation in the water column can also affect the ability to detect eDNA (Harrison et al., 2019). In the present study, transport of eDNA out of the lake by currents or tidal forces may have compromised the ability to detect sawfish eDNA oringiating from individuals inhabiting the lake. However, samples were collected during falling tide in the main channel with the purpose of increasing the detection probability by collecting any eDNA flowing out of the lake and into the ocean. On the other hand, given that eDNA degradation generally occurs within the first two weeks after it has been deposited (reviewed by Harrison, Sunday, & Rogers, 2019), it is plausible that false negative detections may be a result of rapid degradation. Therefore, eDNA detection relies on the target species having been present in the area within the approximate time of sampling. Aside from biological factors, there are technical factors that may also influence detection sensitivity. This includes failure to capture target eDNA in small water volumes or in only few field replicates when sampling in dynamic coastal habitat. Accordingly, survey replication and sampling across multiple time points are important steps to improve the sensitivity of the survey method.

Sawfishes in northern Australia are rare and patchily distributed and capture and sighting records are lower than they were historically (Peverell, 2005; Stevens et al., 2008). In the case of largetooth sawfish, low catch rates have been documented throughout the Northern Territory, even in areas of known sawfish occurrence (Kyne, Pillans, 2017). Additionally, very low capture records of juveniles may indicate the low survival rates of this species during early stages (Kyne et al., 2017). Independent surveys of dwarf sawfish in the Gulf of Carperntaria have also reported low capture rates (Field et al., 2008). Similarly, sawfish eDNA surveys in northern Australia indicated that detections were generally sparse and uneven. For example, approximately 15% of surveyed sites in the Kakadu region were positive for dwarf sawfish eDNA, and positive replicates contained only small amounts of detectable DNA (Cooper et al., *in review*). The survey in this current study was undertaken in December, which precedes the local pupping season of all four Indo-West Pacific sawfishes. Neonate and juvenile largetooth sawfish occupy freshwater sections of rivers and move into estuarine systems when they mature (Thorburn et al., 2007; Thorson, 1982b, 1982a), however, there is no evidence to suggest that the saltwater lake is a nursery habitat for largetooth sawfish. Especially considering there is not a substantial freshwater flow. In addition, seasonality, habitat fidelity, and spatial movements (Kyne et al., 2017) may also affect detection rates. The fact that we did not detect largetooth or dwarf sawfish eDNA could indicate that the species were not present at the Lake during sampling.

5. Recommendations

Conducting eDNA surveys during pupping seasons, when gravid females are expected to return to riverine environments could provide a higher chance of detecting sawfish eDNA. Alternatively, it is also likely that sawfishes are rare in the water adjacent to Groote Eylandt and given that catch rates and sightings are variable, eDNA surveys should ideally be repeated seasonally in order to account for the natural variability of sawfish occurrence. Finally, conducting eDNA sampling shortly after an unconfirmed sawfish sighting could substantiate the utility of the technique for Ranger groups who wish to continue employing eDNA detection methods for species of local importance or conservation concern. These findings are relevant and important for research efforts that aim to reveal crucial baseline information for sawfishes, where conservation efforts are impeded by lack of technologies to reliably monitor them.

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