Resolving the global distribution of sawfishes (family Pristidae) using environmental DNA

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Finishing this was a commitment to myself and those who believed in me.

ABSTRACT

The development of monitoring or population recovery plans for any endangered species should involve understanding the species' true distribution. A major problem with aquatic research and monitoring programs is that species detection is imperfect. This problem is exemplified by sawfishes (family Pristidae), which are highly threatened and have severely declined as a result of their habitat specificity. Sawfishes have a strong affinity to mangrove-lined, shallow, and riverine habitats, throughout all or some of their life. Mangrove habitats and their inhabitants are directly impacted by anthropogenic threats such as development and overfishing, respectively, and, as a result, sawfish populations are sparse, fragmented, and the current global distribution is largely uncertain. Due to lack of contemporary sightings and paucity of research capacity, populations of sawfish are presumed extinct in up to half of their historic range. Moreover, the habitat preference of sawfish in highly turbid habitats that are often in remote locations create challenges for the application of conventional survey methods. Cost and time limitations of survey methods and the lack of resources and capacity in much of their historical range has precluded comprehensive assessment of their contemporary spatial extent. Tools to reliably and accurately detect sawfishes are needed to increase potential of protection and conservation. Characterisation of environmental DNA (eDNA) extracted from water samples has garnered significant appeal as a non-invasive approach for detection of rare and threatened aquatic species such as sawfishes. This thesis demonstrates the utility and challenges of eDNA to detect sawfishes and proposes methods for future development of the tool for detecting rare and threatened species.

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COVID-19 THESIS IMPACT STATEMENT

The emergence of the COVID-19 pandemic and subsequent rounds of Australia-wide and state lockdowns impacted my planned PhD research activities and the timeline to which I was able to complete my research and thesis. Though, as the timing of the pandemic was later during my candidature, the scope of the research was not significantly modified and the standard of the thesis remain largely unimpacted.

Australia went into a national lockdown including the closure of non-essential services on 21 March 2020. The immediate closure of JCU campus and restricted access to laboratories where I had experiments underway had immediate impact on progress. In late April 2020, I was permitted restricted, monitored access to the laboratory. The timeline for completion of the laboratory work was delayed due to necessary health and safety measures, including limits of number of people and social distancing while working in the lab. I completed lab work 7 months later in October 2020, 3 months later than planned and 3 months prior to the end of my RTPS scholarship and planned completion date. Subsequently, my thesis timeline and submission date were modified to reflect these delays and ongoing disruptions given the pandemic.

Furthermore, closure of national borders and the halt of field research globally resulted in modifications of data collection. Specifically, Chapter 6 presents results from a global survey of sawfishes using eDNA, including the analysis of samples collected throughout key nations of the global tropics and subtropics. However, there are nations that I had planned to include in this study, namely Sri Lanka, India, South Africa, and Mozambique, where sampling kits had been sent but sample collection was not possible given the risk presented by the pandemic. As a result, the chapter does not capture the entirety of what was planned for the global survey; however, the exclusion of these nations did not severely impact the research in this chapter.

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LIST OF ACRONYMS

Acronym	Description
12S	Small subunit gene of eukaryotic mitochondrial ribosome
16S	Small subunit gene of prokaryotic ribosomes and eukaryotic mitochondrial ribosomes
18S	Small subunit gene of eukaryotic cytoplasmic ribosomes
ΔRn	Normalised reporter value representing the magnitude of the signal generated during a qPCR reaction, calculated as the experimental reaction value minus to reaction value of the baseline signal generated by the qPCR instrument
ANOVA	Analysis of variance
AUV	Autonomous underwater vehicle
BAC	Benzalkonium chloride
BDT	BigDye™ terminating
BLAST	Basic local alignment search tool
bp	Base pairs
BRUVs	Baited Remote Underwater Videos
BSA	Bovine serum albumin
COI	Cytochrome C Oxidase subunit gene of mitochondrial DNA
CITES	Convention on International Trade in Endangered Species of Wild Fauna and Flora
CMS	Conservation of Migratory Species of Wild Animals
CytB	Cytochrome B gene of mitochondrial DNA
Ct	qPCR cycle number at which amplification reaches a fluorescence threshold above background levels
CTAB	cetyltrimethylammonium bromide
cPCR	Conventional polymerase reaction
ddPCR	Droplet digital polymerase chain reaction
D-loop	Non-coding sequence of the mitochondrial genome
DNA	deoxyribonucleic acid
eDNA	Environmental deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
EEZ	Economic exclusion zone

EPBC	Commonwealth Environment Protection and Biodiversity Conservation
eRNA	Environmental ribonucleic acid
ESA	Endangered Species Act (United States)
FAM	5-carboxyfluorescein
gDNA	Genomic DNA
GF	Glass fibre
GPS	Global Positioning System
IDT	Integrated DNA Technologies
IPC	Internal positive control
ITS	Internal transcribed spacer
IUCN	International Union for Conservation of Nature
IUU	Illegal, unreported, and unregulated; typically in reference to fishing
JCU	James Cook University
MCE	Mixed cellulose ester
NaCl	Polyethersulphone
NADH4	Core subunit of the mitochondrial membrane respiratory chain nicotinamide adenine dinucleotide dehydrogenase complex I
NCBI	National Centre for Biotechnology Information
NGS	Next-generation sequencing
PCR	Polymerase chain reaction
PEG	Polyethylene glycol
PES	Polyethersulphone
PPLPP	‘Preserve, precipitate, lyse, precipitate, purify’
qPCR	Quantitative polymerase chain reaction
Rn	Reaction number
RNA	Ribonucleic acid
SCUBA	Self-contained underwater breathing apparatus
sDNA	Double-stranded synthetic DNA fragments
TED	Turtle exclusion device
Tris HCl	Trisaminomethane hydrochloride
UV	Ultra-violet
VIC	2′-chloro-phenyl-1,4-dichloro-6-carboxyfluorescein

GLOSSARY

Term	Description
Abundance	In ecology, local abundance is the relative representation of a species in a particular ecosystem, usually measured as the number of individuals found per sample
Amplicon	A copy of a short DNA fragment generated during PCR replication
Appendix I	A list of species that are the most endangered among CITES-listed animals and plants. They are threatened with extinction and CITES prohibits international trade in specimens of these species except when the purpose of the import is not commercial, for instance scientific research.
Appendix II	A list of species that are not necessarily now threatened with extinction but that may become so unless trade is closely controlled. International trade in specimens of listed species may be authorised by the granting of an import permit or re-export certificate. No import permit is necessary for these species under CITES.
Appendix III	A list of species included at the request of a Party that already regulates trade in the species and that needs the cooperation of other countries to prevent unsustainable or illegal exploitation. International trade in specimens of listed species is allowed only on presentation of the appropriate permits or certificates.
Assay	An experimental method to find and measure the presence, level, or activity of a biological molecule
Barcode region	Specific gene locus within the genome
Biological replicate	A test performed on a biologically distinct sample representing an individual sampling event, i.e., 5 5L water samples collected from the same riverine habitat
Critically Endangered	A taxon that is considered by the International Union for Conservation of Nature (IUCN) to be facing an extremely high risk of extinction in the wild.

Data deficient	A species that is considered by the International Union for Conservation of Nature (IUCN) as offering insufficient information for a proper assessment of conservation status to be made
Distribution	Is the manner in which a biological taxon or species is spatially arranged in geographic space, often referred to its range
eDNA	A technique based on the isolation, PCR amplification, and sequencing of freely associated DNA in the environment
Endangered	A taxon that is considered by the International Union for Conservation of Nature (IUCN) as very likely to become extinct in their known native ranges in the near future.
Ex situ	Off site or away from the natural location.
Extant	The species is known or thought to very likely occur currently in the area, which encompasses localities within current or recent (last 20–30 years) records where suitable habitat at appropriate altitudes remains
Extinct	The termination of a species, generally considered to be the death of the last individual of the species
Haplotype	A physical grouping of genomic variants or polymorphisms that tend to be inherited together.
IUCN Red List of Threatened Species	A critical indicator of the health of the world's biodiversity, used to inform and catalyse action for biodiversity conservation and policy change, critical to protecting the natural resources we need to survive
In silico	An experiment that is performed on the computer or via computer simulation
In situ	In its original or natural position or place
In vitro	Experiments performed on biological moleculars outside their normal biological context, in an artificial environment such as a laboratory in microtubes
Metabarcoding	Massive parallel sequencing of complex bulk sample for the simultaneous identification of many taxa within the same sample

Oligonucleotide	Short synthetic strands of DNA or RNA
SYBR™ Green	A dsDNA-binding dye that intercalates non-specifically into dsDNA allowing measurement of PCR product
TaqMan™	Allelic specific nucleic acid probe complementary to an internal segment of the target DNA labelled with two fluorescent moieties designed to increase the specificity of quantitative PCE
Technical replicate	A test performed on the same sample multiple times, i.e., the PCR reactions using the same eDNA sample
Threatened	Species that are listed as Vulnerable, Endangered, and Critically Endangered by the International Union for Conservation of Nature (IUCN)
Vulnerable	A taxon that is considered by the International Union for Conservation of Nature (IUCN) as threatened with extinction unless the circumstances that are threatening its survival and reproduction improve.

Chapter 1

General Introduction

1.1. Aquatic species in decline

Anthropogenic impacts are placing growing pressure on aquatic ecosystems, as evidenced by widespread biodiversity declines and an increased number of species under threat (Dulvy et al., 2021; Halpern et al., 2008; He & Silliman, 2019; Hoffmann et al., 2010; Pereira et al., 2012; Worm et al., 2006). This warrants immediate attention to mitigate the risk to the environment and the detrimental societal and economic consequences. However, only in recent years have we witnessed aquatic biodiversity become a part of the global conversation (e.g., Blue Economy, United Nations, 2014; Sustainable Development Goals, Brooks et al., 2015). Declines in aquatic biodiversity have been extensively documented and are heterogeneously occurring throughout the world's rivers (Guohuan et al., 2021; Reid et al., 2019) and oceans (Halpern et al., 2008; Pereira, Navarro and Martins, 2012; Francisco et al., 2021). The vastness of the world's rivers and oceans may cause reason to believe that there is plenty of space for every animal to find safety, yet this vastness presents a challenge to the assessment and conservation of many aquatic species (McCauley et al., 2015). Consequently, and importantly here, the ability to detect and study species in these environments is challenging our ability to protect them (McCauley et al., 2015; Webb & Mindel, 2015).

Understanding the decline of population distributions and occurrence, and therefore their threats and extinction risk, is a core tenet of ecological research and conservation biology.

The global standard for classifying a species' extinction risk is The International Union for Conservation of Nature's (IUCN) Red List of Threatened Species (Hoffmann et al., 2008; Mace et al., 2008). A recent systematic analysis estimated that one-third (32.6%; 391 species) of Chondrichthyes (sharks, skates, rays, and chimeras; herein referred to as sharks and rays) are at risk of extinction (Dulvy et al., 2021). Some populations have declined by more than 95% as a result of overfishing (Dulvy et al., 2014). The status of sharks and rays makes them one of the most threatened vertebrate lineages, second only to amphibians, of which 41% are classified as threatened (Hoffmann et al., 2010). The threatened status of sharks and rays, as it is for most aquatic vertebrates inhabiting both freshwater and marine ecosystems, is driven by direct and indirect exposure to anthropogenic drivers of threat (Davidson et al., 2012; Dulvy et al., 2014; McClenachan et al., 2012; Spatz et al., 2014).

Chondrichthyans are among the oldest and most diverse lineage of aquatic vertebrates, with a history spanning 420 million years (Grogan et al., 2004; Stein et al., 2018) and more than 1200 extant species (Fricke et al., 2018), that occupy an important position in riverine, coastal, and oceanic ecosystems (Ferretti et al., 2010; Heithaus et al., 2008). Today, this lineage of evolutionarily unique vertebrates plays an important role in the global economy and food security, driven by the demand on fisheries for meat and fins and the emergence of globalised trade of shark products (Dulvy et al., 2017). The value of shark fin and meat products in the period 2012−2019 was estimated at US$4.1 billion (Niedermüller et al., 2021). Shark fin consumption throughout South and East Asia is a primary driver for this trade. For example, at the end of the last century, the trade of shark fin through the world's largest shark fin market in Hong Kong suggested that the fins of between 30 and 52 million shark are harvested from the ocean annually (Clarke et al., 2006). Even though the price of fins can be an order of magnitude higher than the price of meat (e.g., US$100 vs US$0.1 per kg), and the fin trade has received significant global attention (Savitz, 2018), the global trade of meat far exceeds fins.

The value of shark and ray meat products comprised almost two-thirds (US$2.6 billion in 2012−2019) of the global trade. This lucrative trade remains largely unregulated and, although the magnitude and growth of the global shark and ray fishing industry are greater than is generally understood by policy makers, or the general public, reversing widespread losses of species may still be possible.

Of all the Chondrichthyes, declines in coastal and euryhaline sharks and rays are most prominent (Dulvy et al., 2021; Grant et al., 2019). A large and increasing proportion of the global human population live close to the coast in overlapping distribution with many shark and ray species. Here, the rapid expansion of fisheries have provided a source of economic value and food security to millions of people, but has also led to international concern regarding the sustainable exploitation and long-term population viability of coastal and euryhaline shark and ray species (Dulvy et al., 2017; Sampson et al., 2015; Stevens et al., 2000; Worm et al., 2006). Elevated exposure of coastal and euryhaline species to threats is owing to their habitat specificity, restricted geographic range, and shallow and narrow depth range, which is exacerbated by the intrinsic biological susceptibility of some sharks and ray species (e.g., late sexual maturity, lengthy pregnancy, low fertility, slow growth, and long lifespan) to overfishing, habitat loss and degradation, climate change, and pollution (Dulvy et al., 2021; Grant et al., 2019; Pardo et al., 2016; Tiktak et al., 2020).

1.2. The pointed end of conservation: Sawfishes

Population declines are a major concern for the loss of genetic diversity and compromised ability of a population to evolve and persist (Domingues et al., 2018). Recently, there has been considerable attention given to a unique family of shark-like rays that are among the most imperilled group of Chondrichthyans globally: sawfishes (Figure 1.3; Dulvy et al.,

2016). Once widespread across shallow, coastal habitats in tropical and subtropical waters (Bonfil et al., 2017; Cuevas et al., 2019; Fernandez-Carvalho et al., 2014; Haque & Das, 2019; Jabado et al., 2017; Leeney, 2016; Leeney, 2017b; Robillard & Séret, 2006; Thorson, 1982), global sawfish populations have experienced historic declines greater than 90% due to unprecedented and unregulated levels of harvest in fisheries and destruction of coastal habitat (Dulvy et al., 2016).

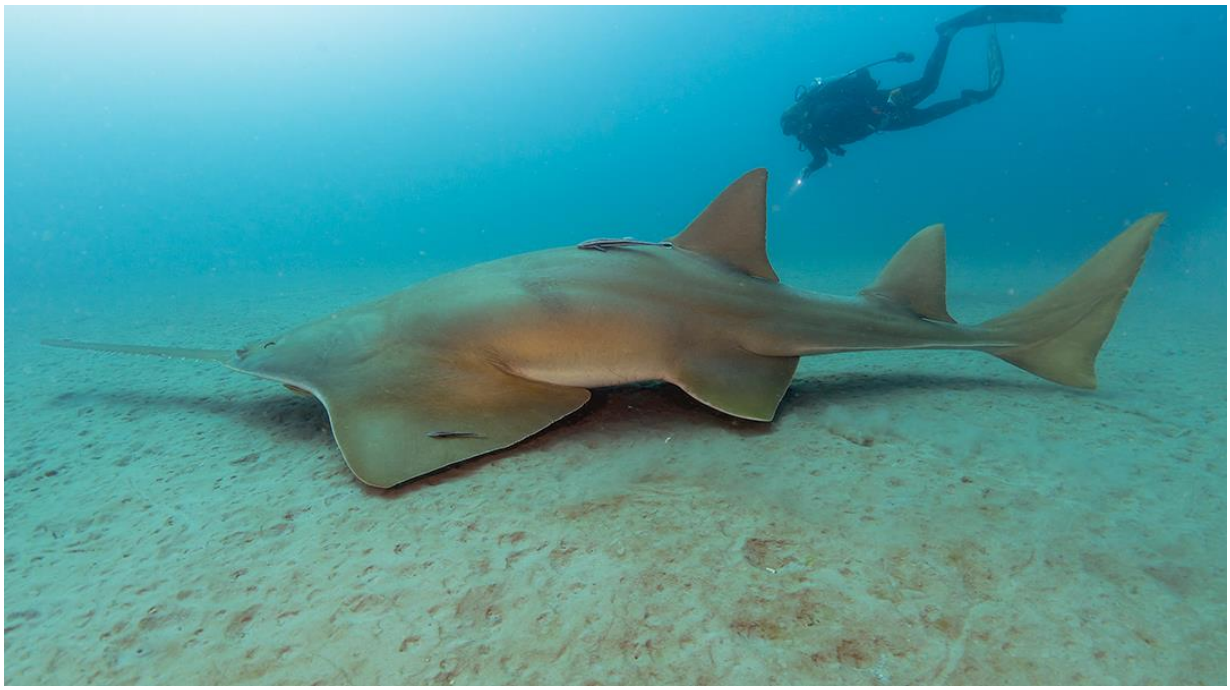

Figure 1.1. Smalltooth sawfish *Pristis pectinata* pictured in front of a diver off the coast of Florida, U.S. Photo credit: Michael Patrick O'Neill.

Sawfishes are identified by their characteristic toothed rostra (often referred to as a 'saw') that can be up to one-quarter of its total body length (Figure 1.2; Wueringer, Squire and Collin, 2009). However, it is this rostrum that exposes sawfish to a greater predisposition of entanglement in fishing nets and lines than any other shark or ray species (Figure 1.2; Simpfendorfer, 2000). When paired with sawfishes' preference for shallow, coastal habitats, capture in fisheries (primarily as bycatch) is the leading reason behind the scale of threat to

sawfishes. Upon capture, rostra are often lethally removed, as are dorsal and pectoral fins and meat. In some countries rostra are retained as culturally important relics or used in medicines; elsewhere, rostra are traded as valuable souvenirs (e.g., rostra have been documented to be sold for over US$1,000; McDavitt & Charvet-Almeida, 2004). Sawfish fins are also traded as an economically valuable commodity (i.e., one of most valued and sought-after fins; Clarke et al., 2007; Fabinyi, 2012; Sadovy de Mitcheson et al., 2018).

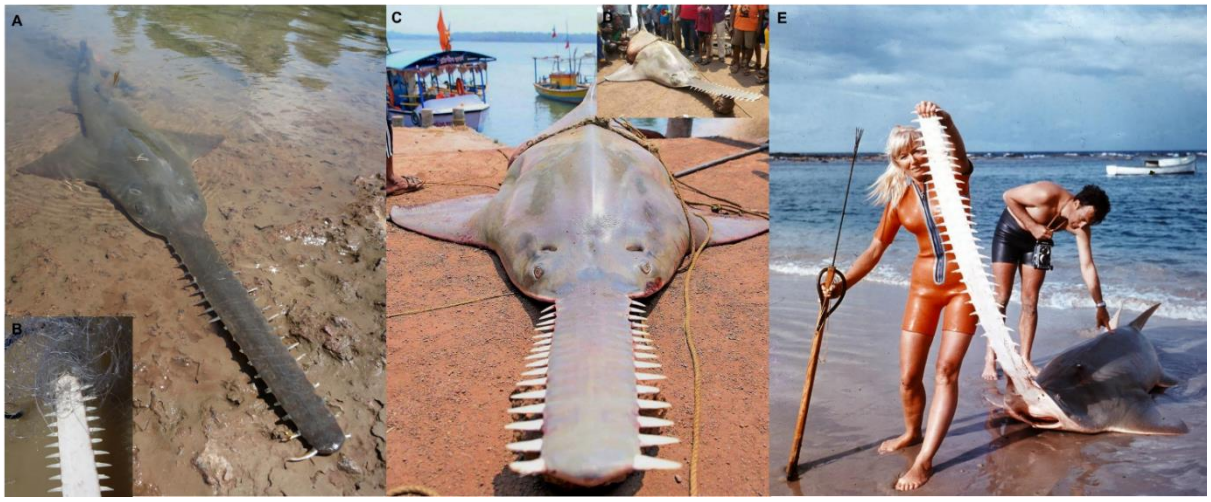

Figure 1.2. A) Juvenile largetooth sawfish (*Pristis pristis*) released after being rescued from a drying floodplain waterhole in northern Australia. Photo credit: Brit Finucci. B) Toothed rostra that is entangled in a gillnet. Photo credit: Peter Kyne. C, D) A 700 kg largetooth sawfish landed in the fishing net of a local fisherman in Maharashtra's Sindhudurg region, India, in 2017. E) Australian shark diver and documentarians, Valerie and Ron Taylor, with a green sawfish (*Pristis zijsron*) captured at Minnewater, New South Wales in 1967.

Although most commercial international trade in sawfish products has been prohibited since 2007 under the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), sawfishes remain one of the most valuable traded wildlife and, in some places where they still persist, are exploited in the absence of adequate fishing restrictions (Dulvy et al., 2016; Fernandez-Carvalho et al., 2014). In 2014, the IUCN Shark Specialist Group issued a call to arms for urgent conservation action by outlining global priorities and

actions that were to be addressed and led by sound scientific research (Harrison & Dulvy, 2014).

The primary motivation of research outlined in this thesis is to rapidly increase our knowledge of the global distribution of sawfishes and improve their conservation outlook by harnessing a novel methodology for a global survey. In the thesis introduction, I delve into the current state of knowledge of sawfish conservation, threats, ecology, and distribution, and review the conventional and emerging methodologies used to study threatened species, with a focus on sawfishes. I then introduce a non-invasive genetic tool, that is the marriage of environmental DNA (eDNA) sampling methodologies with well-established genetic technologies, which has revolutionised the way we study living organisms (Deiner et al., 2017; Huerlimann et al., 2020). Finally, I introduce the key aims of this thesis and summarise the objective of each thesis chapter.

1.2.1. Conservation status of sawfish

The IUCN Red List classifies three species as Critically Endangered (largetooth sawfish *Pristis pristis*, smalltooth sawfish *P. pectinata*, and green sawfish *P. zijsron*) and two species as Endangered (dwarf sawfish *P. clavata* and narrow sawfish *Anoxypristis cuspidata*) (Figure 1.3) (Carlson et al., 2013; D'Anastasi et al., 2013; Kyne, Carlson, et al., 2013; Kyne, Rigby, et al., 2013; Simpfendorfer, 2013). Despite the re-assessment of *A. cuspidata* and *P. clavata* as Endangered from Critically Endangered, due to the availability of new data, populations of these species are still in decline and at high risk of extinction (Dulvy et al., 2016). Given their imperilled state, and owing to the necessity for scientific data collection because of the uncertainty in their current distribution, there has been a surge in research and collaborative conservation efforts, particularly in the United States (e.g., Fearing et al., 2018; Graham et al.,

2021; Lehman et al., 2020; McDonnell et al., 2020; Papastamatiou et al., 2020; Smith et al., 2021; Yan et al., 2021), Australia (e.g., Green et al., 2018; Morgan et al., 2011, 2015, 2021; Whitty et al., 2009; Wueringer, 2017), and, more recently, Central America (e.g., López-Angarita et al., 2021; Valerio-Vargas & Espinoza, 2019), South America (e.g., Cabanillas-Torpoco et al., 2020; Cuevas et al., 2019; López-Angarita et al., 2021; Manir Feitosa et al., 2017), Papua New Guinea (e.g., Grant et al., 2021; Leeney et al., 2018; White et al., 2017), Bangladesh (e.g., Haque et al., 2020; Haque & Das, 2019; Hossain et al., 2015), and East and West Africa (e.g., Braulik et al., 2020; Downing & Leeney, 2019; Everett et al., 2015; Leeney & Downing, 2016; Leeney & Poncelet, 2015).

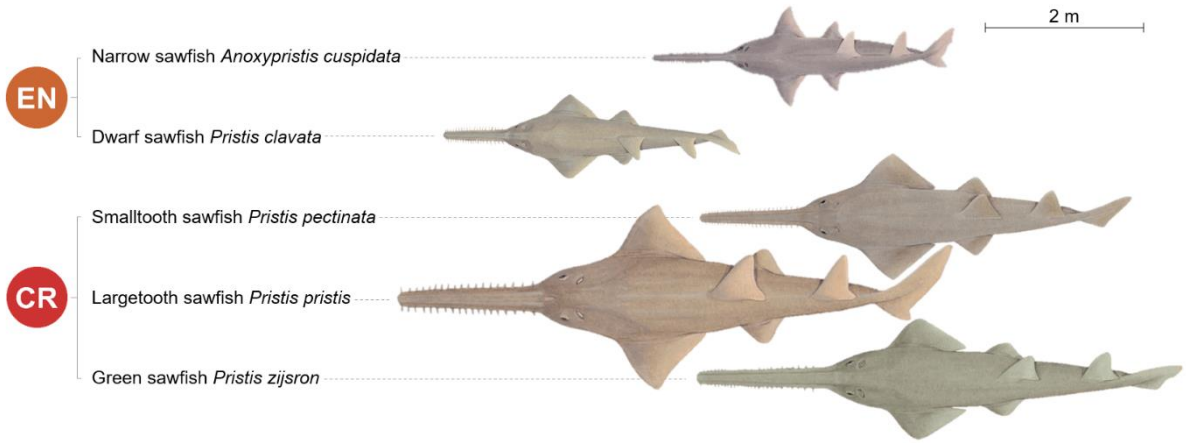

Figure 1.3. Depiction of the five sawfish species along with their IUCN Red List of Threatened Species Status. CR indicates Critically Endangered; EN indicates Endangered. The sawfish illustrations are from Last et al., (2016). Scale bar is approximate and illustrates the maximum total length of adult sawfish of each species.

Despite a ground swell in research effort, current global population status and spatial extent of sawfishes is unclear making conservation efforts challenging in regions where it is needed most (Dulvy et al., 2016; Fernandez-Carvalho et al., 2014). Historically, sawfishes were widely distributed throughout tropical and subtropical coastal regions of the Atlantic, Pacific, and Indian Oceans, recorded in at least 90 countries, but their biological disadvantage that is a low intrinsic rate of population increase following significant declines has diminished the

geographical range in which they are currently found (Figure 1.4; Dulvy et al., 2016; Harrison and Dulvy, 2014; Stevens et al., 2000). It is proposed that sawfishes are now completely absent from 20 nations where they were previously known, and another 43 nations have lost at least one species (Dulvy et al., 2016). Put differently, sawfish are estimated to occur in only 34 nations, but only reliably so in 24 (Dulvy et al., 2016; Harrison & Dulvy, 2014). Overall, in more than 25% of the sawfish geographical range contemporary presence is undefined and in many of these nations the relative extinction risk is high (Figure 1.4; Dulvy et al., 2016; Yan et al., 2021).

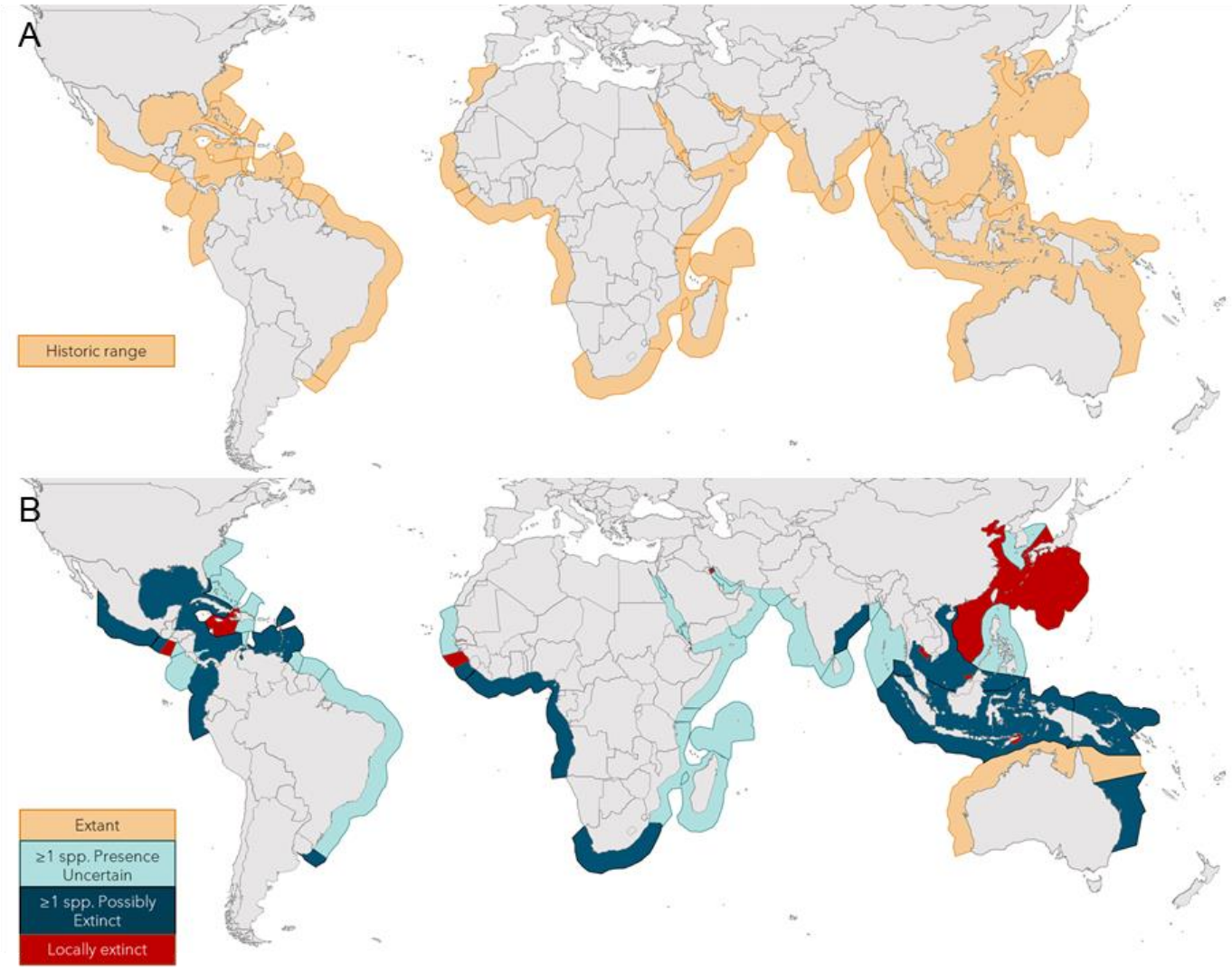

Figure 1.4. Sawfish (family Pristidae) global distribution: A) historical and B) contemporary. Figures data adapted from data presented in Dulvy et al. (2016) & Yan et al. (2021).

As with their contemporary occurrence, national wildlife protection legislation for sawfishes vary considerably across ocean regions (Table 1.1; Downing and Leeney, 2019; Harrison and Dulvy, 2014; Fernandez-Carvalho et al., 2014). Florida, U.S. and northern Australia are considered to host the last remaining viable populations of sawfishes in the world (Dulvy et al., 2016; Morgan et al., 2011). In these two regions, sawfish are strictly protected by legislative measures. Australia alone is home to four of five sawfish species (excluding *P.*

pectinata) and accounts for half of the legislative protection for the extant geographical range of sawfishes in the world (D'Anastasi, 2010; Dulvy et al., 2016). The U.S. provides the only legal protection for extant *P. pectinata* in the world. Protective measures are in place in 16 other countries, in various capacities and for some species only, accounting for 31% of the remaining extant range (Table 1.1; Dulvy et al., 2016). Sawfishes are also listed on Appendix I and II of the Convention on the Conservation of Migratory Species of Wild Animals (CMS), stipulating basic obligations for improving the conservation status of species, but not all nations are parties to the Convention. Existing protective measures are, however, not enough to ensure recovery of sawfish populations given the current state of uncertainty in large parts of their range. Nevertheless, there is momentum building for research capacity and resources in Central and South America, Asia, and Africa, which cover large geographical ranges and where limited scientific understanding of sawfishes existed (Braulik et al., 2020; Haque et al., 2020; Haque & Das, 2019; Juliana López-Angarita et al., 2021; Manir Feitosa et al., 2017; Moore, 2015).

Table 1.1. All five species of sawfish current geographic range status (n = 90 historical range countries and territories; Dulvy et al., 2016). Bold font indicates priority countries for initiating or continuing specialized surveys to determine sawfish status, given their high conservation potential, as outlined by Yan et al. (2021). Asterisks indicates countries with existing sawfish-specific protections. Superscript numbers indicate high priority countries with respect to national protective regulations and whether they don't exist[1]; don't cover all relevant species in a specific manner[2]; or they are not sufficiently enforced[3].

Species name (valid name as at 2013; Faria *et al.*, 2013)	**Common name (Synonyms)**	**Broad Distribution**	**Country and Territory Status**		
			Extant	**Presence Uncertain**	**Possibly Extinct**
Anoxypristis cuspidata	Narrow sawfish (knifetooth sawfish)	Indian Ocean, Western Pacific Ocean	Australia*[,2], Bangladesh*, India*[,3], Indonesia*, Iran[1], Malaysia*, Myanmar[1], Papua New Guinea[1], **Sri Lanka** *n* = 9	Cambodia[1], China, Japan, Republic of Korea, Oman, Pakistan[1], Singapore, Somalia, Taiwan, Thailand, Timor-Leste, Yemen *n* = 12	Vietnam *n* = 1
Pristis clavata	Dwarf sawfish (Queensland sawfish)	Indian Ocean, South-Western Pacific Ocean	Australia*[,2] *n* = 1		India*[,3], Indonesia*[,2], Malaysia*[,2], Papua New Guinea[1] *n* = 4
Pristis pectinata	Smalltooth sawfish	Atlantic Ocean, South-West Indian Ocean	Bahamas[2], Belize, **Cuba[1]**, Honduras[2], Sierra Leone[1], United States* *n* = 6	**Brazil***[,3], **Colombia[1]**, Costa Rica, Dominican Republic, French Guiana[1], Guinea*, Guinea Bissau[1], Guyana, Haiti, Mauritania[3], **Mexico***, Nicaragua*[,2], **Panama[1]**, Suriname[1], Venezuela[1] *n* = 15	Angola, Antigua & Barbados, Benin, Cameroon, Cayman Islands, Congo, Democratic Republic of Congo, Cote d'Ivoire, Dominica, Equatorial Guinea, Gabon, Gambia[1], Ghana, Grenada, Jamaica, Liberia, Montserrat, Nigeria, Senegal*[,3], St. Lucia, St Vincent & Grenadines, Togo, Trinidad & Tobago, Turks & Caicos, Uruguay *n* = 25
Pristis pristis (previous names: *Pristis microdon; Pristis perotteti*)	Largetooth sawfish (freshwater sawfish; Leichhardt's sawfish; common sawfish)	Indian Ocean, Atlantic Ocean, Pacific Ocean	Australia*[,2], Bangladesh*, Belize, **Brazil***[,3], **Colombia[1]**, French Guiana[1], Guinea Bissau[1], Guyana, Honduras[2], India*[,3], Indonesia*[,2], **Madagascar[1]**, Mozambique[1], Nicaragua*[,2], Pakistan[1], **Panama[1]** (E. Pacific), Papua New Guinea[1], Sierra Leone[1], Somalia, Suriname[1] *n* = 20	Antigua & Barbados, Bahamas[2], Brunei, Cayman Islands, Chine, Costa Rica, Cuba[1], Dominican Republic, Granada, Haiti, Jamaica, Kenya[2], Mauritania[3], Montserrat, Myanmar[1], **Panama[1]** (Atlantic), Philippines, **Sri Lanka**, St. Lucia, St. Vincent & Grenadines, **Tanzania[1]**, Trinidad & Tobago, Turks & Caicos, Vietnam, Yemen *n* = 25	Angola, Benin, Cambodia[1], Cameroon, Congo, Democratic Republic of Congo, Cote d'Ivoire, Ecuador[1], Equatorial Guinea, Gabon, Gambia, Ghana, Guatemala, Guinea*, Laos, Liberia, Malaysia*, **Mexico***, Nigeria, Peru, Senegal*[,3], Singapore, South Africa*, Thailand, Togo, United States*, Uruguay, Venezuela[1] *n* = 28
Pristis zijsron	Green sawfish (narrowsnout sawfish)	Indian Ocean, South-Western Pacific Ocean	Australia*[,2], Bahrain*[,3], Eritrea, Indonesia*[,2], Kenya[2], Malaysia*[,2], Papua New Guinea[1], Qatar*[,3], Sudan, Timor-Leste, United Arab Emirates*[,3] *n* = 11	Bangladesh*, Brunei, Cambodia[1], China, Djibouti, Egypt, India*[,3], Iran[1], Iraq, Kuwait, **Madagascar[1]**, Mozambique[1], Myanmar, Oman, Pakistan[1], Philippines, Saudi Arabia, Singapore, Somalia, Sri Lanka, Taiwan, Tanzania[1], Vietnam, Yemen *n* = 24	South Africa*, Thailand *n* = 2

1.2.2. Threats to sawfishes

Significant declines and local extinction of sawfish across their range has been hastened by the expansion and intensification of fishing effort in the past century, widespread availability and use of outboard motors and monofilament nets, and increased exposure of sawfish to fishing activity in shallow, coastal waters (Field et al., 2013; Harrison & Dulvy, 2014; Peverell, 2005; Thorson, 1982a). Sawfish were once a target species of subsistence fisheries throughout Central America, Caribbean, Red Sea, and Persian Gulf (Harrison & Dulvy, 2014; Thorson, 1982b). However, in the past half century the greatest demise of sawfish has been due to incidental bycatch in commercial and recreational fisheries; this threat continues to place pressure on populations (Peverell, 2005; Field et al., 2013). If handled correctly, post-capture mortality of sawfishes can be avoided (Prohaska et al., 2018); however, animals are often killed to avoid damage to the fishing gear or causing injury to the fisher or retained because of the high-value of their shark-like fins, rostrum, and meat (Harrison & Dulvy, 2014). The threat of capture in nets is greatest in developing countries where dense coastal populations rely heavily on subsistence fishing for food and income, but the impact is largely unmeasured as these fisheries are absent from management or monitoring (Davidson et al., 2016; Grant et al., 2021).

Research undertaken in their global safe havens, Australia and the US, indicate that mangrove-lined creeks, rivers, estuaries, seagrass beds, and mudflat ecosystems represent significant habitat for sawfishes, including pupping grounds and nursery areas for their young (Figure 1.5; Carlson et al., 2014; Lear at al., 2019; Morgan et al., 2011, 2015, 2021; Papastamatiou et al., 2015; Peverell, 2005; Poulakis et al., 2013; Simpfendorfer et al., 2011). A reliance on a restricted range of coastal habitats puts sawfish at higher risk to coastal development, habitat degradation, and pollution (Knip et al., 2010; Speed et al., 2010). Coastal development has indirect impacts on sawfishes through its influence on prey availability and

habitat connectivity. For example, *P. pristis* migrate through to the freshwater extent of river systems in northern Australia; therefore, creation of dams or extraction of freshwater for development and industry has the potential to impact connectivity between critical habitats (Whitty et al., 2009). The direct impact of development is habitat loss (e.g., the destruction of estuaries and mangrove forests used as nursery and refuge habitats) (Morgan et al., 2015; Polidoro et al., 2010; Wiley & Simpfendorfer, 2007). Juvenile *P. pectinata*, *P. clavata*, and *P. zijsron* have a demonstrated affinity to mangrove-lined intertidal and estuarine zones for the first 1–3 years of their life, rendering these animals inherently susceptible to loss of this habitat (Morgan et al., 2015, 2021; Wiley & Simpfendorfer, 2007). Globally, there has been a significant reduction in mangrove and seagrass coverage and substantial reduction in biodiversity of every river basin on Earth (Albert et al., 2021; Bunting et al., 2022; Lotze et al., 2006; Polidoro et al., 2010; Short et al., 2011). Mangrove declines of up to 35% have been documented globally in the last three decades, particularly in regions of sawfish historical occurrence, and relative loss is highest in Southeast Asia, the Caribbean, and Central America (Polidoro et al., 2010). Primary drivers of mangrove habitat destruction and removal are coastal development, agriculture, aquaculture, and overexploitation of fisheries, which exacerbate the problem of sawfish habitat loss (Murray et al., 2022; Polidoro et al., 2010).

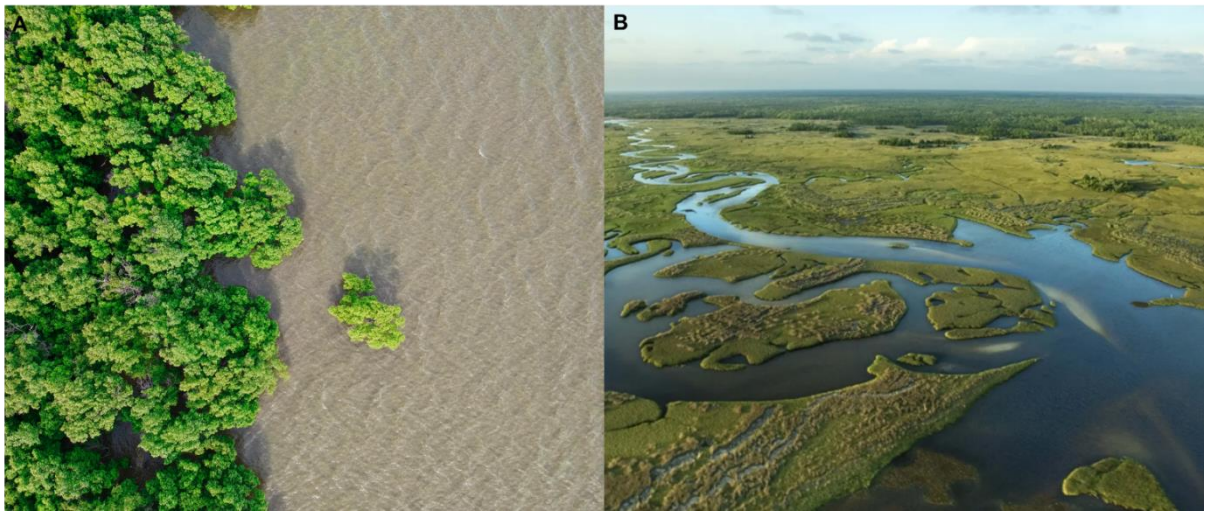

Figure 1.5. Aerial photos of mangrove-lined rivers, which are significant habitat for sawfishes. A) Thailand (photo by Waranont (Joe) on Unspalsh), B) Everglades National Park (photo by Jupiter Images on Getty Images).

1.2.3. Cultural importance

Sawfish and their saws are enmeshed in the cultural heritage of many indigenous communities (Harrison & Dulvy, 2014; Leeney & Poncelet, 2015; McDavitt, 1996; Robillard & Séret, 2006). They are often symbolically depicted in cultural drawings, used in traditional medicine, have mythological meaning, and saws are kept as relics or sold in markets (a phenomenon also seen in western society) (Harrison & Dulvy, 2014; McDavitt, 1996). The cultural value and historical significance of sawfishes in different communities provides important information including their historical range, behaviour, biology, and extent of exploitation (Robillard & Séret, 2006). For example, sawfish art, rostra collections, and mythology in Papua New Guinean culture provide historical accounts of abundance and range in the region (McDavitt, 1996). Sawfish are associated with authority and a symbol of morality in West African culture; on the Atlantic Coast of Panama sawfish are “friends of mankind”; and in Southeast Asia sawfish are embedded in traditional accounts of the spread of religion (Harrison & Dulvy, 2014; McDavitt, 1996). It is thus important to recognize the religious and

cultural merit of threatened species when implementing conservation strategies. Likewise, traditional ecological knowledge should be acknowledged for its value in mapping trends in occurrence and abundance (Leeney & Poncelet, 2015; Robillard & Séret, 2006).

1.3. Sawfish spatial ecology and distribution

Knowledge of the global distribution and habitat use of sawfishes over time has been consolidated from various survey methods (e.g., active and passive acoustic tracking, remote satellite tracking, traditional ecological knowledge surveys, fisher interviews, and fisheries-dependent population surveys) (Braulik et al., 2020; Carlson et al., 2014; Dulvy et al., 2016; Leeney, 2016; Leeney & Downing, 2016; Poulakis et al., 2013) and by harnessing historical records and public encounter databases (Fearing et al., 2018; Fernandez-Carvalho et al., 2014; Manir Feitosa et al., 2017; Wiley & Simpfendorfer, 2010). Data gleaned from historical records, traditional ecological knowledge surveys of fishers, and public encounter databases tell the story of declining capture rates and sightings across the geographical distribution. Where these data are lacking, technological advances have greatly improved the global research capacity, for example, the advent and success of electronic tracking technology (i.e., acoustic and satellite transmitters) for marine animals in the past few decades has enhanced our understanding of animal movements and habitat utilisation (Hussey et al., 2015; Iverson et al., 2019). This has enabled us to learn about the fine-scale spatial ecology of sawfishes that is important for designing surveys and designating areas for conservation or management. Sawfish movement and habitat use studies utilising tracking devices indicate that, in general, the home range and depth profile of sawfishes increases with body size (Carlson et al., 2014; Morgan et al., 2015; Poulakis et al., 2013; Simpfendorfer et al., 2010; Whitty et al., 2009). This is a common biological trait of many coastal shark and ray species as a function of predator avoidance, shifting diet preferences, and environmental tolerances (Knip et al., 2010; Speed et

al., 2010). Juvenile sawfish are likely to occupy shallow riverine and estuarine nursery sites within the first year of life (Morgan et al., 2015; Poulakis et al., 2013; Simpfendorfer et al., 2010; Whitty et al., 2009). Juvenile and natal philopatry is common in many coastal shark species where adult and juvenile populations are spatially segregated and ontogenetic shifts in patterns of movement and habitat use are observed (Chapman et al., 2015; Flowers et al., 2016). In general, adult sawfish utilise both marine and estuarine waters, often associated with mangrove or seagrass habitats within a depth range of 1−100 m, but most frequently less than 10 m depth (Carlson et al., 2014; Guttridge et al., 2015; Wiley & Simpfendorfer, 2010). Studies of *P. pectinata* and *P. pristis* suggest that adults sexually segregate seasonally with male-biased dispersal (Papastamatiou et al., 2015; Phillips et al., 2017; Simpfendorfer et al., 2010), whereas *P. clavata*, *P. zijsron*, and *A. cuspidata* adults exhibit non-sex-biased dispersal (D'Anastasi, 2010; Morgan et al., 2015; Phillips et al., 2017). Adult sawfish that travel over greater distances and traverse marine waters, seemingly occupy high salinity waters more readily than smaller juvenile sawfish. It is unclear whether this is due to a higher salinity tolerance of adult sawfish, or if the occupation of less saline habitats by juveniles in shallow riverine and estuarine habitats confers protection and increased survival. Distribution and habitat use trends are describe in more detail for each species in the following sections.

These data also highlight the limitations in assessing the status of sawfishes. The logistical constraints in travelling to remote locations and in capturing or sighting sawfish concealed within turbid environments is supported by the inefficiency of visual observation and fishing surveys for sawfish (Manir Feitosa et al., 2017; Valerio-Vargas & Espinoza, 2019). Moreover, fishing surveys are costly and require considerable time spent in the field, which is not always practicable in remote, tropical environments that are subject to high, humid temperatures and monsoonal wet seasons. Data retrieved from public encounter databases and ecological knowledge surveys of fishers have provided information on changes in geographical

distribution over time, however, they cannot always be independently used as reliable indicators of presence and distribution trends. These approaches rely on considerable outreach, the48bilityy of the public and fishers to answer truthfully and correctly identify species, and the willingness of people to report encounters and/or respond to surveys, which carries its own challenges and limitations. Recreational and commercial fishers are less likely to report encounters with a protected species. Moreover, the likelihood of retrieving contemporary distribution data in traditional ecological knowledge surveys is unlikely given the rarity of sawfishes in most locations (Leeney & Poncelet, 2015).

1.3.1. Narrow sawfish (*Anoxypristis cuspidata*) *Anoxypristis cuspidata* is the only extant species in the genus. The species has the second largest historical extent of occurrence and is distributed throughout the Indo-West Pacific, from the Red Sea to Australia, and north to Japan (Dulvy et al., 2016; Last et al., 2016). Despite having undergone 30% decline in geographic range and possible local extinction in Vietnam, *A. cuspidata* remains a frequently recorded species in the rest of the Indo-West Pacific, presumably due to its relatively high intrinsic rate of population increase compared to other sawfishes and therefore decreased susceptibility to population disturbances (D'Anastasi, 2010; Dulvy et al., 2016). However, their low genetic diversity compared to other sawfishes is likely to have an impact on resilience of the species to increasing levels of threat (Green et al., 2018).

Ontogenetic changes in habitat use are documented for *A. cuspidata* in a large tropical embayment in northern Queensland, Australia (Adkins et al., 2016). Juveniles frequented the intertidal and subtidal mudflats at depths of 10 m or less (Adkins et al., 2016). Adults are found in inshore regions to depths of 40 m, and less frequently in estuaries (Last & Stevens, 1994; Peverell, 2005). The species is also an abundant bycatch species in northern Australia inshore

and offshore fisheries, with a seemingly widespread distribution compared to all other sawfish species (Field et al., 2013; Peverell, 2005). Peverell (2005) also states that large mature animals are caught predominantly offshore in contrast to high capture rates of juveniles inshore.

1.3.2. Dwarf sawfish (*Pristis clavata*)

Pristis clavata is endemic to the Indo-West Pacific; historically found in the fewest of countries, albeit with the largest coastal zones, including Australia, Papua New Guinea, Indonesia, and India (Dulvy et al., 2016; Last et al., 2016). The species is currently only reliably encountered in northern Australia having undergone a 70% reduction and possible extinction from former range states (Dulvy et al., 2016). *Pristis clavata* occur in shallow coastal waters (0.5−10 m) on sand and mudflats adjacent to mangroves, occasionally penetrating marine reaches of tidal creeks and rivers (Kyne et al., 2013; Last & Stevens, 1994; Morgan et al., 2021; Thorburn et al., 2008).

Findings from fisheries assessments in northern Australia suggest that the abundance of *P. clavata* is low and highly variable and that their distribution is patchy, with catches limited to the shoreline (Field et al., 2013; Peverell, 2005). Habitat utilisation studies of tagged *P. clavata* in northern Western Australia describes movements influenced by large tides (Morgan et a., 2021; Stevens et al., 2008). Individuals move towards inundated mangroves on high tide returning to within 100 m of the previous high tide resting point and away from the shore on the falling tide, remaining in depths < 1.5 m. Movements with the tidal current were generally across coastal mudflats and sand, corresponding with the understanding that *P. clavata* home ranges are restricted to marine water (Thorburn et al., 2008). Habitat and environmental parameters, as described by Thorburn et al., (2008), include silt and sand flats with generally low water clarity, and water temperatures between 25 and 32°C.

1.3.3. Smalltooth sawfish (*Pristis pectinata*)

Historically, *P. pectinata* occurred throughout tropical and subtropical coastal regions of the eastern and western Atlantic Ocean. The most recent assessment by Dulvy et al., (2016) indicates that the highest risk to *P. pectinata* stems from an extreme range contraction (81% decline). Remaining populations are small and fragmented, only reliably found along the coast of southern Florida, U.S and in the Bahamas (Carlson et al., 2014; Graham et al., 2021; Simpfendorfer et al., 2010). Encounters elsewhere are rare, but there are reports in Honduras, Belize, Cuba, Sierra Leone, Guinea-Bissau, and Mauritania (Fernandez-Carvalho et al., 2014).

Presently, *P. pectinata* occurrence is most dense in southern Florida, from the Caloosahatchee River, through Ten Thousand Islands and Everglades National Park, to Florida Bay (Graham et al., 2021). These areas are predominantly shallow mud banks, seagrass beds, and extensive mangrove habitats, with drainage from adjacent estuaries and other sources of freshwater flow (Papastamatiou et al., 2015; Simpfendorfer et al., 2011). Population viability models predict that the *P. pectinata* population can recover in the southern Florida region with continuation of appropriate management and strict legal protection (Carlson & Simpfendorfer, 2015).

The spatial ecology of *P. pectinata* in Florida and the Bahamas has been studied using telemetry and encounter records (Carlson et al., 2014; Graham et al., 2021; Guttridge et al., 2015; Simpfendorfer et al., 2011). Juvenile *P. pectinata* have high site fidelity to mangrove-lined shores and adjacent seagrass habitats, most frequently encountered in March to April in water less than 1 m (Poulakis et al., 2013; Simpfendorfer et al., 2011). This concurs with reports from the Bahamas, where more than two-thirds of *P. pectinata* encounters occur from March to June in shallow water within or adjacent to mangrove-lined creeks (Guttridge et al., 2015).

Acoustic telemetry data indicates that juveniles are more frequently found in specific locations within shallow estuarine nursery habitats, sometimes called 'hotspots', and these localised areas are consistently important for at least the first three years of life (Hollensead et al., 2016; Poulakis et al., 2011, 2013; Simpfendorfer et al., 2010; Simpfendorfer et al., 2011).

Rate of movement and home range size tends to increase with size, for example, neonates exhibit small activity spaces within the 'hotspots', and despite challenges to osmoregulation with fine-scale salinity changes, they will remain in protected habitats presumably to avoid predation (Simpfendorfer et al., 2011). The data indicate that juveniles selectively use depths < 50 cm and salinities between 18 and 24 ppt (Simpfendorfer et al., 2011). To remain in shallow estuarine water, juveniles will utilise diel or tidal driven movements, presumably in order to minimise predation risk (Carlson et al., 2014; Poulakis et al., 2013; Simpfendorfer et al., 2011). Once they've attained a larger size (presumably ≥ 150 cm), juveniles appear to move to mangrove habitats on the marine fringe at depths of 0.5–1 m, possibly pre-emptive of movement further into marine open water (Simpfendorfer et al., 2010). Adults inhabit marine open-water habitats where they are less likely to be seen (Carlson et al., 2014; Waters et al., 2014). Adults are more mobile than juveniles, frequently at depths < 10 m and occasionally at depths of 30−100 m and show seasonal residency to localized areas of Southern Florida (Carlson et al., 2014; Papastamatiou et al., 2015).

Acoustic and satellite tagging of adult and sub-adult *P. pectinata* in Florida Bay reveal seasonal residency and sexual segregation of males and females (Papastamatiou et al., 2015). The nature of seasonal residency is unclear. Adult *P. pectinata* are prevalent during spring before commencing northward summer movements (moving more than 200 km in 28−65 days), predominantly remaining in < 10 m warmer water zones and occasionally performing dives to 100 m (Carlson et al., 2014; Papastamatiou et al., 2015). *Pristis pectinata* are believed

to reproduce biennially, and hence partial migration of a population might occur based on sex, breeding status, and body condition (Papastamatiou et al., 2015).

Fine-scale patterns of movement during periods of residency appear to be related to tidal stage. During flood tides adults remain in shallow (< 2 m), warmer water over seagrass beds or in mangrove-lined channels, and then move to cooler, deeper (> 2 m) water during the slack tide (Papastamatiou et al., 2015). There is no evidence of diel movement patterns. It is suggested that adult *P. pectinata* preferentially occur in waters < 30°C and salinities < 36 ppt, but occasionally they are found in water of 30−34°C (Carlson et al., 2014; Guttridge et al., 2015; Papastamatiou et al., 2015).

1.3.4. Largetooth sawfish (*Pristis pristis*)

Pristis pristis has the largest historical distribution range extending across the eastern and western Atlantic, eastern Pacific, and the Indo-West Pacific (Dulvy et al., 2016; Last et al., 2016). Their contemporary range is estimated to have declined by 61% (Dulvy et al., 2016). *Pristis pristis* are the only sawfish species known to occur in freshwater lakes and floodplain waterholes (Thorson, 1982b; Whitty et al., 2009). At present, they are known to occur in tropical river systems in Africa, India, Southeast Asia, Papua New Guinea, Central and South America, and northern Australia (Last et al., 2016). Encounters have been most frequent in northern Australia and the Lake Nicaragua-Rio San Juan system in Central America, from which originate the majority of information on biology and ecology of *P. pristis* (most literature falls under the former synonyms *P. microdon* and *P. perotteti*) (Lear et al., 2019; Thorburn et al., 2007; Thorson, 1974, 1982b; Whitty et al., 2009, 2017).

The Atlantic/Eastern Pacific Ocean subpopulation of *P. pristis* has a latitudinal distribution in tropical and subtropical shallow, coastal estuarine and freshwater waters of

Central and Southern America and West Africa (Kyne et al., 2013). A recent evaluation of historical records of *P. pristis* in the Atlantic Ocean suggests that the Amazon estuary has the highest remaining abundance, followed by the Colorado-San Juan River system in Nicaragua and Costa Rica, and the Bissagos Archipelago in Guinea Bissau (Fernandez-Carvalho et al., 2014). Conventional tag and recapture studies, including one actively tracked individual using a hydrogen-filled balloon (Figure 1.6), were undertaken by Thorson (1982a), documenting the first known movements of any sawfishes. Recapture rates (56.8%) and locations for 377 tagged *P. pristis* demonstrated residency of individuals, with movements upstream, downstream, and the whole length of the San Juan River system at depths of up to 26 m between 1968 and 1974. Thorson's work first outlined the importance of freshwater habitats for *P. pristis* following observations of sawfishes spending most of their life history, including parturition, in the Lake Nicaragua-Rio San Juan system. To obtain more detailed information on movement patterns, acoustic and satellite tracking methods should be employed to further understand the patterns of habitat use of the Atlantic/Eastern Pacific Ocean subpopulation.

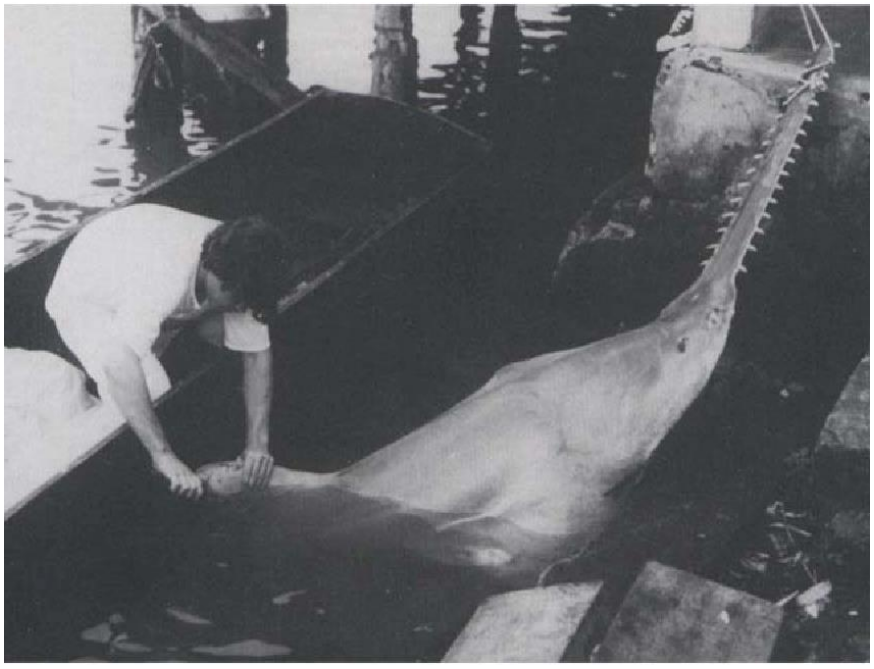

Figure 1.6. Pioneer sawfish researcher, Dr. Thomas B. Thorson, conducted some of the seminal conventional tag and recapture studies on largetooth sawfish *Pristis pristis* in the Lake Nicaragua – San Juan River system, Costa Rica in the late 70s and early 80s. One of the first active tracks of a largetooth was completed by attaching a hydrogen-filled red balloon to a roto-tag.

The Indo-Pacific Ocean subpopulation is comprised of a higher portion of individuals in Australia and a smaller portion of encounters in Papua New Guinea, Indonesia, Malaysia, Cambodia, Vietnam, and the Philippines (Last et al., 2016; White et al., 2017). Northern Australia is arguably one of the most important habitats for *P. pristis*, considered as one of the last viable population strongholds (Morgan et al., 2011; Thorburn et al., 2007; Whitty et al., 2009, 2017). Peak occurrence of *P. pristis* in northern Australian estuaries, creeks, river systems, as indicated by inshore commercial fisheries that target river mouths, corresponds with the monsoonal wet season (Peverell, 2005). This finding is supported by observations that suggest freshwater flows, flooding, and lowering salinity levels at rivers mouths associated with monsoonal weather patterns may be the environmental cue responsible for triggering pupping (Peverell, 2005; Thorburn et al., 2007). Mature *P. pristis* are reported to inhabit marine waters during the post wet season and enter freshwater during the wet season to pup. Phillips et al., (2017) suggests that the reliance on rivers as pupping sites and nursery habitats and hence strong localised natal philopatry for this species is a driver for male-based dispersal, which is not observed in other Australian sawfish populations (i.e., *P. clavata* and *P. zijsron*).

Higher water levels associated with longer duration of the wet season are correlated with strength of recruitment and survivability, as freshwater pools are more accessible to pups providing them with protective habitats (Lear et al., 2019, 2021; Whitty et al., 2009). During the late wet season, pups commence upstream migrations to freshwater pools at depths < 0.5 m, generally using tidal currents to control movements (Thorburn et al., 2007; Whitty et al., 2009). Ontogenetic depth partitioning is observed among pups and larger juveniles (> 1 m), the

latter moving in to deeper waters (> 0.5 m on average), which is a similar behaviour observed for *P. pectinata* (Simpfendorfer et al., 2010; Whitty et al., 2009). Movement patterns of larger juveniles are influenced by lunar phase and light intensity; remaining in very shallow water (< 0.2 m) in the full moon and moving to depths of 1.5 m during the new or half-moon, which is likely linked to predator avoidance. Large predators in tropical freshwater systems include the bull shark *Carcharhinus leucas*, saltwater crocodile *Crocodylus porosus*, and freshwater crocodile *Crocodylus johnstoni*.

1.3.5. Green sawfish (*Pritis zijsron*)

Pristis zijsron occurs in tropical to temperate coastal and offshore habitats of the Indo-Pacific Ocean (Dulvy et al., 2016; Last et al., 2016). Substantial declines of up to 38% are reported across the historic range for *P. zijsron*, with possible localized extinctions in South Africa and Thailand (Dulvy et al., 2016; Everett et al., 2015). National regulation and protective measures seemingly safeguard remaining extant populations of *P. zijsron* in northern Australia, which has some of the last remaining viable populations (Morgan et al., 2015). Regardless, it remains as the most uncommon sawfish recorded in surveys and catch data and there is a distinct lack of data on their biology and global population (Field et al., 2013; Thorburn et al., 2003).

It has been suggested that the southern Pilbara region of Western Australia is one of the most important remaining pupping sites for *P. zijsron* in the world (Morgan et al., 2015, 2017). The estuary and adjacent tidal mangrove creeks of the Ashburton River provide critical nursery habitat. Pupping is presumed to occur in October (early wet season) due to higher encounter rates of pups (Morgan et al., 2015). After an unknown period in the estuarine waters, it is presumed that larger *P. zijsron* move into marine inshore and offshore waters, where they are

infrequently caught in trawl fisheries across northern Australia from <1 to >70 m depth (Field et al., 2013; Peverell, 2005; Stevens et al., 2008). Peverell (2005) reports the occurrence of *P. zijsron* on coastal mud flats and bays in the Gulf of Carpentaria, Northern Australia. One short-term acoustic tracking study documented diurnal movements in a narrow activity space parallel to the shoreline (Peverell & Pillans, 2004). The individual remained in deeper (0.84 m) water during the day and shallow (0.48 m) water at night, highlighting the importance of shallow habitats. In contrast, a single active tracking report from Stevens et al. (2008) documented tidal-influenced movements of a 260 cm individual in a macrotidal mangrove-lined environment, where all movements were generally restricted to depths less than 1.5 m. The limited data on *P. zijsron* preclude any inferences about fine-scale habitat usage and movement patterns of the species and, as such, there is an urgent need for more data in all key life-history stages.

1.4. Environmental DNA and its application to detection of sharks and rays

Conservation management actions are only as good as the data on which they are based. In order to provide the most comprehensive and accurate data possible on past, present, and future changes in species distribution it is necessary to expand and improve technological and methodological approaches. Genetic material that is captured and isolated from environmental samples, termed environmental DNA, or eDNA (detailed in Chapter 2), emerged in the early 2000's as a complementary detection tool with considerable monitoring and conservation appeal (reviewed by Taberlet et al., 2012; Barnes and Turner, 2016). It was dubbed as a 'game changer' for biodiversity sampling (Lawson Handley, 2015) because of the relative ease and non-invasiveness of eDNA sample collection and the subsequent interest as a tool for detecting low density, rare, cryptic, and endangered species (Dejean et al., 2012; Foote et al., 2012; Lehman et al., 2020; Ma et al., 2016; Sepulveda et al., 2019; Weltz et al., 2017; Wilcox et al., 2016). Beneficially, permits for eDNA sample collection are easier to obtain, or sampling may

not require permits because of the non-invasive nature of the sampling, which may otherwise limit studies undertaken on legislatively protected species (e.g., animal ethics approvals to capture animals, human ethics approvals to conduct social surveys, and research permits for sampling in specific regions such as marine parks).

The application of eDNA techniques to sharks and rays is still in its infancy relative to teleost fishes (reviewed by Le Port *et al.*, 2018). The first study, by Simpfendorfer et al. (2016), successfully detected *P. pristis* in freshwater habitats in northern Australia in locations of both known (based on gillnet surveys and traditional ecological knowledge from local indigenous ranger groups) and unknown sawfish presence. Since this study, the efficacy of using eDNA as a monitoring tool has further been demonstrated with species-specific eDNA surveys successfully applied to a range of elasmobranchs across different marine habitats (see Table 1.2.2.), including Maugean skate *Zearaja maugeana* (Weltz et al., 2017), Chilean devil ray *Mobula tarapacana* (Gargan et al., 2017), great white shark *Carcharodon carcharias* (Lafferty et al., 2018; van Rooyen et al., 2021), bull shark *Carcharhinus leucas* (Drymon et al., 2021; Schweiss et al., 2020; van Rooyen et al., 2021), smalltooth sawfish *P. pectinata* (Bonfil et al., 2021; Lehman et al., 2020), blacktip sharks *C. limbatus* (Postaire et al., 2020), and tiger sharks *Galeocerdo cuvier* (van Rooyen et al., 2021). For blacktip sharks, eDNA surveys were shown to yield temporal and spatial results equivalent to extensive fishing surveys and acoustic telemetry (Postaire et al., 2020). Van Rooyen et al. (2021) demonstrated that eDNA detection of shark species involved in human-shark interactions on the east Australian coast could be used as a rapid, cost-effective tool to help shark monitoring programs for bather safety. Together, these studies demonstrated that eDNA is a viable tool for detection and monitoring of species in a manner that is either complementary to other techniques (Postaire et al., 2020) or in regions where it may otherwise be difficult to generate contemporary occurrence and distribution data (Bonfil et al., 2021; Gargan et al., 2017; Lehman et al., 2020).

The characterisation of whole shark and ray communities using the eDNA metabarcoding approach, i.e., non-targeted sequencing of multiple species' eDNA using generic markers, has also been demonstrated. eDNA metabarcoding surveys have been conducted on remote coastline (West et al., 2021) highly urbanised coastlines (Ip et al., 2021), coral reefs and sea grass beds (Bakker et al., 2017; Boussarie et al., 2018; Mariani et al., 2021; Stat et al., 2017; West et al., 2020), and on the subarctic continental shelf (Thomsen et al., 2016). Many metabarcoding studies were initially established and validated for sharks and rays in aquaria (Figure 1.7; Miya *et al.*, 2015) or assays were adapted from validated and published DNA metabarcoding techniques used for marine vertebrate diet analysis (Berry et al., 2017; Deagle et al., 2007). The approach was also validated with baited remote underwater video systems (BRUVs) (Boussarie et al., 2018; Stat et al., 2019) with varying success in detection sensitivity to some species.

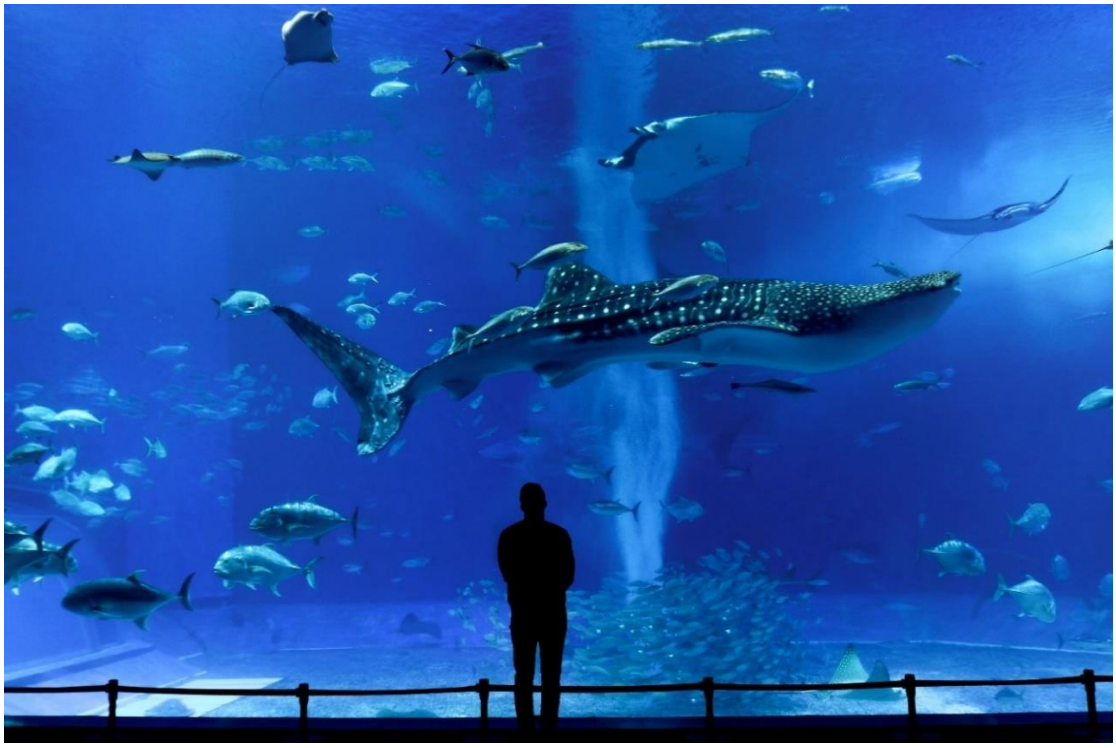

Figure 1.7. One of the first metabarcoding assays for shark and ray eDNA was validated with seawater samples collected from the aquaria pictured above at Okinawa Churaumi Aquarium, Okinawa, Japan. Photo by Susann Schuster on Unsplash.

Table 1.2. Summary of environmental DNA (eDNA) studies as at June 2021 focusing on sharks and rays. Sample collection method includes: number of field replicates, replicate volume, filter pore size and material type, and filtration device, unless precipitation method is stated. Abbreviations: COI, cytochrome c oxidase subunit 1; MCE, mixed cellulose ester; NADH4, core subunit of the mitochondrial membrane respiratory chain nicotinamide adenine dinucleotide dehydrogenase complex I; PES, polyethersulphone; GF, glass fibre; PCR, polymerase chain reaction; ND, not disclosed.

Author	Year	Study title	Species of interest		Geographical location	Ecosystem	Study objective	Sequencing method	Target gene	bp	Sample collection method	Extraction method	Inhibition testing
			Common name	Scientific name									
Miya et al.	2015	MiFish, a set of universal PCR primers for metabarcoding environmental DNA from fishes: detection of more than 230 subtropical marine species	Elasmobranchs and teleosts	-	Okinawa Churaumi Aquarium, Okinawa, Japan	Aquarium	Validation trial	Metabarcoding (Illumina MiSeq amplicon sequencing)	12S	163-185	3× 2 L; 0.7 µM GF filters; vacuum filtration	DNeasy Blood & Tissue Kit (Qiagen)	ND
Simpfendorfer et al.	2016	Environmental DNA detects Critically Endangered largetooth sawfish in the wild	Largetooth sawfish	*Pristis pristis*	Northern Territory, Australia	Riverine	Species-specific presence-only detection	PCR	COI	145	5× 2 L; 20 µM nylon net filters; vacuum pump	ISOLATE II Genomic DNA Kit	ND
Thomsen et al.	2016	Environmental DNA from Seawater Samples Correlate with Trawl Catches of Subarctic, Deepwater Fishes	Elasmobranchs and teleosts	-	Davis Strait, Greenland	Epi/mesopelagic	Biodiversity assessment	Metabarcoding (Illumina MiSeq amplicon sequencing)	12S	100	1× 1.5 L; 0.45 µM nylon filters; vacuum pump	DNeasy Blood & Tissue Kit (Qiagen)	ND
Weltz et al.	2017	Application of environmental DNA to detect an endangered marine skate species in the wild	Maugean skate	*Zearaja maugeana*	Tasmania, Australia	Harbour	Species-specific presence-only detection	qPCR	NADH4	331	2× 10 L; 0.45 µM ND filters; vacuum pump	PowerWater DNA Isolation Kit (QIAGEN; formerly MOBIO)	ND
Gargan et al.	2017	Development of a sensitive detection method to survey pelagic biodiversity using eDNA and quantitative PCR: a case study of devil ray at seamounts	Chilean devil ray	*Mobula tarapacana*	Azores	Seamounts	Species-specific presence-only detection	qPCR	COI	86	1× 3 L; 0.45 µM nylon filters; vacuum pump	DNeasy Blood & Tissue Kit (Qiagen)	ND
Sigsgaard et al.	2017	Population characteristics of a large whale shark aggregation inferred from seawater environmental DNA	Whale shark	*Rhincodon typus*	Qatar, Persian Gulf	Oceanic - pelagic	Population genetics	Metabarcoding (Illumina MiSeq amplicon sequencing)	D-loop 3	412; 476-493	3× 0.5 L; 0.22 µM Sterivex filters; 60 mL syringes	DNeasy Blood & Tissue Kit (Qiagen)	ND

Stat et al.	2017	Ecosystem biomonitoring with eDNA: metabarcoding across the tree of life in a tropical marine environment	Eukaryotes	-	Coral Bay, Western Australia	Inshore; lagoon and bay	Biodiversity assessment	Metabarcoding (Illumina Miseq amplicon sequencing)	*(1) 18S; (2) COI; (3) 16S	(1) 240-420; (2) 304-313; (3) 178-228	1× 1 L; 0.45 µM nylon filters; peristaltic pump	DNeasy Blood & Tissue Kit (Qiagen)	1:10 dilution
Bakker et al.	2017	Environmental DNA reveals tropical shark diversity in contrasting levels of anthropogenic impact	Elasmobranch	-	New Caledonia & Caribbean	Reef	Biodiversity assessment	Metabarcoding (Illumina Miseq amplicon sequencing)	COI	127	1× 4 L; 0.45 µM MCE filters; vacuum pump	PowerWater DNA Isolation Kit (QIAGEN; formerly MOBIO)	ND
Lafferty et al.	2018	Detecting Southern California's White Sharks with Environmental DNA	Great white shark	*Carcharodon carcharias*	California, USA	Inshore	Species-specific	ddPCR	CytB	163	3× 0.5 L; 0.22 µM Sterive filters; 50 mL syringes	DNeasy Blood & Tissue Kit (Qiagen)	
Boussarie et al.	2018	Environmental DNA illuminates the dark diversity of sharks	Elasmobranch	-	New Caledonia	Reef	Biodiversity assessment	Metabarcoding (Illumina Miseq amplicon sequencing)	COI	127	1× 2 L; 0.45 µM MCE filters; vacuum pump	DNeasy PowerSoil Isolation Kit (Qiagen)	ND
Truelove et al.	2019	A rapid environmental DNA method for detecting white sharks in the open ocean	Great white shark	*Carcharodon carcharias*	Pacific Ocean	Oceanic - epi/mesopelagic	Species-specific	Metabarcoding (Oxford Nanopore MinIon and 1D Amplicon sequencing)	12S	170	1× 3 L; 0.2 µM polyvinylidene fluoride filters; ND	PowerWater DNA Isolation Kit (QIAGEN; formerly MOBIO)	ND
Schweiss et al.	2019	Development of highly sensitive environmental DNA methods for the detection of Bull Sharks, *Carcharhinus leucas* (Müller and Henle, 1839), using Droplet Digital™ PCR	Bull shark	*Carcharhinus leucas*	Alabama, USA	Aquarium	Species-specific	ddPCR	ND2	237	6× 1 L; 0.8 µM nylon filters; vacuum pump	DNeasy Blood & Tissue Kit (Qiagen)	ND
Lehman et al.	2019	An environmental DNA tool for monitoring the status of the Critically Endangered Smalltooth Sawfish, *Pristis pectinata*, in the western Atlantic	Smalltooth sawfish	*Pristis pectinata*	Florida, USA	Riverine	Species-specific	ddPCR	ND2	100	3× 1 L; 0.8 µM nylon filters; vacuum pump	DNeasy Blood & Tissue Kit (Qiagen)	ND
Stat et al.	2019	Combined use of eDNA metabarcoding and video surveillance for the assessment of fish biodiversity	Elasmobranchs and teleosts	-	Jurien Bay, Western Australia	Inshore; Reef and seagrass beds	Biodiversity assessment	Metabarcoding (Illumina MiSeq amplicon sequencing)	16S	178-228	3× 0.5 L; 0.45 µM PES filters; peristaltic pump	DNeasy Blood & Tissue Kit (Qiagen)	ND

Bakker et al.	2019	Biodiversity assessment of tropical shelf eukaryotic communities via pelagic eDNA metabarcoding	Eukaryotes	-	Caribbean	Reef	Biodiversity assessment	Metabarcoding (Illumina MiSeq amplicon sequencing)	COI	3113	1× 4 L; 0.45 µM MCE filters; vacuum pump	PowerWater DNA Isolation Kit (QIAGEN; formerly MOBIO)	ND
West et al.	2020	eDNA metabarcoding survey reveals fine-scale coral reef community variation across a remote, tropical island ecosystem	Eukaryotes	-	Cocos (Keeling) Islands	Reef	Biodiversity assessment	Metabarcoding (Illumina MiSeq amplicon sequencing)	(1) 16S – Fish, (2) COI, (3) 16S – Crustacean, (4) 18S	(1) 178-228, (2) 110-241, (3) 90-213, (4) 336-423	6× 1 L; 0.22 µM PES filters; peristaltic pump	DNeasy Blood & Tissue Kit (Qiagen)	ND
Drymon, et al.	2020	Swimming against the flow—Environmental DNA can detect bull sharks (*Carcharhinus leucas*) across a dynamic deltaic interface	Bull shark	*Carcharhinus leucas*	Alabama, USA	Riverine	Species-specific presence-only detection	ddPCR	ND2	237	5× 1 L; 0.8 µM nylon filters; vacuum pump	DNeasy Blood & Tissue Kit (Qiagen)	ND
Postaire et al.	2020	Environmental DNA detection tracks established seasonal occurrence of blacktip sharks (*Carcharhinus limbatus*) in a semi-enclosed subtropical bay	Blacktip sharks	*Carcharhinus limbatus*	Florida, USA	Bay	Species-specific presence-only detection	qPCR	ND2	149	4-6× 2 L; 0.45 µM PES filters; vacuum pump	DNeasy PowerSoil Isolation Kit (Qiagen)	ND
Jensen et al.	2020	Genome-scale target capture of mitochondrial and nuclear environmental DNA from water samples	Whale shark	*Rhincodon typus*	Qatar, Persian Gulf	Oceanic - pelagic	Genome-scale target capture	Target capture (myBait probes, Illumina MiSeq)	whole mitochondrial genome and ~0.1% nuclear genome	80	2× 1 L; 0.22 µM Sterivex filters; 60 mL syringes	DNeasy Blood & Tissue Kit (Qiagen)	ND
Van Rooyen, et al.	2021	Development of an environmental DNA assay for detecting multiple shark species involved in human–shark conflicts in Australia	(1) White shark, (2) Tiger shark, (3) Bull shark	(1) *Carcharodon carcharias*, (2) *Galeocardo cuvier*, (3) *Carcharhinus leucas*	Northern New South Wales, Australia	Inshore	Species-specific presence-only detection	qPCR	(1) D-loop, (2) D-loop, (3) ND5	(1) 128, (2) 92, (3) 228	2× 1 L; 0.22 µM Sterivex filters; 60 mL syringe	DNeasy Blood & Tissue Kit (Qiagen)	TaqMan Exogenous Internal Positive Control
Bonfil et al.	2021	Detection of critically endangered marine species with dwindling populations in the wild using	Sawfishes	*Pristis* spp.	Gulf of Mexico; Caribbean	Inshore, estuarine	Species-specific presence-only detection	PCR	COI	145	(1) 3× 1 L; 5 µM Nuclepore filters; vacuum pump, (2) 3× 15 mL	PowerWater DNA Isolation Kit (QIAGEN; formerly MOBIO), (2)	ND

		eDNA gives hope for sawfishes									precipitation samples	DNeasy Blood & Tissue Kit (Qiagen)	
Budd et al.	2021	First detection of critically endangered scalloped hammerhead sharks (*Sphyrna lewini*) in Guam, Micronesia, in five decades using environmental DNA	Scalloped hammerhead	*Sphyrna lewini*	Guam	Harbour	Species-specific presence-only detection	qPCR	12S	117	10× 10 L; 10 µM nylon filters; diaphragm pump	PPLPP method; Zymo OneStep PCR Inhibitor Removal Kit	TaqMan Exogenous Internal Positive Control; Spiking-dilution
West et al.	2021	Large-scale eDNA metabarcoding survey reveals marine biogeographic break and transitions over tropical north-western Australia	Elasmobranchs and teleosts	-	North-west Western Australia	Mid-shelf, coastal, inshore, and estuarine	Biodiversity assessment	Metabarcoding (Illumina MiSeq amplicon sequencing)	(1) 16S, (2) COI	(1) 178-228, (2) 110-241	4× 1 L; 0.45 µM PES filters; peristaltic pump	DNeasy Blood & Tissue Kit (Qiagen)	ND

Despite the rapid growth in the uptake of eDNA to monitoring aquatic species by researchers, conservation practitioners, and commercial and government organisations alike, its application is not a one-size-fits-all approach. The importance of optimised study design has been emphasised (Barnes & Turner, 2016; Goldberg et al., 2016), that is, that the choice of methodology must be appropriate for the question and consider the influence of the ecosystem being sampled and ecology of the target species (See Chapter 2). The potential of eDNA methods in applied conservation is staggering but is it only with methodological testing that we can begin to understand how these methods can be applied most appropriately to species that are at the edge of extinction.

Resolving the distribution of sawfishes, via confirmation of their presence with traditional monitoring tools, relies on locating and/or catching the animals, which can prove challenging and time-consuming due to their rarity, cryptic habits, ecological specialisation, and potential occurrence in remote and difficult-to-access locations. A comprehensive global survey of sawfishes throughout their historic range will benefit from a reliable and efficient approach that can be used in a variety of settings, including remote and difficult to sample locations with low density species.

1.5. Outline of Thesis

The aim of this thesis is to determine if eDNA can be used as a tool to resolve the contemporary distribution of sawfishes across their historical range. The importance of this work is that on-the-ground conservation and research efforts, and higher-level policy and management decisions must be based on the most up to date evidence of species occurrence. The ability effectively to determine the contemporary range of sawfishes is promise for the ability to conserve the last remaining populations. Accordingly, a large focus of this thesis is

the development and validation of the eDNA methodology, from the field to the laboratory, in a manner that is appropriate for its application to species that are exceptionally rare. In Chapter 2, I inquire how eDNA has been used to study aquatic species, and piece together the challenges, constraints, and unknowns. Equipped with this information, Chapters 3–5 are a series of optimisation and validation experiments, to demonstrate how eDNA can be used optimally and efficiently as tool for surveying the distribution of sawfishes. The final data chapter, Chapter 6, details a survey of sawfishes throughout nations that comprise their historic range. Here, a diverse and skilled team of scientists and conservationists assisted in the collection of eDNA samples following the optimised procedures from previous chapters throughout riverine and coastal ecosystems of the global tropics where sawfish are anticipated to be rare and sparsely distributed.

The thesis is divided into seven chapters, including this General Introduction chapter. The specific aims of each chapter are as follows:

In Chapter 1, the 'General Introduction', the aim was to recover relevant biological and ecological background information on sawfishes to inform the development of a non-invasive genetic survey to study them. This review highlighted fact that sawfishes are rare and highly threatened, live in environments that are equally under threat of disturbances and pollution, there is limited biological and ecological data for all species, and updated information on their occurrence and distribution is needed to urgently inform conservation and management. I then introduce environmental DNA (eDNA) as a tool that has some characteristics that might make it useful in answering questions on sawfish occurrence and distribution, in anticipation of Chapter 2, which is a review of eDNA, and as the foundation to data Chapters 3–5.

In Chapter 2, 'Review of environmental DNA for detection and monitoring of sharks and rays', I reviewed the environmental DNA literature to examine and understand the key

steps in the design, optimisation, and validation of an eDNA survey for the study of rare aquatic species. The purpose being that I could then adequately develop the experiments that comprised the research in the following Chapters of this thesis. The nature of this literature review was a valuable undertaking and some parts of this work can be found in two separate publications. A co-first-authored literature review in *Animal Conservation* and a co-authored book chapter in *Shark Research: Emerging Technologies and Applications for the Field and Laboratory*.

The aim of Chapter 3 'Development, optimisation, and validation of environmental DNA workflow for detection of threatened sawfishes', was to design sensitive and robust species-specific qPCR assays for the five sawfish species and an eDNA preservation and extraction procedure. Assays were developed and tested in the laboratory against a host of tissue samples for target and non-target exclusion species. This was followed by field experiments to validate the assays as suitable for use with eDNA samples. An important part of this Chapter was a paired comparison of eDNA sample preservation and extraction methods that sought to evaluate the efficacy of either method for increased detection sensitivity of sawfishes. This yielded important results that informed the procedure for optimal detection of low-copy sawfish eDNA. This chapter has been published in *Aquatic Conservation: Marine and Freshwater Ecosystems*.

Chapter 4, 'Practical sampling methods inferred from eDNA particle size distribution and comparison of capture techniques of largetooth sawfish (*Pristis pristis*) eDNA', investigated how best to capture eDNA in the environment by comparing the sensitivity and efficiency of water filtration and whole water precipitation techniques at capturing sawfish eDNA. I also characterised eDNA particle size distribution, for which we have a remarkably shallow understanding of in general in the eDNA literature. This the first time this had been done for any Chondrichthyan. Taken together, the data generated in this chapter were

informative for the optimal capture of sawfish eDNA. This chapter has been published in *Environmental DNA*.

The aim of Chapter 5, 'A comparison of survey effort and detection sensitivity of scientific gillnet and environmental DNA methods for sawfish in a global hotspot', compared the efficacy of eDNA and gillnetting, which is a conventional, established monitoring tool, for the detection of sawfishes as a final validation of the newly developed eDNA methodology. This chapter used smalltooth sawfish as the model organism, given their abundance in the global hotspot for the species, Florida, United States. Further to this, the incidence of false negative eDNA detection was assessed in two ways, by examining the level of technical replication required to confidently consider an eDNA sample as negative and by exploring the utility of inhibitor removal kits to minimise the risk of false negative detection from PCR inhibitors that are inherent in eDNA samples. The data generated in this Chapter was the final validation step required to confidently commence the final data Chapter.

Chapter 6, 'Environmental DNA survey of sawfish throughout historical range provides overlapping perspective with current understanding', deployed the optimised eDNA workflow from previous chapters in priority nations throughout the global tropics and subtropics. Due to the simple nature of eDNA sample collection and the ability to deploy complete sampling kits to key in-country collaborators, there was a focus on specific locations where resources and capacity to comprehensively survey for sawfishes were previously limited but there was evidence to suggest the possibility of sawfish occurrence though in very low abundance. The survey was arguably the largest eDNA-based survey to date and the largest survey of sawfishes using a single method. The data generated supports contemporary reports and anecdotal evidence of sawfish persistence in nations that are considered as "lifeboat" and "beacon of hope", which strengthens the case for enacting and enforcing management and protection of the species and habitat.

Finally, Chapter 7 summarises the finding of the research in this thesis, details the limitations of the methodology, and describes future directions as a result of the findings of the PhD research. In addition, I briefly summarise the recent advancements in the field of eDNA that have evolved over the duration of this work and how they are set to strengthen future research.

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Chapter 2

Review of environmental DNA for detection and monitoring of sharks and rays

Parts of this chapter were adapted from my contribution to the following publications:

Huerlimann, R.*, **Cooper, M. K.***, Edmunds, R. C.*, Villacorta-Rath, C., Le Port, A., Robson, H. L. A., Strugnell, J. M., Burrows, D., Jerry, D. R. (2020). Enhancing tropical conservation and ecology research with aquatic environmental DNA methods: an introduction for non-environmental DNA specialists. *Animal Conservation*, 23(6), 632–645. https://doi.org/10.1111/acv.12583

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Le Port, A., Bakker, J., **Cooper, M. K.**, Huerlimann, R., & Mariani, S. (2018). Environmental DNA (eDNA): A valuable tool for ecological inference and management of sharks and their relatives. In J. C. Carrier, M. R. Heithaus, & C. A. Simpfendorfer (Eds.), *Shark Research: Emerging Technologies and Applications for the Field and Laboratory* (pp. 255–283). CRC Press, Boca Raton, Florida, United States. https://doi.org/10.1201/B21842-21

Statement of the Contribution of Others

Nature of assistance	Contribution	Names & affiliations of co-contributors
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	Theoretical	Dr Colin Simpfendorfer, JCU Dr Dean Jerry, JCU Dr Roger Huerlimann, JCU Dr Richard Edmunds, JCU Dr Agnes Le Port, JCU Dr Richard Edmunds, JCU Dr Cecilia Villacorta-Rath, TropWater, JCU Heather Robson, JCU Dr Jan Strugnell, JCU Dr Judith Bakker, University of Salford, United Kingdom Dr Stefano Mariani, University of Salford, United Kingdom
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2.1. A primer to environmental DNA

As the repository of genetic information, deoxyribose nucleic acid (DNA) is inherently associated with all life on Earth. The nucleotide sequence of DNA is the code for information about the identities of organisms, how they function and interact with each other and their environment, and their evolutionary history. DNA can also persist in the environment, wherein it can occur in the medium of nearly all the Earth's ecosystems, including water, soils, and sediments (Ficetola et al., 2008; Ogram et al., 1987; Tablerlet et al., 2012). The discovery that DNA preserved in sediments could allow us to study extinct species instigated the field of 'ancient DNA' (Willerslev & Cooper, 2005). Similarly, microbiologists were using genetic material from soil, water, and permafrost to gain insights into the diversity of microbial populations (DeFlaun & Paul, 1989; Ogram et al., 1987; Willerslev et al., 2003). Subsequently, due to the universal method of sampling genetic material in the environment, the study of extant macro-organisms from environmental samples was founded (Ficetola et al., 2008), termed 'environmental DNA' (eDNA), and has since been applied to the detection of a large range of species across a range of ecosystems (reviewed by Darling and Mahon, 2011; Tablerlet et al., 2012; Rees et al., 2014; Barnes and Turner, 2016; Deiner et al., 2017; Deiner, Yamanaka and Bernatchez, 2021). The first dedicated textbook, '*Environmental DNA: For Biodiversity Research and Monitoring*' was published by Taberlet et al. in 2018.

Environmental DNA sampling is the sampling of genetic material from environmental samples that originates from two different sources: organismal and extra-organismal DNA (definition by Rodriguez-Ezpeleta et al., (2021) adopted for this thesis). This refers to any DNA that is collected from a bulk environmental sample without specifically isolating the target organism(s). I adopt this broad definition because of the indiscriminatory nature of eDNA sampling that results in the capture of a complex mixture of both whole organisms (e.g., microscopic single-cell organisms such as protists, bacteria, and viruses) and extra-organismal

DNA (Figure 2.1). This eDNA soup originates from a variety of sources and in varying proportions. For example, a single litre of seawater can contain over a billion viruses and bacteria (Australian Antarctic Program, 2014), which can comprise up to 95% of the DNA sequenced from an eDNA sample (Figure 2.2A) (Stat et al., 2017). The remaining portion is likely extra-organismal eDNA shed from eukaryotes and organismal DNA from microeukaryotes (Stat et al., 2017). For this thesis, I focus on the extra-organismal component derived from vertebrates.

Vertebrates naturally release genetic material into their local environment, as with all living organisms including humans, as part of cellular turnover, metabolic waste excretion, and reproduction (e.g., broadcast spawning exhibited by some fish and cnidaria) (Torti et al., 2015; Pochon et al., 2017; Taberlet et al., 2018; Andruszkiewicz Allan et al., 2021). This genetic material can be intra-cellular/-organellar, which is DNA within intact or dying cells, organelles, or tissue aggregates originating from living or necrotic biomass (Figure 2.1). Though, Curtis and Larson (2020) found that a crayfish carcass produced no detectable eDNA over 30 days in a freshwater stream, indicating that the half-life of DNA from necrotic biomass is likely short. Alternatively, as the genetic material is released from cells, it becomes extra-cellular/-organellar and either free-floating, solubilised in water, or bound to organic/inorganic detritus or sediment aggregates (Torti et al., 2015).

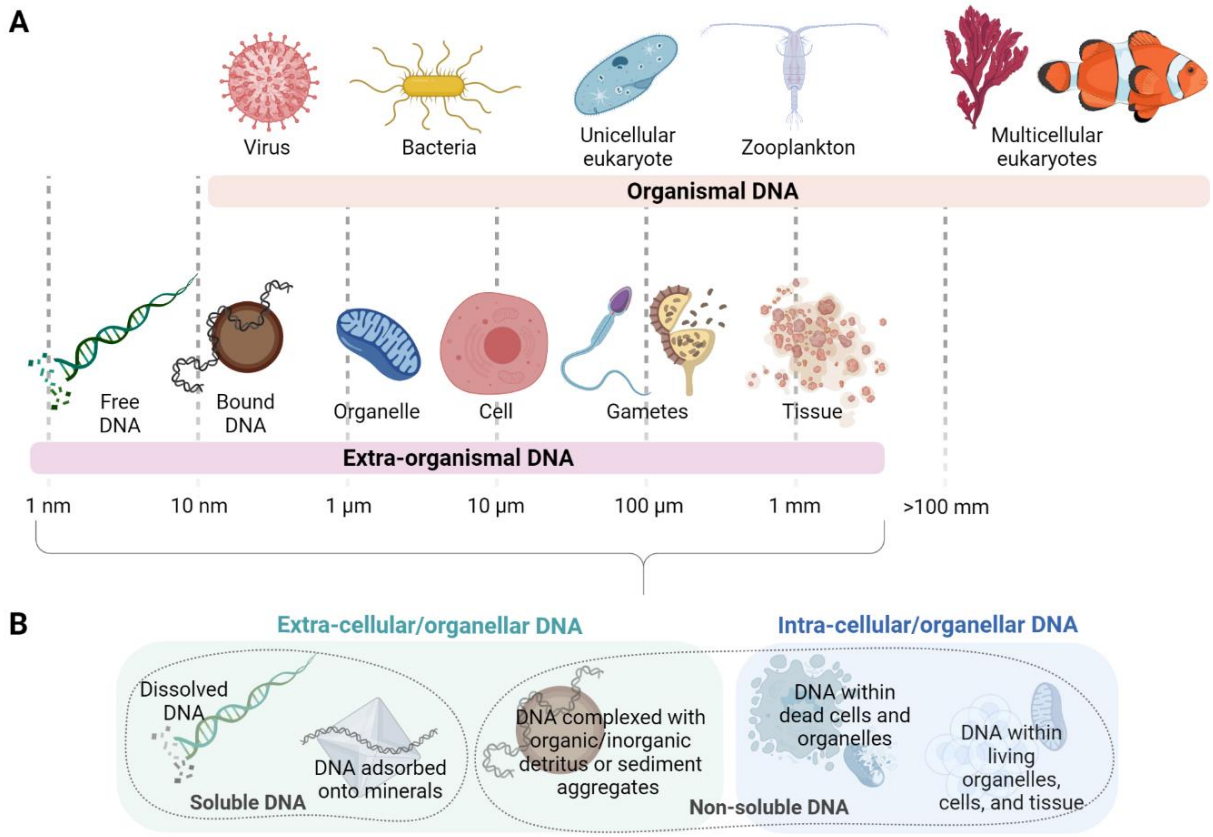

Figure 2.1. a) Types of environmental DNA, including examples of organismal sources and extra-organismal cell-bound and cell-free forms, and approximate size ranges (indicated by dotted line) of each. Adapted from Rodriguez-Ezpeleta et al. (2021). b) The extra-organismal component of eDNA can be intra-cellular/organellar and extra-cellular/organellar simply based on whether it occurs inside or outside cells or organelles, respectively, regardless of the physiological status of the cell (alive, dormant, dead). Soluble DNA consists of dissolved DNA (i.e., fully solubilised in water) and molecules adsorbed onto sediment minerals via electrostatic interaction, which are readily displaced from the sediment matrix by washing with alkaline, phosphate buffers, whereas non-soluble DNA is released only after harsh physical/chemical lysis treatments. Non-soluble DNA includes organically/inorganically complexed DNA (comprised of molecules complexed to insoluble detrital organic/inorganic components, or locked inside sediment aggregates), DNA within structurally intact dead cells, and DNA within living cells). Adapted from Torti, Lever and Jørgensen (2015). Created with BioRender.com.

Collection of eDNA samples (e.g., via filtration of water, collection of bulk soil, drilling of ice cores) is non-destructive, can be resource- and time-efficient, and offers greater opportunity compared to traditional sampling approaches to detect organisms that are not necessarily present at the exact time and place of sampling, but are or were present within the sampling area (Akre et al., 2019; Bonfil et al., 2021; Budd et al., 2021; Fujii et al., 2019; Jerde,

2021; Postaire et al., 2020; Schmelzle & Kinziger, 2016). Environmental DNA can be isolated from a variety of media and studied for a range of purposes (reviewed by Thomsen and Willerslev, 2015), including soil to study associated microbes and organisms (Ogram, 2000); sediment, peat, permafrost or coprolite to study aquatic and terrestrial macro- and microorganismal paleoenvironments (Jørgensen et al., 2011; Willerslev et al., 2003; Wood et al., 2012); faeces to study feeding ecology or microbiota; and, most commonly, water to study micro- and macro-organisms living inside and near water bodies (Ficetola et al., 2008; Ishige et al., 2017; Torresdal et al., 2017).

In aquatic environments, eDNA is generally captured using filtration (i.e., nominal filter pore sizes 0.22−20 μM; Kumar et al., 2020; Majaneva et al., 2018; Schabacker et al., 2020; Turner, Barnes, et al., 2014), or alcohol-salt precipitation (Buxton et al., 2018; Edmunds & Burrows, 2020; Eichmiller et al., 2016; Williams et al., 2017). Filtration has the advantage of being able to process water volumes several magnitudes larger than precipitation (e.g., 0.5−100 L vs 15−30 mL; but also >1,000 L filtered using mesh tow nets (Pedersen et al., 2014; Schabacker et al., 2020; Sepulveda et al., 2019)), which can increase the likelihood of species detection (Muha et al., 2019; Sepulveda et al., 2019). Use of nominal pore sizes ≤10 μM ensures maximized capture of extra-cellular/-organellar eDNA (Jo et al., 2019a; Turner, Barnes, et al., 2014; Wilcox et al., 2015); however, filtration time or rapid filter clogging can become inhibitive (Bonfil et al., 2021; Ip et al., 2021; Robson et al., 2016). As with all samples collected for molecular analyses, regardless of collection method, eDNA samples should be preserved rapidly following collection to protect against further degradation until laboratory-based extraction (see section 2.1.1.3 and review by Lear et al., 2018).

Aquatic eDNA is thought to be mostly present in small fragments, owing to rapid degradation (see section 2.1.3.1; Goldberg, Strickler and Fremier, 2018; Deutschmann et al., 2019; Jo et al., 2019). As such, polymerase chain reaction (PCR) assays designed for the

amplification of eDNA typically target short fragments of gene within the range of 70−300 base pairs (bp) (Table 1.2; reviewed by Taberlet et al., 2018). Gene regions that contain sufficient information within a few hundred base pairs for species identification can be found in both the mitochondrial and nuclear genome. As vertebrate eDNA is present at low concentrations in the water column relative to highly abundant microbial organisms (Stat et al., 2017), mitochondrial DNA (mtDNA) is the primary target since there are substantially more mitochondrial DNA copies (tens to thousands copies) than nuclear DNA copies per cell (Foran, 2006; Robin & Wong, 1988). Mitochondrial DNA genes that are commonly targets of eDNA assays, depicted in Figure 2.2, include cytochrome c oxidase 1 (COI) (Bakker et al., 2017; Gargan et al., 2017; Simpfendorfer et al., 2016; Stat et al., 2017), 12S ribosomal DNA (rDNA) (Budd et al., 2021; Cooper et al., 2021; Miya et al., 2015; Muha et al., 2019; Thomsen et al., 2016), 16S rDNA (Stat et al., 2017; West et al., 2021), ND2 (Lehman et al., 2020; Schweiss et al., 2020), and D-loop (Sigsgaard et al., 2017; van Rooyen et al., 2021). Some studies have suggested the use of nuclear DNA markers, particularly high abundance ribosomal RNA genes such as internal transcribed space (ITS) regions (Jo et al., 2019b; Minamoto et al., 2016; Moushomi et al., 2019; Trujillo-González et al., 2019). The main factor determining which marker to use is the level of target specificity required for the study (Apothéloz-Perret-Gentil et al., 2021; Schenekar et al., 2020; Wilcox et al., 2015). In addition, PCR machines and sequencing platforms currently available for eDNA amplification and sequencing (e.g., ThermoFisher QuantStudio qPCR System and Illumina MiSeq Sequencing System, respectively; Table 1.2) are optimised for short amplicons and short-read capabilities, respectively, limited to a few hundred base pairs, which limits the choice of primers to short barcoding regions. Though, conventional PCR protocols can be optimised for longer fragment lengths.

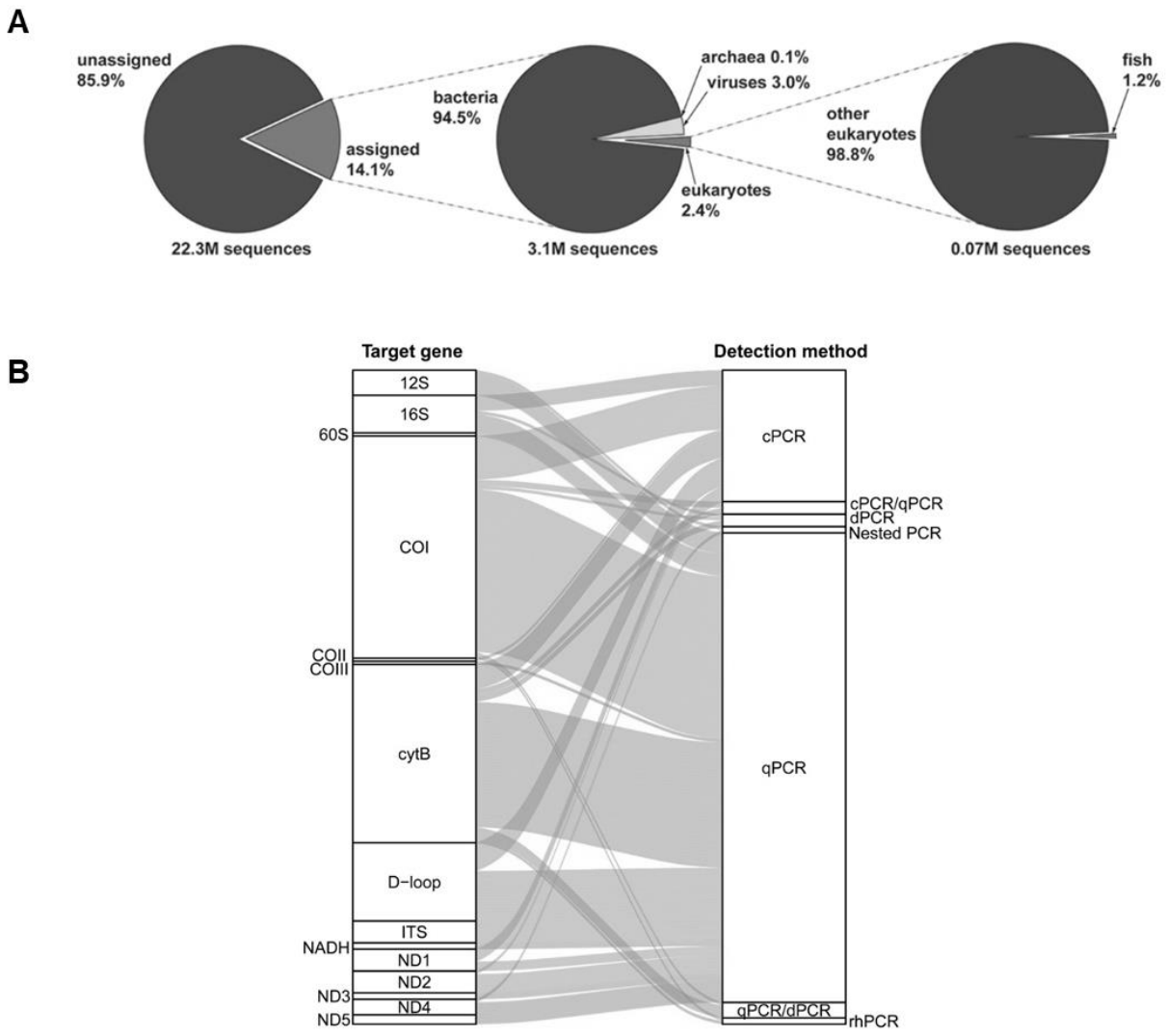

Figure 2.2. Summary of fish eDNA isolated from the aquatic environment. a) From Stat et al., (2017): The percentage of sequences assigned to fish in comparison to other eukaryotes and all other unidentified eDNA sampled in a marine ecosystem. Fish comprised 1.2% of identifiable eukaryote eDNA and 0.0000004% of all unidentified eDNA. b) From Wang et al., (2021): A Sankey diagram of an analysis of peer-reviewed publications containing "environmental DNA" and "fish" in the title, abstract, and keywords (n = 324). The coloured lines are weight based on proportion and link studies using target mitochondrial gene regions for specific markers with a corresponding detection method. Abbreviations: cPCR, conventional PCR; qPCR, quantitative PCR; dPCR, digital PCR; rhPCR, RNase H-dependent PCR.

Analysis of eDNA falls into two primary domains, species-specific (i.e., single species) detection using PCR and community-level (i.e., multiple species) detection using

metabarcoding. Species-specific detection is most common using quantitative PCR (qPCR) and conventional PCR (cPCR) and derivatives such as nested PCR, droplet digital PCR (ddPCR), and RNase H-dependent PCR (rhPCR) are far less common (Figure 2.2). For studies concerning species-specific eDNA detection, the ideal marker will have high specificity to the target species and not closely related taxon (Wilcox et al., 2013; Wilcox et al., 2015) and for broad taxa metabarcoding assessment the marker will contain some specificity (e.g., minimise complementarity with DNA from humans and *E. coli*), but also generality across all populations of the target taxa group (Coghlan et al., 2021; Miya et al., 2015; Nester et al., 2020; West et al., 2021). Though still in its infancy, the target enrichment method based on DNA hybridisation capture rather than PCR has demonstrated improvements in extracting population genomic data from tens of thousands of loci of vertebrate eDNA (Jensen et al., 2021). Though an important and large part of the eDNA research field, the research described in this thesis focuses on species-specific detection using qPCR and as such a review of metabarcoding approaches was not within scope and will not be detailed further.

A significant challenge for assay design and data interpretation for all approaches is the underrepresentation of many aquatic species in genetic databases (see Marques et al., 2021). Genetic databases such as GenBank (Agarwala et al., 2018; https://www.ncbi.nlm.nih.gov) and Barcode of Life Data System (BOLD; Ratnasingham & Hebert, 2007; www.barcodinglife.org) are freely accessible online repositories for nucleotide sequence information and depositing sequences here is often requirement for publishing scientific research. However, not every species is represented and the databases are not strictly curated for correctness. The lack of genetic information for target species can be addressed cheaply by sequencing whole mitochondrial genomes or specific mitochondrial genes and depositing them into publicly available databases. This can be further facilitated through the use of tissue and DNA from vouchered specimens in museums and academic institutions or through strategic collaborations

supported by online platforms such as Otlet (https://otlet.io, Green, Meyer and Fetterplace, 2019).

2.1.1. General considerations for eDNA survey design: in the field

Effective and accurate detection of vertebrate eDNA is dependent on the development of an appropriate sampling design. There is no single eDNA sampling method that fits all target species and environments (Barnes & Turner, 2016; de Souza et al., 2016). Therefore, conducting a pilot study is considered an important first step (Furlan & Gleeson, 2016; Goldberg et al., 2016). Overall, it is important to understand the characteristics of eDNA in the context of local environmental conditions, including the influence of biotic and abiotic factors on DNA degradation and dispersal (see section 2.1.3); and factors related to the target species/community, including life history and ecology (Goldberg, Strickler and Fremier, 2018; Kelly, Gallego and Jacobs-Palmer, 2018; Postaire et al., 2020; Schweiss et al., 2020). These factors can result in variation in detection probability. Currently, the recommended approach is to assess eDNA detection probabilities for the target species given the proposed field and laboratory protocols (Goldberg et al., 2016). Here, preliminary laboratory and aquarium eDNA assays can be applied to test and confirm the sensitivity and specificity of the methodology. Where possible, controlled tank-based experiments can be conducted to further understand eDNA shedding and degradation rates (Turner, Barnes, et al., 2014; Weltz et al., 2017).

A variety of methods have been used to capture, preserve, extract and analyse eDNA; however, due to the sequential nature of eDNA sample processing and lack of gold-standard practices (Goldberg et al., 2016; Pierre Taberlet et al., 2018), methodological decisions must consider the multiple inherent technical challenges discussed below.

2.1.1.1. Contamination management With any method it is imperative to monitor and mitigate contamination risk. Cross-contamination during sample collection and resultant false positive detections can have major consequences on downstream management processes and decisions when unnoticed (Davis et al., 2018). Contamination can occur at any point during an eDNA workflow, from in-field sample collection to transportation or laboratory analyses; however, contamination is most difficult to mitigate during sample collection due to inherent challenges with creating a DNA-free workplace in the field (Champlot et al., 2010; Goldberg et al., 2016). Therefore, it is of critical importance to adhere to strict decontamination procedures and include appropriate field, equipment, and analysis controls to ensure samples are uncontaminated or if contaminated that contamination source can be pinpointed within the workflow (see Goldberg et al., 2016). For example, bleach wipes or tablets, which are low-cost and commercially available, are a practical option for in-field decontamination and liquid bleach can be deactivated by ascorbic acid or UV irradiation prior to disposal. Furthermore, in-depth training is recommended to help mitigate contamination derived from user error, such as use of unclean consumables, equipment, clothing, and gloves.

Access to ample DNA-free water is required for equipment cleaning and generation of field controls. This constitutes a logistical challenge given the weight associated with the water volume required for equipment decontamination and controls. For targeted studies, any water source at a field location (e.g., drinking water or bottled water) free of targeted species DNA can be utilised for equipment decontamination and controls. Conversely, molecular grade water is preferred for metabarcoding studies to avoid introducing pre-existing eDNA into the samples given the common use of universal primers. If unavailable, systemic contamination at any stage of the procedure can be removed bioinformatically during post-sequencing processing (e.g., microDecon; McKnight et al., (2019)); however, this potentially increases cost and reduces limit of detection due to loss of sequencing reads to contamination.

2.1.1.2. Capture Filtration and precipitation are the most commonly used methods to capture eDNA from water. Generally, the precipitation method involves the collection of small volumes of water (e.g., 15−30 mL) (Buxton et al., 2018; Edmunds & Burrows, 2020; Eichmiller et al., 2016; Williams et al., 2017), which are then preserved in-field with the addition of sodium acetate and absolute ethanol (salt and ethanol precipitate nucleic acids from water) or in cold storage, respectively. The whole water preservation method requires few collection tools (i.e., precipitation solution or ice cooler and collection tubes, typically 50 ml falcon tubes). The relative ease of this method is a major benefit for users (Villacorta-Rath et al., 2021). Where the processing of larger volumes of water is required (e.g., for rare species detection), it is advisable to increase the number of biological replicates or, alternatively, use the filtration method. Filtration is more advantageous when dealing with larger bodies of water such as rivers, estuaries, or marine environments (Hinlo et al., 2017; Turner, Miller, et al., 2014). Filtration requires the passage of water through a membrane that captures the eDNA, and generally allows the processing of larger volumes of water (typically 1−10 L) (Kumar et al., 2020; Majaneva et al., 2018; Schabacker et al., 2020; Turner, Barnes, et al., 2014). Filtration can be carried out on-site with a portable filtration system (Thomas et al., 2018), or water samples can be stored on ice or preserved with the addition of the cationic surfactant benzalkonium chloride (BAC) and transported to a laboratory (or equivalent processing facility) for filtration (Sales et al., 2019; Takahara et al., 2020). If not performed in the field, filtration should be undertaken as soon as possible (i.e., within 24 hours) to ensure optimal eDNA recovery (Hinlo et al., 2017; Weltz et al., 2017).

Recent advancements in field-based filtration systems have provided effective alternatives for laboratory-based filtration (Schabacker et al., 2020; Thomas et al., 2018; Yamahara et al., 2019). Field filtration devices such as the ANDe™ pump system (Thomas et

al., 2018), Grover environmental samplers (groverscientific.com.au), mobile peristaltic pumps (Yamanaka et al., 2016), or Sterivex syringes and enclosed filter capsules (Spens et al., 2016), provide a cost-effective and practical alternative to using cold-chain storage of water samples for laboratory-based filtration, circumventing transport of large water volumes back to the laboratory. Notably, autonomous underwater vehicles (AUV) and autonomous monitoring stations fitted with an eDNA sampling and filter preservation system demonstrated a future for eDNA-based monitoring in the absence of human manipulation (Formel et al., 2021; Yamahara et al., 2019).

Alternatives to active filtration and collection methods are also being explored, including deploying simple devices containing granular activated carbon, montmorillonite clay (Kirtane et al., 2020), or filter membranes (Bessey et al., 2021) in the water column to passively bind eDNA, and the collection of tissue from marine sponges, which naturally filter thousands of litres of seawater (Mariani et al., 2019). Lateral flow dipstick eDNA detection has also been trialled as an alternative to active filtration and to provide rapid assessment (i.e., result within several minutes) (Doyle & Uthicke, 2021). Additionally, on-site eDNA extraction and analysis, such as loop-mediated isothermal amplification (e.g., Gene-Z portable gene analyzer; Williams et al., 2017), qPCR amplification (e.g., Franklin™ Biomeme; Thomas et al., 2020), or DNA sequencing (e.g., Nanopore MinION; Truelove et al., 2019), can decrease time between sample collection and results, avoiding preservation and laboratory analyses altogether. However, these methods currently have limited sample number and analytical capabilities.

The filter material type and pore size used for the filtration of eDNA from water samples also varies across studies and can influence detection probabilities (Capo et al., 2020; Deiner et al., 2015; Eichmiller et al., 2016; Hinlo et al., 2017; Li et al., 2018; Liang & Keeley, 2013; Majaneva et al., 2018; Muha et al., 2019; Schabacker et al., 2020; Sepulveda et al., 2019; Wang et al., 2021). The inherent properties of the filter material can affect the binding affinity

of eDNA and, as such, eDNA recovery rates across different studies will differ depending on the type of filter used (Capo et al., 2020; Li et al., 2018; Liang & Keeley, 2013; Majaneva et al., 2018; Muha et al., 2019; Sepulveda et al., 2019). For example, depth filters (e.g., glass fibre) retain particles on the surface and within the filter matrix, whereas surface filters (e.g., nylon net) retain all particles that are larger than the pore size on the filter's surface (Eichmiller et al., 2016). Glass fibre, nylon, cellulose nitrate, mixed cellulose ester, polycarbonate, and polyethersulfone (e.g., Sterivex™) filters are commonly used filter material types (reviewed by Deiner et al., 2015; Goldberg et al., 2016; Wang et al., 2021). Despite reports of differences in eDNA yield and detection probabilities, these studies are often conflicting, have limited replication, and not directly comparable because of the use of different filter pore sizes (reviewed by Kumar, Eble and Gaither, 2020). For example, Lacoursière-Roussel, Rosabal and Bernatchez, (2016) reported that glass fibre filters, which are the most frequently used filter types, performed better than mixed cellulose ester filters for capture of fish eDNA; Capo et al., (2020) reported that Sterivex™ polyethersulfone filters and mixed cellulose ester filters performed better than glass fibre for capture of fish eDNA; and Djurhuus et al., (2017) found no differences in community richness when comparing multiple different filter types (i.e., glass fibre, cellulose nitrate, polyethersulfone, polyvinylidene difluoride, and polycarbonate).

Filter pore size is another important consideration for optimal eDNA capture. Intuitively, larger sample volumes will increase eDNA capture success (Cantera et al., 2019; Schabacker et al., 2020; Sepulveda et al., 2019; Wittwer et al., 2018); however, there is a trade-off between volume, pore size, and eDNA particle retention. Specifically, smaller pore sizes capture more eDNA particles (i.e., both intact intracellular and fragmented extracellular eDNA particles are captured on the filter surface as they are larger than the filters pores), but this limits the total volume that can be filtered due to rapid filter saturation and obstruction of any further filtration (Kumar et al., 2021; Thomas et al., 2018). Conversely, a larger filter pore size allows

for a larger sample volume at a faster filtering rate, but in turn may reduce the amount of eDNA particles captured on the filter (i.e., restricted to capture of clumped or intact cells and organelles or eDNA bound to minerals) (Turner, Barnes, et al., 2014; Wilcox et al., 2015; Barnes et al., 2020). The challenges of filter pore size choice are more difficult for studies employed in turbid ecosystems. Filter pore sizes ranging from 0.45 to 3 µm have been used in studies undertaken in moderately turbid water (Gargan et al., 2017; O'Donnell et al., 2017; Weltz et al., 2017). In highly turbid water, however, even 3 and 5 µm filters quickly become clogged with suspended particulate matter, requiring the use of larger pore sizes of up to 20 µm to minimise clogging and maintain an efficient filtration rate (Ip et al., 2021; Robson et al., 2016). If filter clogging is a frequent occurrence, multiple filters may be used and eDNA extracts pooled for sample replicates or a pre-filtration strategy can be used (Li et al., 2018; Majaneva et al., 2018). Replicate experiments are still needed to effectively determine the most appropriate filter pore size, material type, and filtrate volume in an effort towards standardisation.

2.1.1.3. Preservation Immediate eDNA sample preservation is critical given that eDNA starts to degrade immediately after it is shed from the organism and continues to do so after sample collection (Andruszkiewicz et al., 2017; Barnes et al., 2014; Dejean et al., 2012; Deutschmann et al., 2019; Pilliod et al., 2013b; Sansom & Sassoubre, 2017; Takahara et al., 2020). A study on eDNA recovery rates from 250 mL stream water samples following various combinations of eDNA capture, preservation, and extraction methods found that total DNA copy number significantly decreases within 2 days regardless of storage temperature (20°C, 4°C, -20°C) (Hinlo et al., 2017). Moreover, decay modelling of Maugean skate eDNA showed that the eDNA concentration in some samples fell below detectable limits within 4 hours of sampling (Weltz et al., 2017).

Commonly used ambient-temperature preservation solutions include ethanol, Longmire's solution, Qiagen ATL lysis buffer, or silica beads/gel (reviewed Kumar, Eble and Gaither, 2020). These solutions are widely endorsed as they circumvent the requirement for immediate refrigeration or short-term storage in an ice cooler, which can be impractical for eDNA sample collection at remote study sites. Ethanol is widely available, inexpensive, and can be use straightaway with no preparation and effectively preserves eDNA on filters in the absence of -20°C freezer storage (Allison et al., 2021; Hinlo et al., 2017); however, particular caution is warranted for the use of ethanol as a long-term ambient preservative due to evaporation over time (Allison et al., 2021; Stein et al., 2013). Longmire's solution (Longmire et al., 1992) has been shown to effectively preserve eDNA captured on filter membranes in temperatures up to 45°C for 2 weeks (Renshaw et al., 2014). Drying eDNA samples with silica gel or beads has been shown to be an effective solution-free preservation method for filter samples for up to 1 month (Allison et al., 2021); however, dried DNA may be vulnerable to degradation over time if storage conditions are not kept constant. Recently, Thomas et al., (2019) reported on a commercially available self-preserving eDNA filter housing, which effectively preserved eDNA on filters (via desiccation) contained inside the housing and without cold storage for up to 6 months.

2.1.2. General considerations for eDNA survey design: in the laboratory

2.1.2.1. Extraction The extraction of eDNA from filter samples is most commonly achieved through the use of commercial kits, such as DNeasy Blood & Tissue or PowerWater (QIAGEN, Hilden, Germany), or through the use of in-house solutions, such as cetyl trimethylammonium bromide and chloroform-isoamyl with or without phenol (Figure 2.3) (reviewed by Lear et al., 2018; Kumar et al., 2020; Wang et al., 2021). The advantage of commercial kit extraction methods is that protocols are simple, streamlined, provide

standardisation across research groups and, therefore, yield comparable results (Deiner et al., 2015; Djurhuus et al., 2017). In comparison, in-house extraction methods such as phenol-chloroform-isoamyl (PCI; Deiner and Altermatt, 2014; Turner et al., 2014; Piggott, 2016), cetyl trimethylammonium bromide (CTAB; Turner et al., 2014; Williams et al., 2017), or glycogen aided-sodium chloride-isopropanol precipitation-lyse-PEG precipitation (PPLPP; Edmunds & Burrows, 2020; Budd et al., 2021) are generally cheaper and provide greater eDNA yield than column-based methods, which is particularly advantageous for large-scale studies (Djurhuus et al., 2017; Edmunds & Burrows, 2020). It is also noted that different methods and adaptations to protocols, which is common for groups using commercial kits, can produce differences in eDNA yield and species detectability (Deiner et al., 2015; Djurhuus et al., 2017; Eichmiller et al., 2016; Roose-Amsaleg et al., 2001). One of the most commonly used modifications for DNeasy Blood & Tissue uses three-times the recommended volume of buffer ATL and Proteinase K in the initial step due to improvements in overall eDNA yield from complete saturation of the filter paper with lysis buffer in a 2 mL microtube (see Thomsen et al., 2012). The QIAGEN DNeasy handbook also suggests the addition of 3–5 µg carrier DNA (e.g., poly-dA; Roche, Cat. No.: 10223581001) to the starting material to obtain optimal DNA yield when the sample has less than 5 ng DNA; however, this is not commonly used in eDNA research despite the low yield of eDNA in filter samples. Piggott, (2016) demonstrated that choice of eDNA extraction protocol had the greatest impact on detection probability alongside choice of sampling and PCR strategy. As a result, the importance of testing and optimising workflows for the study objective has been emphasised (Kumar et al., 2020).

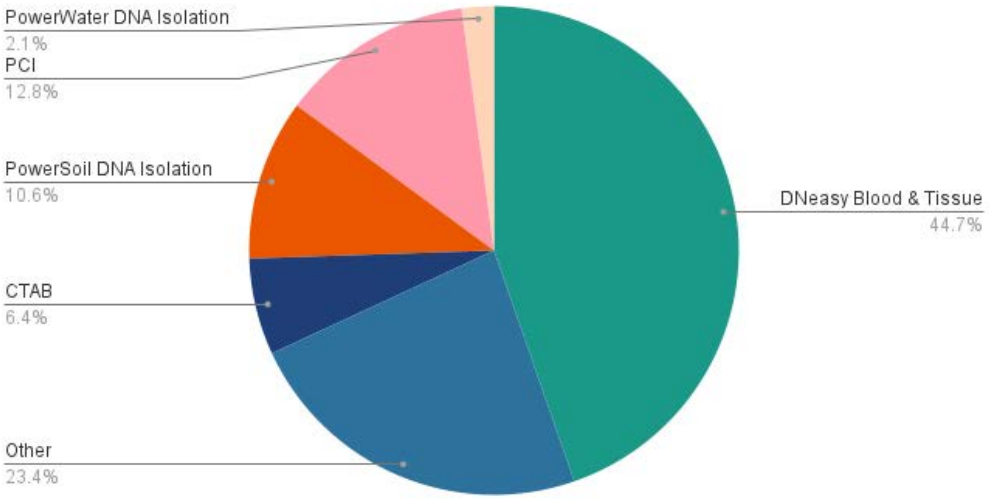

Figure 2.3. Summary of different extraction methods used in a subset of publications (n = 44) that were reviewed in Huerlimann et al., (2020). Publications included in the review included original research articles that answered conservation-based questions or have implications for conservation management.

2.1.2.2. PCR Inhibition As with extraction, multiple different methods have also been used to identify and remove inhibitory compounds that are often found in environmental samples (Budd et al., 2021; Edmunds & Burrows, 2020). Inhibitors that are naturally found in the environment such as debris, fulmic acids, humic acids, and polyphenols can block downstream PCR enzymic reactions if not effectively removed or diluted (Wilson, 1997). In particular, inhibitors interfere with PCR at a molecular level by either completely or partially blocking the production of amplicons. For example, humic acids interact with template DNA and block the action of DNA polymerase or other PCR reagents during each cycle of PCR and as a result may quench double-stranded DNA-binding dyes (e.g., SYBR Green) (Sidstedt et al., 2015).

The occurrence of inhibition during qPCR amplification can be determined by measuring the effect on amplification efficiency. Differences in relative amplification efficiency can be seen in changes in the slope of the exponential amplification curve compared to a non-inhibited control sample, such as the protocol used in the commercially available

exogenous Internal Positive Control Assay (Hartman et al., 2005) or the exogenous DNA standard spiking-dilution method (Cao et al., 2012). Exogenous DNA controls are run in multiplex qPCRs with the field eDNA sample to test for inhibition. If the control DNA fails to amplify or if there is an increase in observed qPCR Ct (i.e., cycle number at which amplification reaches a fluorescence threshold above background levels) relative to a positive control sample, the sample is considered completely or partially inhibited, respectively (Turner et al., 2014; Wilson et al., 2014).

Accordingly, the removal of inhibitors or specialised treatment of inhibited eDNA samples is recommended. The Zymo OneStep™ PCR Inhibitor Removal Kit has been widely and successfully used (Deiner et al., 2018; Djurhuus et al., 2017; Kumar et al., 2021; McKee et al., 2015; Williams et al., 2017) and, less so, the QIAGEN DNeasy PowerClean CleanUp Kit (Budd et al., 2021; Minegishi et al., 2019; Villacorta-Rath et al., 2020). Other common methods for minimising the influence of co-extracted inhibitors include addition of bovine serum albumin (BSA) to PCR chemistry (Deiner et al., 2015; Dejean et al., 2012; Schmidt et al., 2013), using inhibitor resistant DNA polymerase (Schrader et al., 2012), or sample dilution (McKee et al., 2015). However, dilution is not recommended for eDNA studies on rare species given the low eDNA starting concentration, since dilution increases the risk of false negative detections (Schrader et al., 2012). Furthermore, PCR modifications may not completely ameliorate inhibitor effects and their utility will vary with the type of inhibitor present (Lance & Guan, 2019).

2.1.2.3. Assay design and validation Currently, the use of eDNA can broadly be divided into two main approaches, a species-specific approach (i.e., single species) and a community-level approach using metabarcoding (i.e., multiple species). The species-specific

approach, which is a focus of research reported in this thesis, is aimed at detecting a single species in the environment using polymerase chain reaction (PCR) to target and amplify a barcoding region specific to the target species. Amplification of the correct target species is then often confirmed by Sanger sequencing and validation of the sequence to a validated reference sequence (i.e., reference sequences from vouchered specimens can be found on NCBI GenBank). Conventional PCR has historically been used for species-specific eDNA detection (Dejean et al., 2011; Simpfendorfer et al., 2016), though qPCR or ddPCR is now more widely adopted (Pierre Taberlet et al., 2012; Wang et al., 2021). qPCR has the distinct advantage over cPCR of being able to quantitatively evaluate target eDNA copy number because the addition of a probe containing intercalating (e.g., SYBR™ Green) or a minor groove binding (e.g., TaqMan™) fluorescent dye allows the amplification of the target sequence to be monitored in real-time by the qPCR instrument. TaqMan probe-based qPCR is currently the most effective and sensitive tool for species-specific eDNA applications and is utilised in the research described in this thesis. Droplet digital PCR (ddPCR) has also been promoted as an alternative platform given its reported higher tolerance for inhibitors and sensitive detection of very low target copy number, yet the equipment is prohibitively expensive (Hunter et al., 2016).

TaqMan probe-based qPCR assays can improve both specificity and sensitivity of eDNA detection as the use of a probe, in combination with forward and reverse primers, ensures that there are three sequences to anneal with the target template DNA. A probe is a short oligonucleotide intended to hybridise in the DNA target region of interest between two primers that consists of a species-specific DNA sequence, a 5’ fluorescent dye, and a 3’ quencher. During qPCR amplification of the target sequence, the TaqMan probe is degraded by the Taq polymerase, resulting in the separation of the reporter and quencher fluorochrome and the fluorescent signal become detectable by the qPCR machine. Although this approach

has high specificity, sensitivity, and quantification ability, it is limited to detecting only one or a few target organisms at a time (Thomsen & Willerslev, 2015).

The design of TaqMan qPCR-based assays is a three-stage validation process including *in silico*, *in vitro*, and *in situ* testing (Goldberg et al., 2016). During the initial stage, primers and probes are designed using a curated database generated from publicly available sequences and include specificity to the target species and generality across the inherent variability of individuals within the target population. Programs such as Geneious (http://www.geneious.com) and AlleleID® (Primer BioSoft, USA), can be used to assist in primer or probe design. Furthermore, the online tool Primer BLAST (www.ncbi.nlm.nih.gov/tools/primer-blast/) can be used to further assess the specificity of the primers against a wider database. It is also important to incorporate base pair differences within the primer and probe sequences to co-occurring or closely related species (referred to as non-target or exclusion species) (Wilcox et al., 2013; Wilcox et al., 2015). Insufficient primer specificity can lead to over- or under-estimation of species presence and, especially when taxa closely related to the target species are present, cross-amplification or interference of amplification can lead to the generation of false positive and negative errors (Wilcox et al., 2013). In studies where a large number of species co-occur, some of which may be closely related, finding a suitable gene to design a species-specific or even genus-specific primer assay may be challenging. This may require the generation of additional reference sequences due to the lack of, or incomplete occurrence of, data for many species. Furthermore, general good practice for primer design are applicable. These include a primer length between 18 and 25 bp, GC content between 40−60%, melting temperature between 55°C and 6°C, GC-clamp of no more than 3 bp in the last 5 bp, and a minimisation of self and cross primer dimer, palindromes, hairpins and runs longer than 4 bp. Lastly, the length of the fluorescent probe should be between 9 and 40 bp, with a melting temperature between 8°C and 10°C higher than the melting

temperature of the primer pair. Lastly, the 5' end of the probe should be as close as possible to the 3' end of the primer located on the same strand.

Following *in silico* design and testing, *in vitro* tests should be conducted to first confirm optimal qPCR parameters (i.e., annealing temperatures, primer-probe concentrations, and cycle number) and then specificity and sensitivity of the assay to the target species. The latter tests should be completed using either genomic DNA (gDNA) or an artificial oligo of the target and non-target sequences (i.e., IDT gBlock Gene Fragments) and often require testing of multiple primer-probe sequence combinations (Wilcox et al., 2013). Lastly, the final combination of primers and probe should be validated *ex situ* (tank experiments or aquaria) or *in situ* (in the field) to demonstrate the applicability in detecting the target species eDNA (Schweiss et al., 2020).

Finally, an optimised and validated eDNA assay can be utilised with eDNA samples to generate presence-absence data based on the amplification or not of target species eDNA during PCR. These data can be used to interpret the presence of the target species in the environment that is survey.

2.1.3. Sources of uncertainty

Environmental DNA detection methods are reported to be highly sensitive, but like any detection method are prone to errors (Doi et dal., 2019; Ficetola et al., 2015; Jerde, 2021). Most studies identify at least some uncertainty around detections and non-detections due to a paucity of knowledge on shedding, degradation, and transport of eDNA in the environment (detailed below), as well as the choice of eDNA capture and extraction methods, and challenges at the PCR amplification step (detailed above).

2.1.3.1. Shedding The availability of detectable eDNA in environmental samples is reliant on the underlying premise that all aquatic organisms shed genetic material. An earlier study on vertebrate eDNA detection suggest that the most probable origin of eDNA is faecal material (Martellini et al. 2005). It is also true that eDNA originates from cells that are shed from living organisms as a result of cellular turnover and reproduction (Andruszkiewicz Allan et al., 2021; Pochon et al., 2017; Torti et al., 2015). Experimental evidence suggest that eDNA shedding rates in aquatic organisms are also linked to population biomass (Deutschmann et al., 2019; Karlsson et al., 2022; Klymus et al., 2015; Nevers et al., 2018; Stoeckle et al., 2017; Weltz et al., 2017) and species-specific physiological characteristics such as skin properties (e.g., slimy coatings (Ficetola et al., 2008; Jerde et al., 2011), metabolic rates (Klymus et al., 2017), and environmental tolerance (Lacoursière-Roussel et al., 2016; Robson et al., 2016)). However, it is likely that this relationship is much more complex, as studies have observed highly variable eDNA production rates among individuals unrelated to body size and suggest that this variation may be attributable to animal physiology or differences in the source of eDNA (e.g., tissues, cells, faecal debris) that is unevenly dispersed in the water column (Buxton et al., 2017; Laramie et al., 2015; Maruyama et al., 2014; Nevers et al., 2018; Wilcox et al., 2016). For example, Klymus et al., (2015) reported up to a 100-fold variation in day-to-day eDNA concentration in the water from the same fish in a controlled environment. Moreover, studies have suggested that stress and feeding behaviour can influence eDNA shedding rates (Sassoubre et al., 2016), but these behaviours are intertwined with the physiological tolerances (Lacoursière-Roussel et al., 2016). Additionally, the eDNA contribution from different life stages may vary seasonally. For example, strong temporal increases in eDNA concentration have been observed during months associated with seasonal migration and breeding (Buxton et al., 2018; Spear et al., 2015). Despite the growing number of experimental studies, the species-specific nature eDNA shedding means that these data are not widely applicable. As

such, any interpretation of eDNA detection results will benefit from a more complete understanding of the ecology of eDNA.

2.1.3.2. Degradation All environmental conditions have the potential to play a role in eDNA persistence and degradation (Andruszkiewicz Allan et al., 2021; Barnes et al., 2014; Jo et al., 2019a; Strickler et al., 2015). Understanding the interactions of environmental factors controlling degradation is essential to understanding the limits of temporal and spatial inference of eDNA detection results. Once shed from an organism eDNA begins to degrade into small fragments and becomes undetectable within hours to weeks (Dejean et al., 2011; Deutschmann et al., 2019; Tsuji et al., 2017; Weltz et al., 2017). Degradation is the primary mechanism limiting the detection of species using eDNA. However, due to this short lifespan, eDNA detection is thought to provide contemporaneous data on species' presence in the environment. The persistence of eDNA for aquatic taxa has been estimated at 15 to 30 days for freshwater fishes (Dejean et al., 2011; Takahara et al., 2012) and hours to 7 days for marine fishes (Weltz et al., 2017), after which time eDNA concentrations drop below the detection limit. Drivers of eDNA degradation are classified into three categories: (1) the DNA characteristic itself, including length, conformation, and association with membranous material (Taberlet et al., 2012), (2) abiotic environment, including temperature, pH, UV radiation, oxygen, and salinity (Barnes et al., 2020; Pilliod et al., 2013b; Strickler et al., 2015; Turner, Barnes, et al., 2014; Weltz et al., 2017), and (3) biotic environment, including exogenous enzymes and microbial activity (Dejean et al., 2011; Salter, 2018).

Elevated temperature has been shown to increase the biotic degradation rate of eDNA through increased microbial growth and enzymatic activity (Goldberg et al., 2018; Strickler et al., 2015). This microbial-driven degradation rate is highly dependent on the microbial

community composition and abundance and the bioavailability of phosphate, a primary energy source for microbes. Phosphate limitation, which is enhanced by elevated temperatures, has been associated with higher uptake of dissolved eDNA by microbes in marine waters (Salter, 2018). On the other hand, clays and humic acids can bind and protect eDNA from enzymatic breakdown (Eichmiller et al., 2016). The eDNA-binding ability of those agents comes at the cost of reduced extraction efficiency, if extraction methods do not account for the tight binding of DNA to clay particles and humic substances, and increased risk of PCR inhibition and false negatives. At the molecular level, UV irradiation directly disrupts DNA base-pair bonds; however, current evidence regarding UV irradiance influence on eDNA persistence in aquatic environments is inconclusive due to inconsistent findings across only a few studies. While two temperate studies reported an increase in aquatic eDNA degradation with elevated UV exposure (Pilliod et al., 2013a; Strickler et al., 2015), no relationship was reported by Mächler et al., (2018). Moreover, the increased eDNA degradation rate at elevated UV-B exposure reported by Strickler et al., (2015) was concluded to be the result of enhanced microbial growth under neutral pH and elevated temperature.

2.1.3.3. Dispersal Understanding the physical movement of eDNA in the environment is important and essential in inferring presence of the detected organism(s) in space and time (Barnes and Turner 2016). Environmental DNA represents a complex mixture of particles ranging in size and composition, which behave independently and move freely in aquatic environments. Particles of eDNA are likely to be randomly and heterogeneously distributed in the water column as a result of spatial clumping (Furlan et al. 2016). The greater the degree of clumping and uneven dispersal of target DNA the more likely it is that some samples will be negative. As a consequence, detection sensitivity for a given sampling protocol will vary temporally and spatially, between samples and from site to site, depending on the

concentration and dispersion of target DNA (Furlan et al. 2016; Weltz et al. 2017). Since eDNA occurs at very low concentration in the aquatic environment and can be heterogeneously distributed, knowledge of how eDNA moves in the environment through water movement (e.g., currents, eddies, waves) and what interacting external drivers may affect its detectability (e.g.., abiotic and biotic factors involved in eDNA persistence in the environment (Barnes et al. 2014; Jane et al. 2015; Strickler et al. 2015) is crucial for the successful detection of species in their environment. This is especially true for the detection of rare species for which eDNA concentrations are likely to be at their lowest (Takahara et al. 2012), and the risk for false negative errors high.

Long distance transport of eDNA from hundreds of meters to several kilometres have been reported in river systems, and should always be taken into account in eDNA studies in flowing waters (Deiner and Altermatt 2014; Jane et al. 2015). Although it could easily be expected that eDNA would travel even larger distances in highly dynamic systems such as open oceans or flowing rivers than in more stagnant systems such as ponds and lakes (Deiner and Altermatt 2014; Shogren et al. 2016), recent work on a dynamic marine coastline found evidence that eDNA transport was limited enough that DNA metabarcoding methods were able to detect differences among vertebrate communities separated by less than 100 m (Port et al. 2016). Also, Gargan et al. (2017) were able to detect the Chilean devil ray using a targeted eDNA approach at 4 out of 5 remote seamounts sampled around the Azores, consistent with positive visual observations. However, their failure to detect target DNA at a location where this species had been observed highlights the need for further investigations into how eDNA transport and degradation affects species detection in open ocean environments.

2.2. Conclusion

The power of eDNA as a monitoring tool for aquatic species is evident. The simplicity of eDNA sample collection coupled with the power of highly sensitive molecular methods has resulted in the rapid and widespread uptake of the methodology for a variety of purposes in academic research through to biodiversity management agencies. The utility of eDNA for rare and threatened species is especially promising, considering governments and management agencies spend thousands of dollars each year on monitoring.

Ultimately, the utility of eDNA as a tool for monitoring aquatic species is dependent on the ability to 1) detect the species in their environment through an optimised and validated eDNA workflow that is highly sensitive to low copy number and 2) the modification of the eDNA study approach to external factors, such as the physiology and space use of organisms, as well as the state and fate of eDNA in the environment. To this end, further study is required in order for the full potential of the methodology to be met.

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Chapter 3

Development, optimisation, and validation of an environmental DNA workflow for detection of threatened sawfishes

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3.1. Abstract

Sawfishes, a family of shark-like rays identified by their characteristic toothed rostra, are among the most threatened and rare elasmobranch species. Tools to reliably and accurately detect sawfish are needed to increase potential of protection and conservation. Characterisation of environmental DNA (eDNA) extracted from water samples has garnered significant appeal as a non-invasive approach for detection of rare and threatened aquatic species such as sawfishes. However, literature indicates that 1) the design primer and probe sets must be highly sensitive and specific to permit rare eDNA detection and 2) eDNA preservation and extraction workflows affect eDNA yield, which in turn can affect the ability to detect species if they are rare in the environment being surveyed. A suite of species-specific TaqMan quantitative PCR (qPCR) assays were developed and optimised for the 12S mitochondrial gene of all five sawfishes. In order to identify novel primer-probe sets with maximum species-specificity and utility for multiplexing, two separate assay design approaches were employed. *In vitro* comparison of TaqMan qPCR assays for sawfish demonstrated that assays were only specific when both primer and probe binding regions contained more than 3 base pair mismatches to closely related, sympatric species. Paired eDNA filter sample halves, collected from turbid tropical coastal waters in Northern Territory, Australia, were used to compare preservation and extraction workflows for detection of *Pristis clavata*, *P. pristis* and *Anoxypristis cuspidata*, species known to occur in the region. Paired filter halves were preserved in either Longmire's solution or ethanol. Longmire's preserved filter halves were extracted using a novel glycogen-aided precipitate-lyse-precipitate protocol. This workflow retained higher concentration of total eDNA and contained *P. clavata* eDNA in samples from three out of twenty sites. In contrast, ethanol preserved filter halves extracted using a commercial column-based extraction method yielded no detections. This study demonstrates that laboratory workflow requires

careful consideration, especially when the starting concentration of rare species eDNA is potentially low in the environment being targeted and detection success can have important conservation outcomes.

3.2. Introduction

Pressures on tropical coastal, estuarine, and riverine ecosystems are increasing, as evidenced by widespread declines and an increased number of species under threat (Davidson et al., 2012; Dulvy et al., 2021; Grant et al., 2019; Murray et al., 2022). These ecosystems are disproportionately affected by higher levels of anthropogenic impact (Halpern et al., 2008; He & Silliman, 2019). The true extent of threatened and rare species that occur at tropical latitudes remains unclear due to high levels of data deficiency. Elasmobranchs (sharks and rays) are considered one of the most poorly understood and threatened groups (Dulvy et al., 2021). Reliable cost-effective solutions are urgently needed to reduce dependence on resource-intensive and invasive sampling methods, such as fishing, baited remote underwater video (BRUVs), and fisheries-dependent surveys, if we are to mitigate their risk of extinction.

Sawfishes (family Pristidae) are amongst the most threatened marine fishes in the world and face a significant risk of extinction (Dulvy et al., 2016). Historically, sawfish populations have been negatively affected by inshore fisheries and coastal development, to which they are disproportionately exposed compared to other elasmobranchs because of their euryhaline and estuarine generalist life history traits (Grant et al., 2019). Sawfishes were once found widespread through tropical and subtropical regions of the Atlantic, Pacific, and Indian Oceans; however, contemporary presence is unknown in more than 25% of the sawfish global historical geographic range (90 countries and territories) (Dulvy et al., 2016; Yan et al., 2021). Following drastic declines in population numbers, sawfishes were listed as either Endangered (dwarf sawfish, *Pristis clavata*; narrow sawfish, *Anoxypristis cuspidata*) or Critically Endangered (largetooth sawfish, *Pristis pristis*; green sawfish, *Pristis zijsron*; smalltooth sawfish, *Pristis pectinata*) on the IUCN Red List of Threatened Species (Carlson et al., 2013; D'Anastasi et al., 2013; Kyne, Carlson, et al., 2013; Kyne, Rigby, et al., 2013; Simpfendorfer, 2013). Despite significant conservation and management efforts, the ability to resolve the uncertainty of

occurrence and implement effective safeguards is limited by the difficulty of detecting sawfishes in the coastal and riverine habitats where they are known to occur (Dulvy et al., 2016; Everett et al., 2015; Haque et al., 2020; Leeney, 2016, 2017; Yan et al., 2021)

Techniques that can rapidly and reliably detect threatened or rare species, such as environmental DNA (eDNA) methods, are necessary for accurate population assessment and monitoring. The use of eDNA to detect rare and threatened aquatic species is promoted by conservation geneticists as a vanguard approach to address conservation and management issues (Barnes & Turner, 2016; Thomsen & Willerslev, 2015). eDNA is the sum of DNA shed from multiple organisms that, when isolated from an environmental sample (e.g., filtration of seawater), provides a molecular signature of all organisms within that environment (Taberlet et al., 2012). Beneficially, eDNA sample collection is non-invasive and does not require permits that can otherwise limit studies undertaken on legislatively protected species. To date, eDNA surveys have facilitated detection of a range of elasmobranchs with different levels of conservation concern, including largetooth sawfish (Simpfendorfer et al., 2016), Maugean skate *Zearaja maugeana* (Weltz et al., 2017), Chilean devil ray *Mobula tarapacana* (Gargan et al., 2017), great white shark *Carcharodon carcharias* (Lafferty et al., 2018), bull shark *Carcharhinus leucas* (Schweiss et al., 2020), smalltooth sawfish (Lehman et al., 2020), and blacktip sharks *Carcharhinus limbatus* (Postaire et al., 2020). For blacktip sharks, eDNA surveys were shown to yield temporal and spatial results equivalent to extensive fishing surveys and acoustic telemetry (Postaire et al., 2020), highlighting the potential of eDNA for monitoring shark populations. Environmental DNA surveys have also recovered population level information for the whale shark *Rhincodon typus* (Dugal et al., 2021; Jensen et al., 2021; Sigsgaard et al., 2017), documented rare and threatened sharks and rays across vast coastlines (West et al., 2021) and on coral reefs (Bakker et al., 2017; Boussarie et al., 2018) via metabarcoding.

Since the inception of eDNA as a detection tool, significant effort has been expended to increase the robustness, sensitivity, and specificity of targeted eDNA assays for detection programs (Furlan et al., 2015; Wilcox et al., 2013) and, as a result, eDNA-based surveys provide an increased potential for successful detection of rare species of conservation concern. However, the variety of eDNA workflows available can complicate the selection of approaches for studying specific taxa in different environments (Huerlimann et al., 2020; Lear et al., 2018; Shaw et al., 2017; Taberlet et al., 2018). In general, eDNA workflows are chosen and optimised to suit the study objective. For example, presence-absence surveys that target rare or low abundance species require optimisation for maximum eDNA recovery and detection sensitivity (Piggott, 2016), while studies aimed at relative abundance quantification (i.e., biomass estimate) require optimisation for precise and consistent eDNA recovery (Deiner et al., 2015). Regardless of study aim, it is imperative to ensure eDNA preservation and extraction workflows are optimised for minimal risk of post-collection degradation as this can lead to false negative detections. This is particularly relevant when starting eDNA concentrations are low in the environment being surveyed.

The extraction of eDNA from filtered samples is most commonly achieved through the use of commercial kits, such as DNeasy Blood & Tissue or PowerWater (QIAGEN, Hilden, Germany), or through the use of in-house solutions, such as salt-alcohol precipitation and phenol-chloroform-isoamyl alcohol phase separation (reviewed by Kumar et al., 2020 & Lear et al., 2018). The advantage of commercial kit extraction methods is that protocols are simple, streamlined, provide standardisation across research groups and, therefore, yield comparable results. In comparison, in-house extraction methods are generally cheaper and provide greater eDNA yield than column-based methods, which is particularly advantageous for large-scale studies (i.e., large sample size) targeting rare species (i.e., low-copy eDNA) (Natarajan et al., 2016; Renshaw et al., 2014). It is also noted that different methods and adaptations to protocols,

which is common for groups using commercial kits, can produce differences in eDNA yield and species detectability (Deiner et al., 2015, 2018; Djurhuus et al., 2017; Eichmiller et al., 2016; Roose-Amsaleg et al., 2001). As a result, the importance of testing and optimising workflows for the study objective has been emphasised. A novel glycogen-aided precipitation-lysis-PEG precipitation protocol was recently validated for eDNA detection of tropical aquatic and amphibious species using both filtered and unfiltered water samples (Edmunds & Burrows, 2020). The glycogen-aided precipitation workflow circumvents the use of acutely harmful chemicals (e.g., phenol and chloroform) and provides an alternative to commercial column-based kits for eDNA extraction from unfiltered and filtered samples; however, has yet to be directly compared with column-based extraction for detectability of eDNA of rare species from filter samples.

The efficiency of different eDNA preservation methods have also been tested on filter samples. Submersion in ethanol or lysis buffer solutions containing salts (e.g., Tris-HCl, EDTA) and detergents (e.g., SDS) are effective preservation mediums when cold-chain storage is logistically impractical or unachievable (reviewed by Shaw, Weyrich, & Cooper, 2017). Minamoto, Naka, Moji, & Maruyama, (2015) demonstrated effective preservation of eDNA stored in ethanol at ambient temperature for several days; however, dangerous goods regulations and rapid evaporation of ethanol in ambient temperatures above 30°C limit the practicality of ethanol preservation in remote and/or tropical sampling locations. Moreover, the effectiveness of ethanol for preserving eDNA samples stored under tropical temperatures (e.g., >30°C) and remote conditions (e.g., >2 weeks transit) have yet to be empirically tested. Drying eDNA samples with silica gel or beads have also demonstrated efficacy for preserving eDNA in filter samples at ambient temperature (Majaneva et al., 2018); however, dried eDNA samples may be vulnerable to degradation over time if storage conditions are not kept constant making them a less attractive preservation option. Longmire's solution (Longmire, Maltbie, & Baker,

1997) was recently shown to effectively preserve eDNA in filter samples subjected to ambient and temperatures above 40°C for two to six weeks (Edmunds & Burrows, 2020; Renshaw et al., 2014), and seamlessly integrates into the PPLPP workflow. Longmire's solution can be made in-house at relatively low cost, is non-hazardous, and can be transported without restriction. It is apparent that tests comparing the efficacy of workflows for the detection of rare, threatened species is important for improving confidence in results gleaned from eDNA surveys.

The aim of this study was to improve detectability of sawfish eDNA through optimising filter sample preservation and extraction. Ethanol preservation coupled with a commercial column-based extraction (DNeasy Blood and Tissue Kit, QIAGEN) workflow was directly compared to the more recently described glycogen-aided precipitation workflow (Edmunds & Burrows, 2020). More specifically, total eDNA yields and quantitative PCR (qPCR) detection rates obtained from paired filter sample halves collected in remote river and estuarine environments in Van Diemen Gulf and Darwin Harbour, Northern Territory (NT), Australia were compared across workflows. To ensure optimal species-specificity of qPCR assays, a suite of TaqMan-based assays were designed, *in silico,* and *in vitro* validated for Indo-West Pacific sawfish species. I provide a recommendation for an optimal eDNA preservation and extraction workflow for enhanced detectability of rare species.

3.3. Methods

3.3.1. Assay design

3.3.1.1. Tissue samples and sequence generation All currently available mitochondrial nucleotide sequences for sawfishes (Table 3.1), as well as closely-related, sympatric elasmobranch species (i.e., exclusion species; Table 3.2), were obtained from NCBI

GenBank nucleotide database (National Center for Biotechnology Information; Agarwala et al., 2018) and compiled into a reference database using Geneious 10.2.6 software (http://www.geneious.com). For assay design, the mitochondrial *12S* ribosomal RNA gene (*12S*) was selected based on high interspecific sequence divergence.

To supplement available *12S* nucleotide sequence data for assay design, 21 tissue samples were obtained from previously sampled sawfish populations in northern Australia (Table 3.1), as well as exclusion species (giant guitarfish *Glaucostegus typus*, $n = 1$; bottlenose wedgefish *Rhynchobatus australiae*, $n = 1$; tiger shark *Galeocerdo cuvier,* $n = 1$). Samples were stored in ≥95% ethanol solution. Genomic DNA was extracted from tissue samples using standard cetyltrimethylammonium bromide (CTAB) protocol (Doyle & Doyle, 1987) with a 12-hour incubation period. To do this, an approximately 5 mm^2 piece of tissue (i.e., fin clip or muscle biopsy) was dissected from the original sample using sterile scissors and forceps and transferred a new 2 mL microcentrifuge tube (Axygen, California, US) where it was cut into smaller pieces to increase surface area for tissue digestion. To each sample, 700 µL CTAB buffer (2% (w/v) cetyltrimethylammonium bromide, 100 mM Tris HCl pH 8, 20 mM EDTA, 1.4 M NaCl) and 10 µL of proteinase K (20 mg/mL) enzyme was added, and tubes were vortexed briefly before overnight digestion for 12 hours at 65°C. To extract the nucleic acid, 700 µL of chloroform-isoamyl was added, tubes vortexed and centrifuged for 10 min at 16,000 g. Approximately 600 µL of the aqueous layer was carefully transferred to a new 2 mL microcentrifuge tube with 600 µL of chloroform-isoamyl and vortexed and centrifuged as before for a second clarification. Approximately 600 µL of the aqueous layer was transferred to a new 2 mL microcentrifuge tube with 600 µL of cold (-20°C) isopropanol and mixed by inversion. Samples were incubated at room temperature for 30 min and the precipitant was pelleted for 30 min at 16,000 g at 4°C. The pellet was washed twice with 1 mL ≥70% ethanol for 10 min at 16,000 g at 4°C. Extracted DNA from each sample was eluted in 100 µL MilliQ

water (Barnstead, ThermoFisher Scientific, Victoria, Australia). Following extraction each sample was briefly vortexed before 1 µL sub-samples were taken for quality check using NanoDrop 2000 Spectrophotometer (ThermoFisher Scientific, Victoria, Australia) and quantification using Qubit Fluorometer (ThermoFisher Scientific, Victoria, Australia). Extracted samples were stored at -20°C.

A 465–468 bp fragment was amplified using end-point PCR and previously published *12S* primers (Phillips, Chaplin, Morgan, & Peverell, 2009). Specifically, PCR was carried out in a SimpliAmp Thermal Cycler (ThermoFisher Scientific, Victoria, Australia) with 2 µL of template, 2× MyTaq Red Mix (Bioline Reagents, Alexandria, Australia), 0.8 µM forward (12SF: 5'-CAAACTAGGATTAGATACCC-3') and reverse (12SR: 5'CACTTACCATGTTACGACTT-3') primer (Phillips et al., 2009), and adjusted to a final volume of 20 µl with PCR-grade water. PCR cycling conditions consisted of an initial denaturation step at 95°C for 5 min, followed by 30 cycles of 95°C for 30 s, primer annealing at 55°C for 30 s, and extension at 72°C for 30 s, with a final extension at 72°C for 7 min. PCR amplicons were sent to the Australian Genome Research Facility (AGRF; Brisbane, Australia) for paired-end BDT (BigDye™ terminator) labelling reaction, clean-up and Sanger sequencing. Sequences were downloaded, aligned, trimmed, and final consensus sequence compared to all other compiled 12S sequences in Geneious 8. All tissue samples used in this study were provided by collaborators from previous studies conducted under appropriate scientific sampling permits and were stored and shipped dry, or in ethanol using appropriate CITES permits.

Table 3.1. A list of sawfish species, country of origin, and NCBI (National Center for Biotechnology Information) accession numbers for all available *12S* mitochondrial gene sequences that were used in assay design. Accession numbers for sawfish and exclusion species partial *12S* sequences that were generated for this study are also provided, including

country of origin. Tissue samples were provided by collaborators from previous studies conducted under appropriate scientific sampling permits and were also used for in vitro assay testing. UN denotes unknown.

Species	Accession Number	Country of origin	Purpose
Sequences available on NCBI			
Anoxypristis cuspidata	KP233202.1	Australia	Assay design
	AF447988.1	UN	Assay design
Pristis zijsron	EU784162.1	Australia	Assay design
Pristis clavata	JN184072.1	UN	Assay design
	KF381507.1	Australia	Assay design
	EU784161.1	Australia	Assay design
Pristis pristis	SRX821566	UN	Assay design
	EU784160.1	Australia	Assay design
	LC020849.1	UN	Assay design
Sequences generated from the tissue bank obtained for this study			
Anoxypristis cuspidata	MN795516	Papua New Guinea	Assay design
	MN795517	Papua New Guinea	Assay design
	MN795518	Papua New Guinea	Assay design
	MN795519	Papua New Guinea	Assay design
	MN795520	Papua New Guinea	Assay design
	MN795521	Papua New Guinea	Assay design
	MN795522	Papua New Guinea	Assay design; *in vitro* testing
	MN795523	Papua New Guinea	Assay design; *in vitro* testing
Pristis zijsron	MN795535	UN	Assay design; *in vitro* testing
	MN795534	Australia	Assay design; *in vitro* testing
Pristis clavata	MN795526	Australia	Assay design; *in vitro* testing
	MN795527	Australia	Assay design; *in vitro* testing
Pristis pristis	MN795528	Australia	Assay design; *in vitro* testing
	MN795529	UN	Assay design
	MN795530	Papua New Guinea	Assay design
	MN795531	Papua New Guinea	Assay design
	MN795532	Papua New Guinea	Assay design
	MN795533	Papua New Guinea	Assay design; *in vitro* testing
Pristis pectinata	MN814039	United States	Assay design; *in vitro* testing
	MN814040	United States	Assay design
	MN814041	United States	Assay design; *in vitro* testing
	MN814042	United States	Assay design; *in vitro* testing
Galeocerdo cuvier	MN795524	Australia	Assay design; *in vitro* testing
Glaucostegus typus	MN795525	Australia	Assay design; *in vitro* testing
Rhynchobatus australiae	MN795536	Australia	Assay design; *in vitro* testing

Table 3.2. Species list used to create a reference database, including common name, scientific name, and relevant synonyms. Mitochondrial gene sequences of sawfishes and sympatric and/or closely related elasmobranch species (referred to as exclusion species) were retrieved from NCBI (National Center for Biotechnology Information) Genbank nucleotide database on 23 January 2017. The number of each sequence is recorded here. The number of mitochondrial genes and genomes is also listed.

Common name	Genus	Species	Synonyms	Cyt b	COI	12s	ND2	ND4	18S	16S	Complete Mitochondrial Genome
Pacific eagle ray	*Aetobatus*	*laticeps*			23		2				1
Spotted eagle ray	*Aetobatus*	*ocellatus*	*Aetobatus guttatus, Myliobatus ocellatus*				1				
Bull ray	*Aetomylaeus*	*bovinus*	*Myliobatus bovina, Pteromylaeus bovina, Pteromylaeus bovinus*								
Narrow sawfish	*Anoxypristis*	*cuspidata*	*Pristis cuspidata*	4	7	1	5	1			1
Eastern shovelnose ray	*Aptychotrema*	*rostrata*	*Raja rostrata*				1	23			
Pigeye shark	*Carcharhinus*	*amboinensus*			61			28			2
Bronze whaler	*Carcharhinus*	*brachyurus*			12	1	1				
Spinner shark	*Carcharhinus*	*brevipinna*			108	4	1	50		1	2
Creek whaler	*Carcharhinus*	*fitzroyensis*			2		1	1			
Blacktip reef shark	*Carcharhinus*	*melanopteris*			47	2	3				2
Common blacktip sharl	*Carcharhinus*	*limbatus*									
Bull shark	*Carcharhinus*	*leucas*	*Carcharias leucas*		43	3	1	10			3
Spot-tail shark	*Carcharhinus*	*sorrah*			125	2	4	30			
Australian swellshark	*Cephaloscyllium*	*laticeps*	*Scylloium laticeps*		1		1				
Colares stingray	*Fluvitrygon*	*colarensus*	*Fontitrygon colarensis, Dasyatis colarensis*								
Marbled whipray	*Fluvitrygon*	*oxyrhynchus*	*Himantura oxyrhyncha, H. krempfi, H. oxyrhynchus, Trygon oxyrhynchus*				1				
Roughbaack whipray	*Fluvitrygon*	*kittipongi*	*Himantura kittipongi*				1				
White-edge whipray	*Fluvitrygon*	*signifer*	*Himantura signifer*	1			1				
Daisy whipray	*Fontitrygon*	*margarita*	*Dasyatis margarita, Trygon margarita*				1				
Pearl whipray	*Fontitrygon*	*margaritella*	*Dasyatis margaritella*				1				
Tiger shark	*Galeocerdo*	*cuvier*			6						
Tope shark	*Galeorhinus*	*galeus*		1	23	1	2	1		1	
	Glaucostegus	*granulatus*					1				5

	Glaucostegus	*typus*			4		1			1	6
	Glaucostegus	*cemiculus*	*Rhinobatos cemiculus*		2		1				200
Ganges shark	*Glyphis*	*gangeticus*									2
Northern river shark	*Glyphis*	*garricki*	*Glyphis sp. C*	1	8		1	1			
Speartooth shark	*Glyphis*	*glyphis*	*Carcharias glyphis, G. sp. nov. A*	11	3		1	1		1	
Irrawaddy river shark	*Glyphis*	*siamensis*	*Carcharias siamensis*	8			1				1
Australian butterfly ray	*Gymnura*	*australis*	*Pteroplatea australis*		3	1		1			
Californian butterfly ray	*Gymnura*	*marmorata*	*Pteroplatea marmorata*		3		2	1	1		
Longsnout butterfly ray	*Gymnura*	*crebripuncta ta*	*Pteroplatea crebripunctata, P. rava*								
Estuary stingray	*Hemitrygon*	*fluviorum*	*Dasyatis fluviorum*		2		1		1		
Southern stingray	*Hypanus*	*americanus*	*Dasyatis americana*				1	1			
Large-eye stingray	*Hypanus*	*marianae*	*Dasyatis marianae*					1			
Atlantic stingray	*Hypanus*	*sabinus*	*Dasyatis sabina, Trygon sabina*		15		1			1	
Bluntnose stingray	*Hypanus*	*say*	*Dasyatis say, D. sayi, Raja say*		11	1					
Coffin ray	*Hypnos*	*monopterygi us*			6			1			
Blackspotted whipray	*Maculabatis*	*astra*	*Himantura astra*								
Round whipray	*Maculabatis*	*pastinacoide s*	*Himantura pastinacoides, H. pareh, Trygon pastinacoides*		33	3	2	1			1
Brown whipray	*Maculabatis*	*toshi*	*Himantura toshi*		2	1					
Chindwin cowtail ray	*Makararaja*	*chindwinensi s*			8		4	17	1	1	
Giant manta ray	*Mobula*	*birostris*	*Manta birostris, Manta hamiltoni, Raja birostris*		23		1	1			
Reef manta ray	*Mobula*	*alfredi*	*Manta alfredi, Deratoptera alfredi, M. fowleri*								
Gummy shark	*Mustelus*	*antarcticus*			8		1		2	1	2
Common eagly ray	*Myliobatis*	*aquila*	*Raja aquila*								
Sharptooth lemon shark	*Negaprion*	*acutidens*		2	7	2	1				
Broadnose sevengill shark	*Notorynchus*	*cepedianus*	*Squalus cepedianus*		21		3	2		1	2
Discus stongray	*Paratrygon*	*aiereba*			5						2
Cowtail ray	*Pastinachus*	*sephen*	*Dasyatis sephen, Himantura fluviatilus, Hypolophus sephen, Raja sephen*	1	1						
Broad cowtail ray	*Pastinachus*	*ater*	*Pastinachus atrus, P. sephen, Taeniura atra*								
Hortle's whipray	*Pateobatis*	*hortlei*	*Himantura hortlei*								

Antenna ray	*Plesiotrygon*	*iwamae*		14	40	1					2
Rough freshwater stingray	*Potamotrygon*	*constellata*	*Taeniura constellata*								
False reticulate freshwater stingray	*Potamotrygon*	*humerosa*		1	6						
Ocellate freshwater stingray	*Potamotrygon*	*motoro*	*P. pauckei, Taeniura motoro*		4						
Marajo freshwater stingray	*Potamotrygon*	*ocellata*	*Trygon ocellata*		2						
Reticulate freshwater stingray	*Potamotrygon*	*orbignyi*	*P. dumerilii, P. humerosa, Trygon orbignyi*	1	3	1	1				1
Schroeder's freshwater stingray	*Potamotrygon*	*schroederi*		3	1	1	1	4			
Whitespotted freshwater stingray	*Potamotrygon*	*scobina*		6			2			1	1
Dwarf sawfish	*Pristis*	*clavata*		1		1	2	2			92
Green sawfish	*Pristis*	*zijsron*									
Smalltooth sawfish	*Pristis*	*pectinata*		4	7	1	2		1	1	
Largetooth sawfish	*Pristis*	*pristis*	*P. microdon, P. perotteti, P. zephyreus, Squalus pristis*				1	1			
Shovelnose huitarfish	*Pseudobatos*	*productus*	*Rhinobatos productus*								
	Pseudobatos	*lentiginosis*	*Rhinobatos lentiginosis*								
American cownose ray	*Rhinoptera*	*bonasus*	*Raja bonasus, Rhinoptera lalandii, Rhinoptera affinis*								
Australian cownose ray	*Rhinoptera*	*neglecta*			1		1				
Milk shark	*Rhizoprionodon*	*acutus*									
Australian sharpnose shark	*Rhizoprionodon*	*taylori*									
Whitespotted guitarfish	*Rhynchobatus*	*australiae*									
Giant guitarfish	*Rhynchobatus*	*djiddensis*									
Broadnose wedgefish	*Rhynchobatus*	*springeri*	*Rhynchobatus sp. nov. B*	1	1		1				1
Smoothnose wedgefish	*Rhynchobatus*	*laevis*	*Rhinobatus laevis*	1			1				
Scalloped hammerhead shark	*Sphyrna*	*lewini*			3	1	1		1		
Winghead shark	*Sphyrna*	*blochii*									
Great hammerhead shark	*Sphyrna*	*mokarran*						1			2
Piked dogfish	*Squalus*	*acanthias*		4			1				
Atlantic chupare	*Styracura*	*schmardae*	*Himantura schmardae*		3		1	1			2

Bullseye round ray	*Urobatis*	*concentricus*	*Urolophus concentricus*				2	1				
Yellow round ray	*Urobatis*	*jamaicensis*	*Urolophus jamaicensis*									
Tumbes round ray	*Urobatis*	*tumbesensis*										
Freshwater whipray	*Urogymnus*	*dalyensis*	*Himantura dalyensis*		23		2					1
Giant freshwater whipray	*Urogymnus*	*polylepsis*	*Himantura chaophyra, H. polylepsis, Trygon polylepsis*				1					
Mangrove whipray	*Urogymnus*	*granulatus*	*Himantura granulata, H. ponapensis, Trygon granulata*									
Tubemouth whipray	*Urogymnus*	*lobistomus*	*Himantura lobistoma*	4	7	1	5	1				1
Dwarf round ray	*Urotrygon*	*nana*					1	23				

For closely related exclusion species lacking tissue samples to assist *in vitro* assay specificity testing, double-stranded synthetic DNA fragments (sDNA; gBlocks™; Integrated DNA Technologies Pty Ltd, New South Wales, Australia) were synthesised. In addition, sDNA fragments were synthesised for target sawfish species for determination of assay limit of detection (LOD). Species-specific sDNA fragments (180−190 bp) were created based on a consensus of *12S* nucleotide sequences for each species and contained a mid-sequence modification (8–12 bp reversal) to permit differentiation of sDNA and gDNA amplicons via Sanger sequencing as a cross-contamination control (Table 3.3). Dried sDNA pellets were resuspended following manufacturer instructions. Stock concentrations were subsequently measured using Quantifluor® dsDNA system with a Quantus™ Fluorometer (Promega Corporation, Wisconsin, USA) and converted to copies per µL using double-stranded molecular weight (http://www.bioinformatics.org/sms2/dna_mw.html), specific nucleotide sequence, and Avogadro's constant (6.022×10^{23}).

Table 3.3. Sequences used to construct synthetic DNA fragments from partial *12S* mitochondrial gene region of sawfishes and other elasmobranchs used for specificity testing. Bold sequence denotes location of nucleotide sequence reversal.

Species	Sequence	Nucleotide base pairs
Pristis pristis	TTCAAACCCAAAGGACTTGGCGGTGCCTCAGACCCACCTAGAGGAG CCTGTTCTATAACCGATAATCCCCGTTAAACCTCACCACTTCTTGCCA TCAACCGCCTATATACC**TGCTGCCG**CAGCTCACCCCATGAGGGAAC AAAAGTAAGCAAAATGAACTAACCTTCAATACGTCAGGTCGAGGTGT AGCGAATGAAGTGGAAAGAAATGGGCTACATTTTCTCCTAAGAAAAA ACGAACAGTATGATGAAAAACTACTT	260
Pristis zijsron	ACCCAAAGGACTTGGCGGTGCCTTAGATCCACCTAGAGGAGCCTGT TCT**AAATAGCCA**ATTCCCCGTTAAACCCCACCACTTCTTGCTATCAA CTGCCTATATACCGCCGTCGTCAGCTCACCCCATGAGGGGTTAAAA GTAAGCAAAATGAATCTATCTTCAATACGTCAGGTCGAGGTGTAGCG AATGAA	192
Pristis clavata	GCTTCAAACCCAAAGGACTTGGCGGTGCCTTAGATCCACCTAGAGG AGCCTGTTCTATAACC**CCTAATAG**CCGTTAAACCTCACCATTTCTTG CTATCAACCGCCTATATACCGCCGTCGTCAGCTCACCCCATGAGGG AACAAAAGTAAGCAAAAAGAACCCACCTTCAATACGTCAGGTCGAGG TGTAGC	192
Pristis pectinata	CCCAAAGGACTTGGCGGTACCTTAGATCTCTCTAGAGGAGCCTGTT CTATAACCGATAA**AATTGCCCCT**ACCTCACCATTCCTTGCTATTAACC GCCTATATACCGCCGTCGTCAGCTCACCCCATGAGGGAGTAAAAGT	192

	AAGCAAAATGGACTCATTCTCCAATACGTCAGGTCGAGGTGTAGCGA ATGAA	
Anoxypristis cuspidata	GTGCCCCAGACCCACCTAGAGGAGCCTGTTCTATAACCGATAATCC CCGTTAAACCTCACCACTTCTTGCCACTAACCGCCTATATACCGCCG TCGTCAGCTCACCCCAT**AAAACAAGGGAG**GTAAGCAAAATGGATTA ACCTCCAACACGTCAGGTCGAGGTGTAGCGAATGAAGTGGA	180
Galeocerdo cuvier	GTATCCCATACCCACCTAGAGGAGCCTGTTCTATAACCGATAATCCT CGTTTAACCTCACCATTTCTTGCCACTACCGTCTATATACCGCCGTC GTCAGCTCACCCTGT**AAAATCAGGAAG**GTAAGCAAAAAGAATAAAA CTTCAAAACGTCAGGTCGAGGTGTAGCAAACGAAATGGGAAGAAAT GGG	189
Rhinobatos lentiginosus	GTGCCTCAGACCCACCTAGAGGAGCCTGTTCTATAACCGATAATCCC CGTTAAACCTCACCACTTGTTGCCAACAACCGCCTATATACCGCCGT CGTCAGCTCACCCCAT**AAAATAAGGGAG**GTAAGCAAAATGAACTCA CCTTCAATACGTCAGGTCGAGGTGTAGCGAATAAAGTGGA	180
Rhinobatus cemiculus	GTACCCCAGACCCACCTAGAGGAGCCTGTTCTATAACCGATAATCCC CGTTAAACCTCACCACTTCTTGCCATCAACCGCCTATATACCGCCGT CGTCAGCTCACCCCAT**AAAACAGGGGAG**GTAAGCAAAATGGGCCC ACCCCAATACGTCAGGTCGAGGTGTAGCGAATGAAGTGGAA	180
Etmopterus sentosus	GTACTCCAGACCCCCCTAGAGGAGCCTGTTCTATAACCGATAATCCC CGTTAAACCTCACCACTTCTTGCTAACTCCGCCTATATACCGCCGTC GTCAGCTCACCCTAT**AAAAATAGGAAG**GTAAGCAAAAAGAATTACCT CCCATACGTCAGGTCAAGGTGTAGCGAATGAAGTGGAAA	180
Rhynchobatus australiae	GTGCCCCAGACCCCCCTAGAGGAGCCTGTTCTATAACCGATAATCC TCGTTTAACCTCACCACTTCTTGCCATTAACCGCCTATATACCGCCG TCGTCAGCTCACCCCAT**AAAACAGGGGAG**GTAAGCAAAATGAATTA AACCTCCAACACGTCAGGTCGAGGTGTAGCGAATGAAGTGG	180
Rhynchobatus djiddensis	GTGCCCCAGACCCCCCTAGAGGAGCCTGTTCTATAACCGATAATCC TCGTTAAACCTCACCACTTCTTGCCATTAACCGCCTATATACCGCCG TCGTCAGCTCACCCCAT**AAAACAAGGGAG**GTAAGCAAAATGAATTA AACCTCCAATACGTCAGGTCGAGGTGTAGCGAATGAAGTG	179

3.3.1.2. *In silico* validation Assay optimisation involved design and comparison of two assay types that differed in number of base pair mismatches in either primer- or probe-binding regions to closelyrelated sympatric species. The two assay design approaches were used to test and ensure species-specificity of final assay. The first assay utilised a "universal sawfish" outer primer pair that targeted a 113 bp region of *12S* conserved across all five sawfish species in conjunction with a species-specific TaqMan probe (one per species) that targeted a species-specific *12S* region (i.e., probe-driven assays; relies on specificity of the probe to target). The second approach used the same five species-specific probes but each probe was used in conjunction with a species-specific primer pair that targeted 110–179 bp regions of *12S* (Table 3.4). These species-specific primers were designed to maximise 3' bp mismatches (i.e., ≥1 per primer) between target and non-target species (i.e., primer-driven assays; Wilcox et al. 2013), with the exception of *P. zijsron* and *P. clavata* as these two assays utilise the same

forward primer. Species-specific probes were labelled with 5' fluorophore (VIC or FAM) and a 3' minor groove binding non-fluorescent quencher (MGB-NFQ). Existing primers for *Pristis* spp. (Simpfendorfer et al., 2016) were not considered for this study as they are not species-specific.

Specificity of candidate primer and probe sequence to target sawfish nucleotide *12S* sequence was initially verified *in silico* using NCBI PrimerBLAST (Ye et al., 2012), BLASTn (Altschul et al., 1990) and AmplifX 1.7.0 (https://inp.univ-amu.fr/en/amplifx-manage-test-and-design-your-primers-for-pcr). Primer pair melting temperature and dimer scores were assessed using a combination of Primer3 (Untergasser et al., 2012), OligoAnalyzer (Owczarzy et al., 2008), and AmplifX 1.7.0. to best understand primer performance because scores varied between programs.

Table 3.4. Primer and TaqMan probe sequence information for the two assay types designed for detection of sawfish eDNA in environmental samples.

Target	Oligo	Sequence (5'–3')	[Final] (nM)	T_m (°C)	GC content (%)	Oligo length (bp)	Amplicon length (bp)
Elasmobranch-generic primer	F	AGAGGAGCCTGTTCTATAACCG	500*	60.7	50	22	112
	R	ATTCGCTACACCTCGACCT	500*	58.1	52.6	19	
Anoxypristis cuspidata	F	TGCCCCAGACCCACCTAGA	500	61.9	63.2	19	114
	R	CCTGACGTGTTGGAGGTTAATC	500	59	50	22	
	P	VIC-TTCTTGCCACTAACCG-MGBNFQ	250		74.1	17	
Pristis clavata	F	GGTGCCTTAGATCCACCTAGAG	300	59.4	54.5	22	111
	R	CTGACGTATTGAAGGTGGGTTCT	300	60.8	47.8	23	
	P	FAM-CATTTCTTGCTATCAACC-MGBNFQ	250		38.9	18	
Pristis pristis	F	GTGCCTCAGACCCACCTAGA	300	60.6	60	20	179
	R	CATCATACTGTTCGTTTTTTCTTAGGAG	300	59.1	59.1	28	
	P	VIC-AAATGAACTAACCTTCAATACG-MGBNFQ	250		31.8	22	
Pristis zijsron	F	GGTGCCTTAGATCCACCTAGA	500	58.3	52.4	21	115
	R	CGACCTGACGTATTGAAGATAGAT	500	58	58	24	
	P	FAM-CCCACCACTTCTTGCTAT-MGBNFQ	250		50	18	
Pristis pectinata	F	GGCGGTACCTTAGATCTCTCTAG	300	59	52.2	23	119
	R	CGACCTGACGTATTGGAGAATG	300	58.9	50	22	
	P	FAM-CCTTGCTATTAACCGCC-MGBNFQ	250		52.9	17	

3.3.1.3. *In vitro* validation and optimisationThe specificity of each assay was tested *in vitro* against ~100 ng gDNA or ~10,000 copies sDNA derived from target or exclusion species using end-point PCR and qPCR. End-point PCR assays were first used to validate that the species-specific primer pairs successfully amplified only the target sawfish species. Triplicate PCR assays were run using SimpliAmp Thermal Cycler (Life Technologies, ThermoFisher Scientific, Victoria, Australia) and each reaction contained 1 µL template, 5 µL 2× MyTaq™ Red Mix (Bioline Pty. Ltd., Alexandria, Australia), 0.4 µM forward and reverse primer and adjusted to a final volume of 10 µl with MilliQ water. A standard 3-step cycling profile was used: 95°C for 1 min, followed by 30 cycles of 95°C for 15 s and 60°C for 15 s and 72°C for 10 s. Subsequently, 1 µL of the final product was run on a 1.5% agarose gel stained with GelGreen (Biotium Inc., California, USA) alongside 2 kB ladder (EasyLadder I, Bioline Pty. Ltd, Alexandria, Australia) for visual confirmation of correct amplicon size. Each qPCR assay was then tested for species-specificity when used with its respective species-specific probe (Table 3.4). Triplicate qPCR assays were run in adjacent wells on a 96-well plate using

the QuantStudio3 qPCR system (Life Technologies, ThermoFisher Scientific, Victoria, Australia). Each 20 µL reaction contained 5 µL template, 10 µL 2× TaqPath ProAmp Multiplex Master Mix (Applied Biosystems, ThermoFisher Scientific, Victoria, Australia), 0.9 µM forward and reverse primer, 0.25 µM probe and adjusted to 20 µL with MilliQ water. Cycling conditions consisted of initial denaturation at 95°C for 10 min, followed by 50 cycles of 95°C for 15 s and 60°C for 1 min. QuantStudio Analysis Software version 1.4.2 was used to analyse threshold cycle value (C_t) based on manually determined baseline and threshold fluorescence values. Any amplicons produced from exclusion species gDNA or sDNA template were Sanger sequenced bidirectionally for verification using the same primers.

Probe-driven assays amplified exclusion species gDNA or sDNA during qPCR *in vitro* tests and, as such, this precluded further *in vitro* testing against target sawfish gDNA or sDNA and *in situ* validation (Table 3.5). All primer-driven assays demonstrated specificity to the target sawfish species across all *in vitro* validation tests and were therefore used in *in situ* validation.

Table 3.5. Summary of in vitro validation results of primer-driven and probe-driven assays against exclusion species gDNA and sDNA, including amplification success depending on cumulative number of base-pair mismatches between primer and probe binding regions to non-target template. Only three closely-related exclusion species examples included here for demonstration. * not sympatric; ** historically sympatric.

Assay target	Primer-driven assays			Probe-driven assays		
	Exclusion species	No. of mismatches	Amplification (Y/N)	Exclusion species	No. of mismatches	Amplification (Y/N)
Anoxypristis cuspidata	*Rhynchobatos australiae*	5	N	*R. australiae*	1	Y
	P. pristis	7	N	*R. djiddensis*	1	Y
	P. clavata	14	N	*Galeocerdo cuvier*	2	Y
Pristis clavata	*P. zijsron*	6	N	*P. zijsron*	2	Y
	P. pristis	7	N	*Glaucostegus cemiculus**	2	Y
	A. cuspidata	14	N	*A. cuspidata*	4	Y
Pristis pectinata	*P. zijsron**	10	N	*R. djiddensis**	2	Y
	*P. clavata**	10	N	*P. clavata**	2	Y

	*P. pristis***	13	N	*Rhynchobatos australiae**	3	N
Pristis pristis	*P. zijsron*	13	N	*P. lentiginosus***	1	Y
	P. clavata	9	N	*P. clavata*	3	Y
	A. cuspidata	11	N	*R. djiddensis**	4	N
Pristis zijsron	*P. clavata*	5	N	*P. pristis*	2	Y
	P. pristis	8	N	*R. australiae*	2	Y
	*P. pectinata**	11	N	*A. cuspidata*	3	Y

Primer concentrations were adjusted for primer-limiting reactions and ease of future multiplexing and were tested using qPCR conditions as described above. Forward and reverse primer concentrations were independently varied (36 combinations; final concentrations of 50, 100, 300, 500, 700, 900 nM) with constant probe concentration (250 nM) and fixed gDNA template amount that represented low concentrations expected to be captured in eDNA field samples (0.1 ng). The primer combination that resulted in the lowest C_t value while maintaining high end-point fluorescence and target specificity and sensitivity was selected for each assay.

Amplification efficiency (E) and LOD were determined for each assay using standard curves generated by 8-point $\log_{10}$ (10^8 to 10 copies per assay) and 3-point log2 (5, 2.5, and 1.25 copies per assay) serial dilution of species-specific sawfish sDNA to ensure each assay could amplify the entire dynamic range of potential eDNA concentrations (MIQE Guidelines; Bustin et al., 2009). Estimates of E were calculated from the serial dilution series using QuantStudio Analysis Software; however, it is acknowledged that *in vitro* assay efficiency using sDNA template might not be indicative of *in situ* performance given potential presence of inhibitors in field collected samples (Svec et al., 2015).

3.3.2. Environmental DNA sampling

3.3.2.1. Study area and species Large tropical river systems in Van Diemen Gulf and estuarine channels in Darwin Harbour, NT, Australia, which are dominated by mangroves and

tidal flats, are known important habitat for sawfishes. These habitats are characterised by macrotidal ranges (7+ m) that result in the movement of large volumes of water and sediment, as well as distinct dry and wet seasons that drive large changes in freshwater input and salinity annually. During monsoonal rainfall (≈1,700 mm per year), substantial freshwater flows bring high sediment loads that are trapped within the coastal boundary (Blondeau-Patissier et al., 2017).

Capture surveys and field observations indicate that, while now rare, all four Indo-West Pacific sawfish species utilise shallow estuarine and coastal habitats of Van Diemen Gulf and Darwin Harbour, with some species also occupying tidal rivers, and *P. pristis* extending into freshwater habitats (Devitt et al., 2015). In general, sawfish have limited, tidally influenced movements and have high site fidelity to a restricted range within coastal environments. During the moving tide, sawfish are relatively active and presumably feeding on invertebrates and small fish (Stevens et al., 2008).

3.3.2.2. Filtration and preservation Triplicate samples were collected at 20 sites throughout Darwin Harbour, Shoal Bay, Adelaide River, West Alligator River, and South Alligator River, NT during 11–15 December 2017 (Table 3.6; Figure 3.1). Collection was undertaken prior to the onset of the monsoonal wet season and sites were dominated by tidally influenced marine and estuarine salinities. One freshwater sample was collected in the Adelaide River and the South Alligator River, with the remaining 18 samples collected within the estuarine, intertidal river reaches, or marine waters. All samples were filtered on the boat with the exception of Casuarina Beach, Shoal Bay, which were collected in sterile 500 ml HPDE bottles and filtered approximately 1 hr later onshore due to poor sea conditions. Sample sites within each location were selected based on prior knowledge of sawfish sighting or capture

and suitable habitat. Moreover, samples were collected from shallow waters (average water depth at sample sites was 0.8 metres, Table 3.6; Lowrance HDS 12 transducer, Oklahoma, US) located along the river edge, or on sand or mud banks. Field sites were accessed by boat due to the risk of encountering dangerous wildlife (e.g., crocodiles) when sampling from shore. Ambient day time temperature averaged ≥ 30°C (min. 23.0°C and max. 38.7°C; Jabiru Airport, bom.gov.au) and water surface temperature ranged between 33–35°C (data not shown; Lowrance HDS 12 transducer, Oklahoma, US). Water at all sites was flowing due to large tidal influence and contained high amounts of visible suspended particulate matter. Total filtrate volume at each site varied depending on site-specific rate of filter clogging (Table 3.6).

The filtration system set-up included two ≈ 1 m lengths of vinyl hosing (10 mm internal diameter; Pope, Adelaide, Australia), serving as input and output hosing, that were connected to a rechargeable battery-operated diaphragm pump (Grover® Scientific, Queensland, Australia) at the respective input and output valves. The distal end of the input hosing was connected to a 3D-printed filter cartridge via a fitting on the base of the cartridge. The filter cartridge cover could be detached to insert and remove filter papers (47 mm nylon net filter paper; Merck, Darmstadt, Germany). Water was filtered through the filter paper via the filter cartridge and flowed through the input hosing to the pump via positive displacement. The output hosing directed the filtrate from the pump to a 10 L graduated bucket to measure the final filtrate volume. Filtered water was returned to the water from the bucket on completion of filtering.

Triplicate surface water samples were collected at each site by submerging the filter cartridge affixed to a GoPro extension pole just below the water surface (e.g., within the top 30 cm) for the duration of filtration. For each replicate, maximum water volume (i.e., until filter clogging) was filtered (Table 3.6). Nominal filter pore size 20 μm was considered for this study based on prior field experience in the region (Simpfendorfer et al. 2016). Specifically,

20 µm filters accommodate the high turbidity (e.g., Figure 3.1D) that was not possible to filter with ≤10 µm nominal filter pore size due to rapid filter clogging. However, in the marine reaches of sampling locations where water was less turbid (i.e., the seabed was viewable from the water surface), 5 or 10 µm filter pore sizes were used arbitrarily instead of 20 µm in order to maximise eDNA capture (Turner et al., 2014). Prior to water sampling at each site, a field blank was collected by filtering 250 ml of bottled spring water. Following filtration, each filter was folded and cut in half using sterilised equipment and each half was transferred into a 2 ml microtube containing either Longmire's solution or non-denatured 96% ethanol preservative. Samples were stored at ambient temperature during the survey period and transported back to Molecular Ecology and Evolution Laboratory (MEEL) at James Cook University where Longmire's preserved samples were stored at ambient temperature until extraction (approx. 22°C) and ethanol preserved samples were stored at 4°C until extraction.

Table 3.6. Description of eDNA sample collection sites, including detail on nominal filter pore size (µM), total filtrate amount (L), and detection results for each preservation and extraction workflow (Ethanol-DNeasy vs Longmire's-precipitation). Pc denotes *Pristis clavata* (dwarf sawfish); Ppr denotes *P. pristis* (largetooth sawfish); Ac denotes *Anoxypristis cuspidata* (narrow sawfish). NA indicates the site was not tested for presence of the corresponding species.

Location	Site no.	Latitude	Longitude	Habitat description	Average water depth (m)	Environment type	Pore size (µM)	Total filtrate amount (L)	Number of positive detections per filter replicate				
									Ethanol-DNeasy		Longmire's-precipitation		
									Pc	*Ppr*	*Pc*	*Ppr*	*Ac*
Darwin Harbour	1	-12.617569°	130.946910°	Mud flat, mangrove stands	2.3	Estuarine	20	16	-	-	-	-	NA
	2	-12.593419°	130.868010°	Sand flat, mangrove stands	1.6	Estuarine	20	3	-	-	-	-	NA
Shoal Bay	3	-12.339600°	130.944920°	Sandy beach	1	Marine	5	3	-	-	2	-	NA
	4	-12.339019°	130.883320°	Sandy beach	0.7	Marine	5	1.5	-	-	-	-	NA
Adelaide River	5	-12.265931°	131.283260°	Sand flat	0.7	Marine	20	12	-	-	-	-	NA
	6	-12.426511°	131.305390°	Mangrove edge	0.5	Estuarine	20	3	-	-	-	-	NA
	7	-12.681839°	131.334560°	Mud flat	0.7	Fresh	20	12	NA	-	NA	-	NA
South Alligator River	8	-12.614250°	132.450240°	Mangrove edge	0.9	Fresh	20	2.2	NA	-	NA	-	NA
	9	-12.523190°	132.412170°	Mangrove edge	0.6	Estuarine	20	6	-	-	-	-	-
	10	-12.458100°	132.420840°	Mangrove edge	0.4	Estuarine	20	2.6	-	-	-	-	-
	11	-12.383839°	132.371500°	Sand flat	0.5	Estuarine	20	2	-	-	-	-	-
	12	-12.225950°	132.377660°	Sand flat	0.6	Marine	20	15	-	-	-	-	-
	13	-12.204281°	132.415040°	Mud flat	0.6	Marine	20	6.3	-	-	2	-	-
	14	-12.157511°	132.435550°	Sand flat	0.7	Marine	10	3.1	-	-	-	-	-
	15	-12.134089°	132.376170°	Sand flat	0.4	Marine	10	4.2	-	-	-	-	-
West Alligator River	16	-12.213200°	132.311360°	Mangrove edge	0.6	Marine	20	4.2	-	-	-	-	-
	17	-12.189789°	132.265880°	Sand flat	0.4	Marine	20	15	-	-	1	-	-
	18	-12.232411°	132.285060°	Mud flat, mangrove stands	0.5	Marine	20	3.3	-	-	-	-	-
	19	-12.289519°	132.259120°	Mangrove edge	0.8	Estuarine	20	3.5	-	-	-	-	-
	20	-12.330669°	132.232510°	Mangrove edge	0.6	Estuarine	20	7	-	-	-	-	-

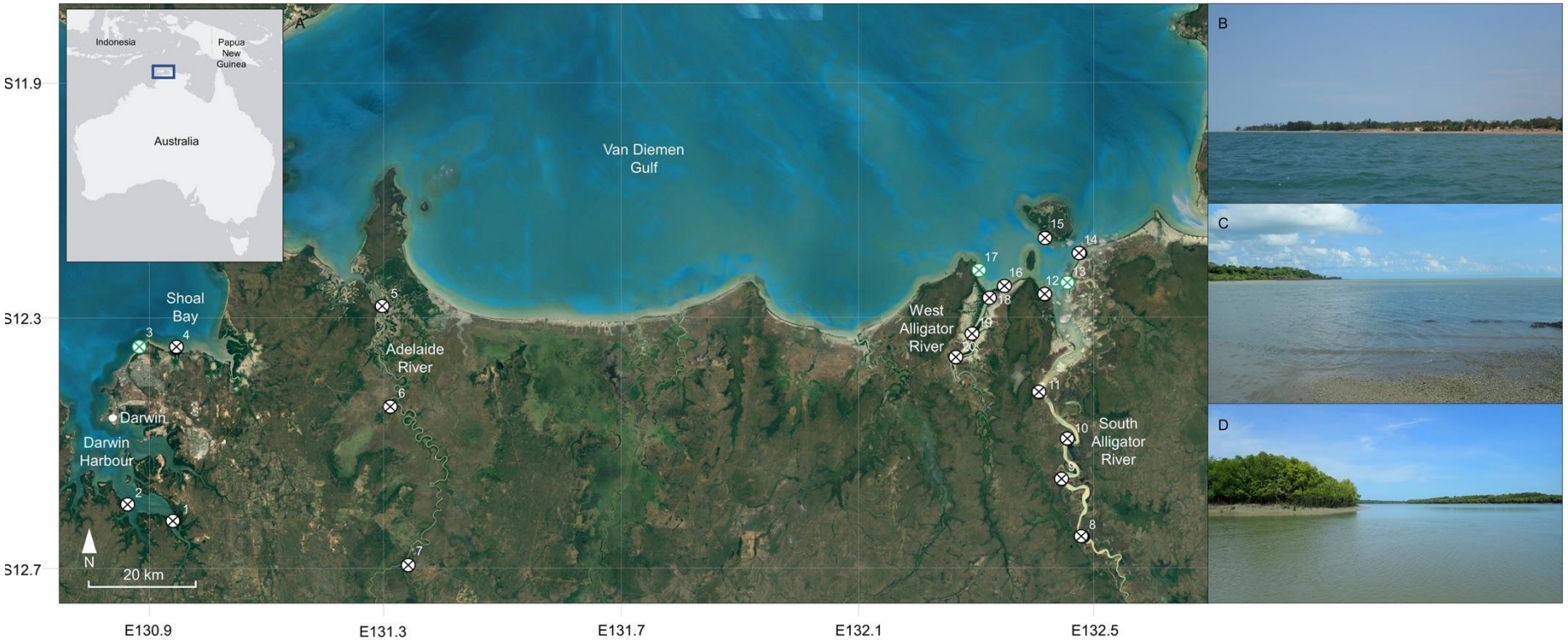

Figure 3.1. A) Satellite image of Darwin Harbour and Van Diemen Gulf, Northern Territory, Australia. Blue box inside greyscale map (top left) indicates approximate position of the satellite image within Australia. Circles denote sampling sites with green circles indicating sites with confirmed positive detections for dwarf sawfish *Pristis clavata* eDNA. Site numbers (corresponding to Table 3.6) are provided adjacent to each circle. Photos on the right, B) Micket Creek, Shoal Bay (site 3); C) River Mouth West, West Alligator River (site 17); D) Brooke Creek, South Alligator River (site 12), were taken at the time of eDNA sample collection and are sites that tested positive for dwarf sawfish presence.

3.3.2.3. Pre-departure, field, and laboratory decontamination and controls All laboratory procedures were completed within the Molecular Ecology and Evolutionary Laboratory (MEEL), at James Cook University, Townsville, where there are dedicated, physically separated rooms for low-copy DNA, gDNA extraction, pre-PCR, and post-PCR processes. The MEEL layout enabled a sequential, unidirectional workflow that minimised the risk of cross-contamination. Extraction, qPCR preparation, and amplification of gDNA from shark and ray tissue samples (including sawfish) took place in the gDNA extraction, pre-PCR, and post-PCR rooms, respectively. Extraction and qPCR preparation of eDNA samples took place in the low-copy DNA room, where plates were sealed prior to loading into QuantStudio qPCR system in the post-PCR room. Tissue, gDNA, sDNA, and eDNA were stored in separate boxes at -20°C within MEEL. Enzymes were stored at -20°C within the pre-PCR and eDNA rooms. As sDNA contained ≈$3e^9$ copies per µL of target sequence, it was treated as a post-PCR product and therefore sDNA serial dilutions were prepared and loaded into test plates in the post-PCR room prior to qPCR.

Lab benches in all rooms were decontaminated prior to use by wiping with 10% bleach, reverse osmosis (RO) water, and finally with 70% ethanol. The low-copy DNA lab benches and floor were decontaminated at the start and end of each day, and liberally throughout extraction processes, with freshly mixed 10% bleach followed by a wipe or mop with RO water. Lastly, negative controls were included with each extraction batch (extraction blank) and on each qPCR plate (no template control) to monitor for any laboratory-based contamination.

Prior to field trip departure, filtration equipment (10 mm vinyl hosing, 3D-printed filter cartridges, and rechargeable-battery operated eDNA pump; Grover® Scientific, Queensland, Australia), storage bins, plastic forceps, and scissors were decontaminated with 10% *w/v* sodium dichloroisocyanurate (bleach tablets) for 20 min before thoroughly rinsing with RO water. Filter cartridges, forceps, and scissors were then UV sterilised in a DNA-free PCR

cabinet (Airstream®, Esco Pte. Ltd., Singapore). Screw-top microcentrifuge tubes (2 mL; Sarstedt, Germany) were UV sterilised and filled with 1.5 mL DNA-free Longmire's solution (Longmire's solution (100 mM Tris pH 8, 100 mM EDTA pH 8, 10 mM NaCl, 0.5 % SDS; Longmire et al., 1997) or non-denatured 96% ethanol solution. Reusable items (e.g., filter cartridges, forceps, and scissors) were only used once per day and were bleached and rinsed with drinking water, at the end of each sampling day. Between sampling sites, the pump, hosing, and workbench were wiped with 10% bleach followerd by RO water and gloves changed.

3.3.3. Laboratory procedures

3.3.3.1. eDNA extraction, inhibitor removal, and quantification Longmire's-preserved filter halves were extracted using a glycogen-aided precipitation extraction (Longmire's-precipitation combination; Edmunds and Burrows, 2020) with modification for extraction from filter papers stored in 2 ml microtubes. Briefly, Longmire's solution was transferred from each 2 mL field tube to a DNA-free 15 mL LoBind® (Eppendorf South Pacific Pty Ltd, New South Wales, Australia) conical tube, diluted up to 5 mL with UltraPure distilled water (ThermoFisher Scientific, Victoria, Australia), and eDNA precipitated at 4°C overnight in 7 mL isopropanol, 10 µL glycogen (20mg/mL; Merck, Victoria, Australia), and 1.7 mL 5M sodium chloride. Precipitant was pelleted for 90 min at 3,270 g (SX4750 rotor, Allegra X-12 R, Beckman Coulter Inc., Indianapolis, US) and 600 µL of pH 10 lysis buffer solution containing 0.8 M guanidine hydrochloride and 0.5% TritonX (Lever et al., 2015) was added with the precipitant to the original field tube containing filter paper half for freeze-thaw-lysis treatment and subsequent incubation at 50°C for ≥3 h. eDNA was then precipitated at 4°C overnight in two volumes polyethylene glycol (PEG) precipitation solution (1.6M sodium chloride, 30% PEG) and 5 µL glycogen (20 mg/mL) followed by 14,000 g for 30 min

(Centrifuge 5430R, Eppendorf South Pacific Pty Ltd, New South Wales, Australia). Extracted eDNA from each sample was eluted in 100 µL UltraPure distilled water (ThermoFisher Scientific, Victoria, Australia). Ethanol-preserved filter halves were extracted using DNeasy Blood & Tissue kit (QIAGEN, Hilden, Germany) (Ethanol-DNeasy) with the following modifications to manufacturer protocol: 1) samples were shaken in bead beater (Mini-BeadBeater-96, BioSpec, Oklahoma, USA) without beads following addition of 540 µL ATL buffer and 60 µL Proteinase K (to ensure filter half was completely submerged), 2) overnight incubation at 56°C, 3) 600 µL AL Buffer, 4) 600 µL ethanol, and 5) final elution with two consecutive additions of 50 µL AW Buffer (Thomsen et al., 2012).

The eluted eDNA of paired filter samples was then purified of co-extracted inhibitors using OneStep™ PCR Inhibitor Removal Kit (Zymo Research Corp, California, USA) following manufacturer protocol. Purified eDNA was collected in 2 mL LoBind® tubes to minimise potential loss of low-copy DNA due to biochemical or electrostatic retention (Lecerf & Le Goff, 2010). Following purification each sample was briefly vortexed before 5 µL sub-samples were taken for quantification of recovered eDNA from each paired filter half using the Quantus™ Fluorometer dsDNA System (Promega Pty Ltd Australia). Extracted and purified eDNA samples were stored at -20°C until qPCR.

3.3.3.2. Detection of sawfish eDNA by qPCR Species-specific TaqMan qPCR assays were used to detect presence of *P. clavata*, *P. pristis*, and *A. cuspidata* eDNA. Specifically, Ethanol-DNeasy filter halves were tested for presence of *P. clavata* (except for the two freshwater sites as the species is not found in fresh water) and *P. pristis*, but not for *A. cuspidata* due to the limited volume of eluted eDNA remaining. Longmire's-precipitation filter halves collected from freshwater sites were tested exclusively for *P. pristis* eDNA presence,

while estuarine and marine filter halves were tested for *P. pristis*, *P. clavata*, and *A. cuspidata* (sites 9–20 only) (Table 3.6). The *P. zijsron* assay was not tested *in situ* due to the limited volume of eluted eDNA remaining. The *P. pectinata* assay was also not tested *in situ* as this species only occurs in the Atlantic Ocean.

Assays were performed in six replicate 10 µl reactions per treatment following optimised reaction and thermocycling conditions derived from *in vitro* tests. Each reaction contained 3 µl of total eluted eDNA (18% of elution screened per assay per treatment). Reactions were run in 96- or 384-well plates and contained triplicates of no-template controls, extraction blanks, and low-copy sDNA standards (10, 5, 2.5, 1.25 copies per reaction). All plates were analysed using QuantStudio™ Design and Analysis Software as described above for *in vitro* tests.

Putative positive amplicons were re-amplified using end-point PCR with same primers. Each 25 µL reaction contained 2 µL post-PCR product, 12.5 µL 2× MyTaq Red Mix, 0.5 µM forward and reverse primer and adjusted to final volume with MilliQ water. Thermocycling followed MyTaq Red Mix standard cycling conditions using a SimpliAmp Thermal Cycler and 1 µL PCR product was visualised on 1.5% agarose gel (see above) alongside 1 ng and 5 ng λ DNA (Genesearch Pty. Ltd., Arundel, Australia). All amplicons were sent to the Australian Genome Research Facility (AGRF Pty Ltd, Brisbane, Australia) for clean-up and bidirectional Sanger sequencing verification. Species-specificity of each sequence was confirmed using BLASTn searches against the entire NCBI nucleotide database. Detections were considered true positives if the following selection criteria were met (modified after Trujillo-González, Edmunds, Becker, & Hutson (2019) for TaqMan assays without dissociation curve generation): 1) ≥1 technical replicate exhibited amplification that crossed fluorescence threshold within 50 cycles, 2) amplicon produced by subsequent end-point PCR exhibited a single band of expected

size, 3) BLASTn search of sequences matched target species with ≥99% pairwise identity, and 4) corresponding negative controls exhibited no amplification.

3.3.4. Statistical analysis

A linear mixed effects model was used to test for differences in total eDNA yield as a result of workflow or its interaction with different filtrate volumes, because filtrate volume is expected to influence the total amount of eDNA captured on a filter membrane. Site and pore size were treated as random variables. The *p* values are based on *t*-tests using Satterthwaite's method. To satisfy heterogeneity and normality assumptions, the dsDNA yield data were $\log_{10}$ transformed. All statistical analyses were performed in R (version 1.1.453) (R Development Core Team, 2016).

3.4. Results

3.4.1. Assay design and validation

Primer and probe binding regions were identified between base pairs 600 and 850 on sawfish *12S* gene alignments (Figure 3.2). Primer and probe sequences, annealing temperature, and final optimised concentration are shown in Table 3.4.

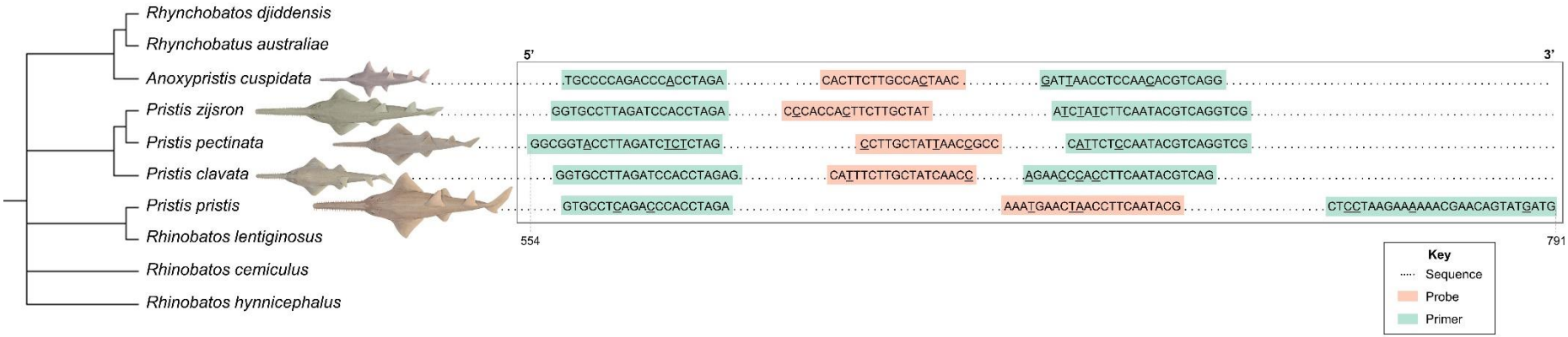

Figure 3.2. Alignment diagram showing the relative position of oligonucleotides for each sawfish qPCR assay developed in the present study. Oligonucleotides are shown in 5' to 3' orientation (including reverse primers, which are shown in reverse-complement to the oligonucleotides used in the assays). Numbers below the box indicate nucleotide positions on sawfish complete *12S* gene alignments. Nucleotides that are dissimilar to other sawfish sequences are underlined. The proportional tree was created in Geneious 10.2.6. using *12S* sequences and thorny lanternshark (*Etmopterus sentosus*) as an outgroup (Figure S3.1). The five sawfish illustrations displayed in the figure are from Last et al. (2016).

In vitro validations of the primer-driven assays exhibited no amplification of tested exclusion species, confirming that ≥3 mismatches in primer and/or probe sequence were sufficient to eliminate false positive detection (Table 3.5). Mismatches were primarily in primer-binding regions; probe design was limited to inclusion one or two base pair mismatches to non-target template (Table 3.5). Theoretically, mismatches should not permit probes to anneal to non-target template and therefore prevent a fluorescent signal; however, all five probe-driven assays exhibited amplification of gDNA or sDNA from tested exclusion species (Table 3.5). Specifically, amplification was generated from exclusion species template for all five probe-driven assays when one and two mismatches were present within probe sequence. The amplification curve was on average 3.178 cycles later than the target species amplification curve, with template concentration constant at ~10,000 copies (Table 3.7). No amplification was observed for probe-driven assays when species-specific probes contained ≥3 mismatches to *12S* sequence of exclusion species. Due to the potential risk of template competition between exclusion species eDNA and low-copy target sawfish eDNA, all probe-driven assays were excluded from *in situ* testing.

Table 3.7. Summary of cycle threshold (Ct) qPCR data from *in situ* validation experiments of probe-driven assays. Probes containing ≤2 base pair mismatches exhibited amplification of non-target species. The location of base pair mismatches are denoted in bold.

Target	Mean Ct	Non-target species	Mean Ct	ΔCt	Probe nucleotide sequence
P. clavata	26.226	*R. cemiculus*	29.461	-3.235	CA**C**TTCTTGC**C**ATCAACC
P. pristis	25.885	*R. lentiginosus*	29.272	-3.387	AAATGAACT**C**ACCTTCAATACG
P. pectinata	25.476	*R. australiae*	28.334	-2.858	**T**CTTGC**C**ATTAACCGCC
		R. diddjensis	27.942	-2.466	**T**CTTGC**C**ATTAACCGCC
P. zijsron	25.701	*E. sentosus*	29.317	-3.616	C**T**CACCACTTCTTGCTA**A**
		R. cemiculus	27.754	-2.054	C**T**CACCACTTCTTGC**C**AT
		R. diddjensis	27.724	-2.024	C**T**CACCACTTCTTGC**C**AT
		R. australiae	28.048	-2.347	C**T**CACCACTTCTTGC**C**AT
A. cuspidata	24.073	*G. cuvier*	28.615	-4.542	TTCTTGCCACT-ACCG
		R. australiae	28.567	-4.494	TTCTTGCCA**T**TAACCG
		R. diddjensis	28.066	-3.994	TTCTTGCCA**T**TAACCG

Species-specific primer and probe binding regions were identified between base pairs 550 and 800 on sawfish *12S* gene alignments. *In silico* analyses indicated that candidate primers and probes contained between 5 and 11 cumulative mismatches to the next-closest relative sawfish sequence (Table 3.5). All *in vitro* specificity validation tests using exclusion species gDNA samples showed no evidence of qPCR amplification and confirmed that primers and probes were species-specific. Primer and probe sequences, annealing temperature, and final optimised concentration are shown in Table 3.4. All extraction and (q)PCR negative controls tested negative in all assay validation test plates. Assay-specific 95% LOD tests indicated that the assays could detect 1.25–5 copies per reaction (Table 3.8). Species-specific standard curve efficiencies, R^2 ranges, 95% LODs, and detection rates are provided in Table 3.8.

Table 3.8. TaqMan qPCR assay efficiency range (%), correlation coefficient range (R^2), 95% limit of detection (LOD), and low-copy detection success rates for each species-specific assay designed for the detection of sawfish eDNA in environmental samples.

Assay Target	Reaction efficiency (%)	R^2	95% LOD (copies reaction^{-1})	2.5 copies reaction^{-1} (Detection success; %)	1.25 copies reaction^{-1} (Detection success; %)
Anoxypristis cuspidata	109.79-129.49	0.992-0.998	2.5	100	87.5
Pristis clavata	102.41-113.87	0.995-0.997	5	83.33	66.67
Pristis pristis	114.81-117.69	0.992-0.998	1.25	100	100
Pristis zijsron	105.96-106.27	0.998-0.999	2.5	100	75
Pristis pectinata	96.19-106.00	0.998-0.999	5	70.83	66.67

3.4.2. Effect of preservation and extraction on total eDNA yield and detection success

There was a significant effect of preservation and extraction workflow on the total amount of eDNA retained on paired filter halves (n = 60 per treatment; Figure 3.3), with no evidence of interaction of filtrate volume. The Longmire's-precipitation workflow yielded a greater total amount of eDNA (average 6.54 ± 0.68 ng/µl across 60 filter replicates) than that

obtained from the Ethanol-DNeasy workflow (2.40 ± 0.38 ng/µl; $\beta = 1.27$, $t(95) = 8.172$, $P < 0.0001$) (Figure 3.3).

The Longmire's-precipitation workflow had the greatest detection rate. Dwarf sawfish *P. clavata* were detected only in Longmire's-precipitation filter halves collected at the mouth of the small, perennial Micket Creek, Shoal Bay, at the mouth of West Alligator River, and at Brooke Creek in the mouth of South Alligator River (Figure 3.2Figure 3.1; Table 3.6). Paired filter halves processed using Ethanol-DNeasy workflow showed no positive detections for *P. clavata*. *P. pristis* and *A. cuspidata* were not detected in either filter half (Table 3.6).

Detected eDNA was approximately 1–3 copies per reaction based on extrapolation from *P. clavata* sDNA standard curve. Sequenced amplicons confirmed all seven *P. clavata* detections from Longmire-precipitation filter halves to be positive in that returned sequences exhibited 100% pairwise identity with species-specific *12S* nucleotide sequence. Absence of field and laboratory contamination was confirmed by the lack of amplification observed for all field controls, extraction blanks, and template controls associated with filter halves processed using Ethanol-DNeasy and Longmire's-precipitation workflows.

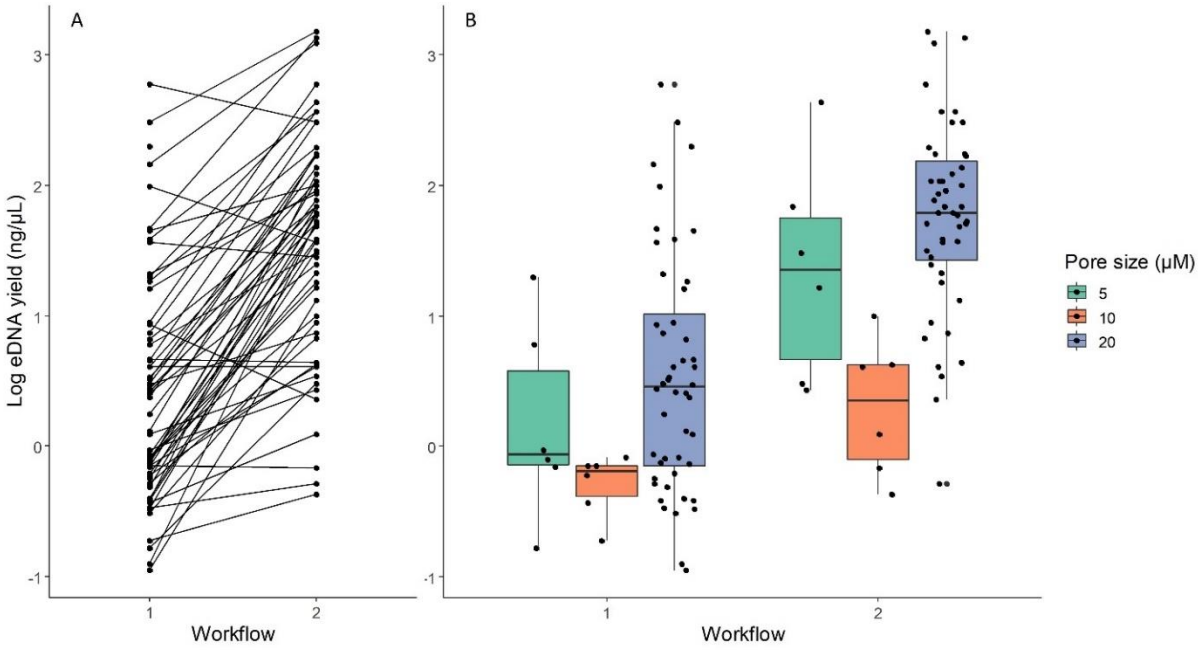

Figure 3.3. Comparison of log total eDNA yield (ng/µl) from two preservation and extraction workflows using paired filter halves (n = 60 pairs). A) Paired filter halves were exposed to ethanol preservation and DNeasy Blood & Tissue extraction (ethanol-DNeasy; 1) or Longmire's buffer preservation and glycogen-aided precipitation extraction (Longmire's-precipitation; 2) workflows. Each point represents the log total eDNA yield, measured using Quantus™ Fluorometer dsDNA System (Promega Pty Ltd Australia), from each filter half in the paired comparison (connected by black lines), where each filter sample was independently collected. Statistical results indicate that the Longmire's-precipitation workflow outperforms the ethanol-DNeasy workflow in retaining total eDNA ($\beta = 1.27$, $t(95) = 8.172$, $P < 0.0001$. B) Data grouped by filter pore size for visualisation, but the effect of pore size on total eDNA yield was treated as a random variable in a linear mixed model due to the uneven spread of sample numbers, and therefore statistical differences in yield due to pore size are not tested here. Points are horizontally jittered.

3.5. Discussion

Detection of rare and threatened species in coastal environments using eDNA as a survey tool presents a cutting-edge approach to answer fundamental questions about occurrence and distribution (Huerlimann et al., 2020). However, the optimal design of eDNA workflows for rare and threatened species, particularly in regards to preservation and extraction methodologies, is a ubiquitous concern for most eDNA users. For sawfishes, advancements in detection methods are needed to improve monitoring efforts and enhance application of effective safeguards. This study reports differences in total eDNA yield (>2-fold) and species-

specific detectability (seven versus zero) obtained following the processing of paired eDNA filter samples using two discrete workflows (Longmire's-precipitation versus ethanol-DNeasy, respectively). This study also reports the design and *in silico* and *in vitro* validation of a suite of TaqMan assays for the detection of five species of sawfish as well as the *in situ* validation of *P. clavata* assay. Positive *P. clavata* detections were observed in filtered water samples collected on the shallow edges (≤1 m depth) of river mouths in Van Diemen Gulf and sand flats of Darwin Harbour, which coincides with the known habitat use of this species (Thorburn et al., 2003; Stevens et al., 2008; Field et al., 2013). Overall, the results reveal that there is significant potential to reduce the rate of false negative detections of rare, threatened species by utilising an optimal combination of preservation method and extraction workflow. The Longmire's preservation and glycogen-aided precipitation protocol used here is a positive methodological advancement that has the potential to assist scientists and conservation practitioners with targeted eDNA surveys of sawfishes and other rare or threatened taxa.

3.5.1. Influence of eDNA preservation and extraction workflow on detection sensitivity

In general, recommendations for the design of eDNA studies highlight the importance of workflow optimisation for the target species (Goldberg et al., 2016; Piggott, 2016; Deiner et al., 2018; Tsuji et al., 2019). However, there is no consensus on best-practice preservation and extraction workflow for single-species surveys, because environments of target species' vary and only a few studies have compared the efficacy of existing protocols (Renshaw et al., 2014; Turner et al., 2014; Deiner et al., 2015; Eichmiller, Miller & Sorensen, 2016). This study demonstrates the efficacy of the Longmire's-precipitation workflow over ethanol-DNeasy. The increase in total eDNA yield and positive *P. clavata* detections in the Longmire's-precipitation workflow could be due to improved cell lysis efficiency of Longmire's solution and/or

guanidinium hydrochloride- and TritonX-based alkaline lysis buffer solution used in the glycogen-aided precipitation workflow (Lever et al., 2015; Natarajan et al., 2016). Specifically, improved cell lysis efficiency can increase eDNA release from suspended particles, including cells, organelles, and other contaminants such as sediments and humic acids, that otherwise retain bound eDNA (Natarajan et al., 2016; Deiner et al., 2018). In contrast, the reduced eDNA yield in ethanol-DNeasy filter halves could be due to competitive binding of particulates and commonly found humic substances to silica-based spin columns, which can result in the loss of eDNA during the extraction step (Lloyd, MacGregor & Teske, 2010). It is also noted that a number of DNeasy Blood & Tissue protocol modifications exist across eDNA literature (reviewed by Adrian-Kalchhauser & Burkhardt-Holm, 2016) that were not empirically tested here. One of the most commonly used protocol modifications, which uses three-times the recommended volume of buffer ATL and Proteinase K in the initial step, was used in this study (see Thomsen et al., 2012). The QIAGEN DNeasy handbook suggests the addition of 3–5 µg carrier DNA (e.g., poly-dA; Roche, Cat. No.: 10223581001) to the starting material to obtain optimal DNA yield when the sample has less than 5 ng DNA; however, this was not tested here and is not commonly used in eDNA research despite the low yield of eDNA in filter samples. The use of glycogen as an inert co-principate in the glycogen-aided precipitation protocol could also improve eDNA yield during DNeasy spin-column extraction (Li et al., 2020). Moreover, the other possible workflow combinations were not empirically tested here (e.g., Longmire's-DNeasy and ethanol-precipitation) and, therefore, should not be disregarded until rigorously tested. Regardless, ethanol preservation presents logistical limitations that non-hazardous Longmire's does not (e.g., transportation restrictions) as well as other limitations being that ethanol cannot be assimilated into column-based extraction protocols because it requires removal as the first step during sample processing. As such, future studies are encouraged to test modifications of commercial spin-column based extraction using filter samples preserved

in Longmire's, as well as extraction of ethanol-preserved filter samples using glycogen-aided precipitation, in both controlled experiments and *in situ* studies that target sawfishes and other rare or threatened taxa.

Extraction protocols that yield high eDNA concentration are also more likely to co-extract high concentrations of DNA-bound inhibitors (Wilson, 1997). Here, inhibitor removal using Zymo OneStep™ PCR Inhibitor Removal Kit likely assisted with removal of eDNA-bound particles from extracted eDNA and, in doing so, reduced qPCR inhibition by dissolved and co-extracted compounds (Mckee, Spear & Pierson, 2015; Djurhuus et al., 2017; Williams, Huyvaert & Piaggio, 2017; Deiner et al., 2018). However, it is suggested that future eDNA studies should consider testing efficacy of chosen inhibitor removal method at removing site-specific inhibitors, for example, through use of exogenous internal positive controls (Hartman, Coyne & Norwood, 2005) or spiking-dilution protocol (Cao et al., 2012). Other common methods for overcoming the influence of co-extracted inhibitors include addition of bovine serum albumin (BSA) to PCR chemistry, using inhibitor resistant DNA polymerase (Schrader et al., 2012), or sample dilution (McKee, Spear & Pierson, 2015). However, dilution is not recommended for eDNA studies on rare species given the low eDNA starting concentration, since dilution increases the risk of false negative detections (Schrader et al., 2012).

3.5.2. Influence of eDNA and species' ecology on detection sensitivity

Given the brief lifespan of eDNA in the aquatic environment (e.g., generally 10 to 50 hours) due to degradative forces of elevated UV, pH, and temperature-induced microbial utilisation of dissolved eDNA (Strickler, Fremier & Goldberg, 2015; Eichmiller, Best & Sorensen, 2016; Collins et al., 2018; Salter, 2018), positive detections may be contemporary and spatially conservative. Single-species and metabarcoding eDNA studies in marine systems

have reported strong local eDNA signals either matching target species habitat use or sightings (Simpfendorfer et al., 2016; Thomsen et al., 2016; O'Donnell et al., 2017), even despite the influence of tidal currents (Baker et al., 2018; Kelly, Gallego & Jacobs-Palmer, 2018). However, turbid aquatic waterbodies where sawfish are known to occur contain humic acids, sediments (soil and clay), and mineral complexes that bind eDNA and offer protection from degradation for weeks (Turner, Uy & Everhart, 2015) to years (Thomsen & Willerslev, 2015; Parducci et al., 2017) depending on the sediment characteristics and depth of the deposited DNA sample (Levy-Booth et al., 2007). In this study, *P. clavata* eDNA was detected in samples collected at the surface of shallow waters (average depth 0.8 m) so as to avoid eDNA that accumulated in the bottom-surface sediment and ensure samples were collected at sites within the depth range of the species (Stevens et al., 2008). However, positive detections of eDNA may be confounded by eDNA that has persisted in the sediment for weeks or years (Turner, Uy & Everhart, 2015; Parducci et al., 2017). It is therefore suggested that information gleaned from eDNA methods can be used to direct more intensive research efforts to visually confirm the presence of species of conservation concern.

There are also factors related to species' ecology that could explain the lack of positive detections in either workflow. Not all species are always detected using eDNA where they are known to occur (Sasso et al., 2017), making inference of negative detection difficult. Moreover, sawfish occurrence in the Northern Territory is now patchy and capture or sighting records are lower than they were historically (Peverell, 2005; Stevens et al., 2008). Seasonality, habitat, and spatial movements could also affect detection rates. The current sampling was undertaken in December, which precedes the local pupping season of all four local sawfishes. In Northern Australia, these species typically pup during the monsoonal wet season (February to April) until the beginning of the dry season (May) (Peverell, 2005). More specifically, gravid female *P. pristis* enter estuaries and river mouths to pup with neonates then move upstream into

freshwater habitats where they remain for several years (Peverell, 2005; Thorburn et al., 2007); therefore, the likelihood of this species being present in the sampling area is reduced outside of the pupping season. In contrast, short-term tagging and tracking data for *P. clavata* showed only localised movement patterns and apparent site-fidelity with a preference for very shallow waters (Stevens et al., 2008). A pattern of residency could increase the likelihood of its occurrence in the study area during the sampling period, and therefore its detection. Similarly, neonate and adult *A. cuspidata* were likely absent from the estuary where pupping is expected to occur (Peverell, 2005). The distribution of adult *A. cuspidata* in offshore benthopelagic habitat also renders this species' eDNA less likely to be detected in filter samples collected in shallow, coastal habitats outside pupping season (Peverell, 2005).

3.5.3. Considerations for eDNA sampling in turbid coastal and riverine environments

Coastal, estuarine, and riverine environments at tropical latitudes present a methodological challenge for eDNA sampling. The high velocity of tidal water and seasonal river flow due to monsoonal rainfall heterogeneously disperses eDNA and suspends high amounts of sediments in the water column, which, as in this study, cause rapid filter clogging and limit filtration volumes. One method to counter rapid filter clogging and increase filtrate volume is the pre-filtering of water samples through membranes with large pore sizes (Wilson et al., 2014; Robson et al., 2016; Majaneva et al., 2018); however, given that pre-filtration in turbid environments using a 20 μm pore size filter can increase filtration times by 16-fold (Robson et al., 2016), this becomes a considerable constraint when sample sites are remote (e.g. furthest sample sites in this study were ≈ 8 hours from the nearest boat ramp), expeditions are time-limited, and tropical weather conditions can be hindering. Pre-filtration was not considered for this study. Turner et al. (2014) suggested that eDNA capture rates in turbid

environments can be maximised by increasing both pore size and filter volume, and Robson et al. (2016) suggested that filtering more than 4 L using 20 µm pore size would improve capture rates. Based on this study, detection of eDNA from a rare sawfish species was possible when ≥3 or 6 L water was filtered through 5 or 20 µm pore size filters, respectively. However, the effect of using different pore sizes across sites was not tested and we cannot differentiate between site specific effects of detection given that pore size and filtrate volume was not standardised.

There are other technical factors to consider that may influence detection of any species. This includes failure to capture: 1) species-specific eDNA using large pore sizes (≥10 µm) because eDNA particle sizes are most abundant from 1–10 µm (Turner et al., 2014); 2) a sufficient volume of eDNA due to filter clogging, as filtrate volume has been shown to be important for accurate assessment (Cantera et al., 2019); or 3) low-copy eDNA within screened qPCR template volume because of the stochastic sampling effect induced during aliquot of the eDNA eluate for qPCR (Mächler et al., 2016). Accordingly, biological and technical replication (i.e., ≥3 field replicates and ≥6 qPCR replicates, respectively), volume and pore size standardisation, and sampling across multiple time points may be important to consider for future studies to improve interpretation. Furthermore, sampling protocols should be standardised prior to application by conservation practitioners interested in monitoring or management programmes using eDNA. To this end, a pilot study that optimises sample collection methods with consideration for the target environment (i.e., remoteness, turbidity), species (i.e., rarity), and time-efficiency (i.e., single- or multi-day expeditions, sample replication) is an important step.

3.5.4. Recommendation for assay design and future compatibility

Reliable detection of rare species using environmental DNA is dependent on the development of highly specific qPCR assays (Budd et al., 2021; Wilcox et al., 2013). For the two assay design approaches, despite the presence of primer-probe mismatches against the tested exclusion species, the assays did not always lead to species-specific amplification. Assay specificity is not only influenced by the presence of base pair mismatches in the primer and/or probe sequences to non-target sequence, but by the position of base pair mismatches (Kutyavin et al., 2000; Wilcox et al., 2013). Specifically, TaqMan minor groove binding (MGB) probes form highly stable DNA duplexes with the target DNA, which enables MGB probes to be shorter than regular probe regions and therefore more specific to target sequence and sensitive to single base pair mismatches (Kutyavin et al., 2000). Target specificity is increased when mismatches are in or near the 3' MGB binding region (Kutyavin et al., 2000; Whiley & Sloots, 2005). Here, each species-specific probe included a least a single mismatch in the terminal 2−4 base pairs (3' end) for the tested exclusion species and non-target sawfish species. However, when paired with the generic primer set ('Elasmobranch-generic primer'; Table 3.4), which contained zero mismatches amongst sawfishes and 1−4 mismatches to tested exclusion species in the reverse primer, the assays revealed non-specific amplification. Here, mismatches were only located the 5' end of the reverse primer. Wilcox et al., (2013) demonstrated that primer mismatches were more influential when positioned at the 3' end than the 5' end of primers. It is therefore likely that the position of mismatches in the generic primer assays designed here impacted specificity. On the contrary, the species-specific primers designed here, which contained 4 or more base pair mismatches in the forward and reverse sequences for non-target sawfish and exclusion species, successfully amplified only target genomic or synthetic DNA. Furthermore, *P. zijsron* and *P. clavata* assays share the same forward primer sequence, due to

limited interspecific variation at this position in the *12S* gene, but both assays successfully excluded the other species.

The assays developed here were optimised using sequence data and genomic DNA derived from the core populations of *P. clavata*, *P. zijsron*, *P. pristis*, and *A. cuspidata* in northern Australia and/or Papua New Guinea and *P. pectinata* in south-eastern United States. To ensure specificity of the primer and probe sequences, the assays were cross tested *in silico*, and *in vitro* if tissue was available, with other sawfish species as well as other elasmobranch species that were considered to be closely related or sympatric throughout their historical geographic range. For future use of the assays to detect sawfishes outside of the respective geographic ranges tested here, it is recommended that *in vitro* tests are performed with sawfish genomic DNA (gDNA) derived from individuals from the targeted region. Where access to contemporary samples is not possible, due to the rarity of specimens or permit restrictions, the utilisation of gDNA or sequence information generated from historical collections is encouraged (Fearing et al., 2018).

3.6. Conclusion

Methodological advancements in the reliability and cost-effectiveness of eDNA methods provide an opportunity to greatly contribute where previous applications have been unsuccessful or impracticable. When appropriate preservation and extraction workflows are utilised, the detection of rare or threatened species such as sawfishes can be achieved non-invasively and, ultimately, circumventing capture and handling stress. In addition to improved detection success and thus minimisation of false negative detections, the Longmire's-precipitation workflow is especially advantageous where field locations require an effective ambient temperature sample storage medium and enables researchers to decrease extraction

costs (precipitation: $3.63 per filter sample versus QIAGEN DNeasy Blood & Tissue kit: $7.60 per filter sample; price in Australian dollars). These findings are relevant and important for researchers and conservation practitioners undertaking eDNA studies in remote or tropical environments (i.e., flexibility in sample storage) and for species that are exceptionally rare or of high conservation concern (i.e., lower costs allow budget to stretch further). Continued fine-tuning of eDNA workflows is important given the potential advantages eDNA offer over other conventional field sampling methods. This is especially evident for the eDNA-based research conducted in the tropics, which comprises a small portion of the total eDNA literature (Huerlimann et al. 2020), but faces the greatest threats to biodiversity.

3.7. Supplementary Information

Data S3.7.1 Primer dimer analysis

Primers and probes were checked for the likelihood of dimerisation, either of the reciprocal primer/probe (heterodimer) or to itself (homodimer). The primer dimer check was completed using the freely available online software PrimerDimer (http://www.primer-dimer.com/) (Johnston et al., 2019; Lu et al., 2017). The software predicts the likelihood of dimer artefacts, particularly extension dimers that have the potential to dimerise during amplification and therefore the most problematic. Extension dimers are formed as a result of high complementarity at the 3'end of the relevant oligonucleotides and are the most prominent dimer-forming pairs that are thermodynamically stable enough for extension and subsequent amplification during PCR (Lu et al., 2017).

Primer dimer analysis was completed following the software instructions for paired analysis. Specifically, primer and probe sequences were copied into the text in FASTA format using the guided software interface. Paired dimer analysis was selected and a downloadable dimer structure report generated.

The dimer structure report provides scores and visualisation of the dimer formation for all possible dimer events (Table S3.1). The score generated in PrimerDimer is based on the stability between the binding of two primer or probe pairs, i.e., Saint Lucia's minimum free-energy calculation. The lower the score, the more stable the bond and therefore the more likely the pair are to form a dimer. The three lowest scores were estimated for dimer pairs 6, 7, and 12 (-3.72, -7.43, and -3.11, respectively). However, the prediction of primer dimer does not always equate to what is observed. Inspection of agarose gel for the presence of primer dimer "clouds" showed that the incidence was either not present or low when the assays were tested with genomic or synthetic DNA of the target species (data not shown), which is not unexpected.

Table S3.1. Paired primer dimer analysis, including score (kcal/mol) and visualisation of the 3' extension dimer formation, for each assay primer and probe pair. Analysis was completed using the freely available online software PrimerDimer (http://www.primer-dimer.com/) (Johnston et al., 2019; Lu et al., 2017). Pairs that were not complementary and therefore no likelihood of dimerisation are not shown here. Bold values indicate the three lowest scores and therefore the highest potential for dimerisation.

	Oligonucleotide #1	Oligonucleotide #2	Score (kcal/mol)	Dimer formation
1	*Anoxypristis cuspidata* Forward	*Anoxypristis cuspidata* Forward	-0.27	5'> TGCCCCAGACCCACCTAGA >3' |||| 3'< AGATCCACCCAGACCCCGT <5'
2	*Anoxypristis cuspidata* Forward	*Anoxypristis cuspidata* Reverse	-2.81	5'> CCTGACGTGTTGGAGGTTAATC >3' || 3'< AGATCCACCCAGACCCCGT <5'
3	*Anoxypristis cuspidata* Probe	*Anoxypristis cuspidata* Probe	-2.67	5'> TTCTTGCCACTAACCG >3' || 3'< GCCAATCACCGTTCTT <5'
4	*Pristis pristis* Forward	*Pristis pristis* Forward	-0.28	5'> GTGCCTCAGACCCACCTAGA >3' || || 3'< AGATCCACCCAGACTCCGTG <5'
5	*Pristis pristis* Forward	*Pristis pristis* Reverse	-0.94	5'> CATCATACTGTTCGTTTTTTCTTAGGAG >3' || || | 3'< AGATCCACCCAGACTCCGTG <5'
6	*Pristis pristis* Probe	*Pristis pristis* Probe	**-3.72**	5'> AAATGAACTAACCTTCAATACG >3' || 3'< GCATAACTTCCAATCAAGTAAA <5'
7	*Pristis pectinata* Forward	*Pristis pectinata* Forward	**-7.43**	5'> GGCGGTACCTTAGATCTCTCTAG >3' |||| 3'< GATCTCTCTAGATTCCATGGCGG <5'
8	*Pristis pectinata* Reverse	*Pristis pectinata* Probe	0.0	5'> CGACCTGACGTATTGGAGAATG >3' | | || | 3'< CCGCCAATTATCGTTCC <5'
9	*Pristis zijsron* Forward	*Pristis zijsron* Forward	-0.27	5'> GGTGCCTTAGATCCACCTAGA >3' |||| 3'< AGATCCACCTAGATTCCGTGG <5'
10	*Pristis zijsron* Forward	*Pristis zijsron* Reverse	-0.92	5'> CGACCTGACGTATTGAAGATAGAT >3' | || | ||| 3'< AGATCCACCTAGATTCCGTGG <5'
11	*Pristis zijsron* Reverse	*Pristis zijsron* Probe	-2.46	5'> CGACCTGACGTATTGAAGATAGAT >3' |||| 3'< TATCGTTCTTCACCACCC <5'
12	*Pristis clavata* Forward	*Pristis clavata* Reverse	**-3.11**	5'> CTGACGTATTGAAGGTGGGTTCT >3' | || ||| 3'< GAGATCCACCTAGATTCCGTGG <5'
13	*Pristis clavata* Forward	*Pristis clavata* Probe	0.0	5'> GGTGCCTTAGATCCACCTAGAG >3' | ||| | | 3'< CCAACTATCGTTCTTTAC <5'
14	*Pristis clavata* Reverse	*Pristis clavata* Probe	-2.5	5'> CTGACGTATTGAAGGTGGGTTCT >3' || || 3'< CCAACTATCGTTCTTTAC <5'

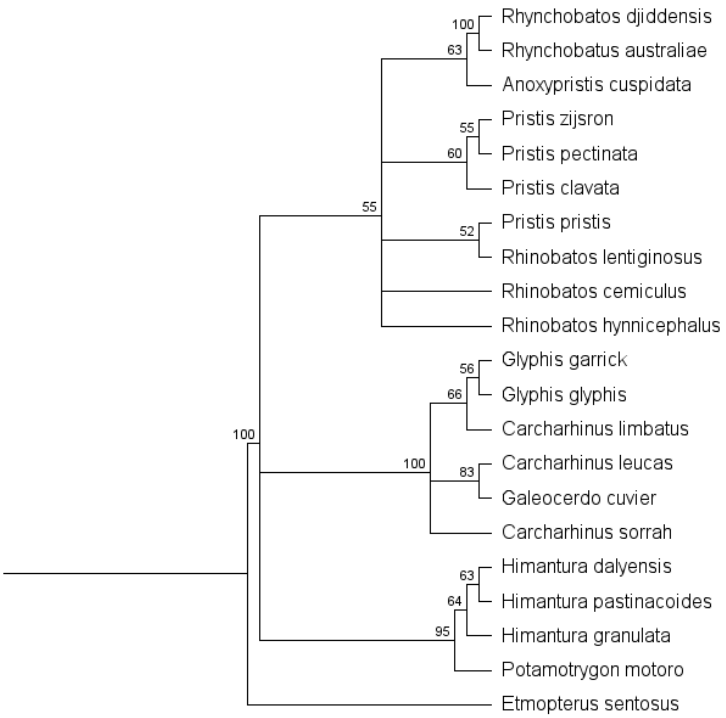

Figure S3.1. Phlyogenetic Tree for Assay Design and Generation of Figure 3.2. Sequences (*12S* rDNA) from twenty-one closely related exclusion species, which were used for design and *in silico* analysis of sawfish TaqMan assays, were aligned and cropped to the assay target region (base pairs 554' to 791'). A Neighbor-Joining phylogram tree was built in Geneious 10.2.6. The tree was built using genetic distance model 'Tamura Nei' and branches are in proportional, decreasing order with thorny lanternshark (*Etmopterus sentosus*) as an outgroup.

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Chapter 4

Practical sampling methods inferred from eDNA particle size distribution and comparison of capture techniques of largetooth sawfish (*Pristis pristis*) eDNA

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4.1. Abstract

Environmental DNA (eDNA) methods are increasingly applied in the marine environment to identify species and describe communities. However, its application to address questions for elasmobranchs is still in its infancy. To establish widely applicable eDNA techniques for elasmobranchs, I used the Critically Endangered largetooth sawfish (*Pristis pristis* Linnaeus, 1758) as a model species for assessing eDNA particle size distribution and comparing the detection sensitivity of filtration and precipitation methods. Water samples (1 L) collected from a tank containing one largetooth sawfish were sequentially filtered through five membranes of decreasing pore size (20, 10, 5, 1.2, and 0.45 μm). The proportion of sawfish eDNA within each size class was determined through quantitative real-time PCR (qPCR) using a species-specific TaqMan probe and primers. A linear mixed-effects model (lme) showed that the 1.2 and 20 μm filters captured most of the eDNA particles present in the sampled water. Additionally, whole water samples (0.375 L) were preserved in Longmire's buffer and compared to filtration. Filtration using 0.45 μm pore size was more sensitive to capture of largetooth sawfish eDNA than filtration with 20 μm filter or water precipitation. However, water precipitation was more efficient when accounting for volume of water processed. These results provide options for best capture and preservation of elasmobranch eDNA.

4.2. Introduction

Management and protection programs are only as good as the data used to determine them. The occurrence and distribution of many rare and threatened aquatic species remains difficult to determine due to vastness of the ocean making much of it inaccessible (McCauley et al., 2015; Webb & Mindel, 2015) and the inefficiency of many traditional survey methods (Shaw et al., 2016; Sigsgaard et al., 2015; Simpfendorfer et al., 2016; Smart et al., 2015). The development of novel technologies is needed to assess populations and improve interventions for management and conservation more rapidly. Non-invasive survey methods are favourable for detection of threatened species, given the threat that invasive sampling methods pose on individual survivorship (Hermosilla et al., 2015) and it does not require capture of the target species. Environmental DNA methods have been applied to detect a variety of threatened species, and have demonstrated success where traditional, invasive sampling methods are otherwise ineffective (Shaw et al., 2016; Sigsgaard et al., 2015; Simpfendorfer et al., 2016; Smart et al., 2015). All organisms naturally release DNA into their local environment through excretion, epidermal shedding, reproduction, or post-mortem decay, which can be isolated from filtered or whole water samples (Taberlet et al., 2012). This eDNA can be isolated and subsequently screened for the target species DNA using real-time quantitative PCR (qPCR) (Bylemans et al., 2017; Erickson et al., 2016; Levi et al., 2019; Lugg et al., 2018). Although eDNA methods are increasingly being used in the aquatic environment, the interpretation of eDNA data can be imperfect due to the myriad of factors that influence detectability (Furlan et al., 2015; Harrison et al., 2019).

Factors that influence detectability of aquatic eDNA particles include its state and fate. Aquatic environmental DNA is presumed to be made up of a complex mixture of intact cells, organelles, and DNA fragments dissolved in water or bound to particles (Sassoubre et al., 2016; Turner, Barnes, et al., 2014). Also, eDNA is degraded through the synergistic effect of biotic

and abiotic factors (Harrison et al., 2019; Huerlimann et al., 2020; Jo & Minamoto, 2021). An additional aspect driving differences in eDNA detectability is that eDNA sampling approaches differ in the size of particles that they target (i.e., whole water precipitation vs filtration, or filtration with different filter pore sizes). As a result, improving our knowledge of eDNA in its natural state and how best to capture and preserve it in samples before arrival at the laboratory will improve detection of target species. This is particularly relevant when dealing with rare species, when eDNA concentration in the natural environment might be lower than that of more abundant species.

Studies on the state and fate of eDNA target organellar (mitochondrial) genome regions (i.e., for bony fishes; Barnes et al., 2020; Jo et al., 2019; Sassoubre et al., 2016; Turner et al., 2014; Wilcox et al., 2015), because these occur at high copy numbers and organelles are abundantly distributed within a single cell (Martellini et al., 2005). The size of organelles, specifically mitochondria, is therefore useful information for the design of eDNA surveys that aim to detect mitochondrial gene fragments, especially when adopting a filtration eDNA capture method. The size of mitochondrial-derived particles that are captured is likely to vary, depending on the tissue source, development stage, or water temperature-dependent degradation (Jo, Arimoto, et al., 2019a, 2019b; Takeuchi et al., 2019). Current literature suggests that mitochondrial-derived eDNA particles from bony fish are most abundant between the 1.2 and 10 µm size class (Jo, Arimoto, et al., 2019a; Sassoubre et al., 2016; Turner, Barnes, et al., 2014; Wilcox et al., 2015), and that this eDNA is therefore most likely still contained within its organelle, cell, or is clustered together in clumps (Furlan et al., 2015).

The use of eDNA to survey elasmobranchs (sharks and rays) is a growing field given the advantages that this method possesses over traditional ones for sampling species of high conservation concern (i.e., one-third of sharks, skates and rays are threatened with extinction; Dulvy et al., 2021). For example, qPCR-based eDNA detection is highly sensitive and specific

to the target species, particularly those of low abundance, and is non-invasive circumventing risks to species susceptible to capture-induced stress (Budd et al., 2021; Lehman et al., 2020). Despite this, there are still fewer studies on eDNA of this taxon (Budd et al., 2021; Lehman et al., 2020; Postaire et al., 2020; Schweiss et al., 2020; Simpfendorfer et al., 2016; Weltz et al., 2017) compared to bony fishes. Elucidating the efficacy of eDNA field surveys, including an increased understanding of the physical state of eDNA in water, will lead to more robust and reliable detection of elasmobranch eDNA. It is anticipated that eDNA survey data on the contemporary occurrence and distribution of threatened shark and ray species will become an asset to practitioners designing tailored management and conservation interventions for shark and ray species.

In this chapter, the particle size distribution of aqueous mitochondrial-derived eDNA from the Critically Endangered largetooth sawfish *Pristis pristis* is assessed. Differences in sensitivity and efficiency of three eDNA capture methods with respect to capture of intra- and extra-cellular eDNA of largetooth sawfish is also assessed. Studies of largetooth sawfish eDNA characteristics and dynamics may be directly transferrable to eDNA studies of other elasmobranch species and especially sawfish relatives of the group Rhinopristiformes, which are of high conservation concern (Dulvy et al., 2016; Kyne et al., 2020).

4.3. Materials and Methods

4.3.1. Experimental set up

Environmental DNA was sampled from an outdoor saltwater tank at James Cook University, Cairns, Australia (-16.816658°, 145.687867°) on 5 September 2019 using individual sterile polyethylene bottles (described below). The tank contained one largetooth sawfish (*Pristis pristis*) and several small reef fish (green chromis *Chromis viridis*, longfin

bannerfish *Heniochus acuminatus*, and blue tang *Paracanthurus hepatus*). The individual largetooth sawfish (male, approx. 1 m total length) had been housed in the tank for five months prior to sample collection (April 2019) and was fed a diet comprised exclusively of mullet (family Mugilidae). Capture, handling, and husbandry of the sawfish in this study was according to James Cook University animal ethics A2584.

The water volume was 33,048 L and the total amount of water for the whole tank system was 75,000 L, which was filtered using biological and mechanical filtration, including sand, wool, live rock, protein skimmers, ozone, algae scrubbers, and mangroves. The water was collected from Trinity Inlet, Cairns, and was pre-filtered prior to use in the tank. Trinity Inlet is not expected to be inhabited by largetooth sawfish as indicated by discontinuity of records for the species along the Queensland coast, excluding Princess Charlotte Bay, in the past two decades (Wueringer, 2017). On the day of sampling, the tank water pH was 8.2, salinity was 35 ppt, and water temperature was 24.5–26.5 °C.

4.3.2. Particle size fractionation

A size fractionation experiment was conducted to understand the particle size distribution of naturally occurring aquatic particles. Evidence suggests that the majority of macro-organismal eDNA is efficiently captured by filter pore sizes between 1–10 µm (Turner, Barnes, et al., 2014). Additionally, studies in turbid waters have proven that target species eDNA can be effectively captured using 20 µm filter pore sizes (Robson et al., 2016). Therefore, I tested filter pore sizes ranging from 0.45–20 µm. The filter series included three types of nylon net filters (20, 10, and 5 µm nominal pore sizes, 47 mm diameter; Merck). Nylon net was the only filter material type available for these larger pore size filters at the time of this study (see also Turner et al., 2014). As a result, I selected nylon membrane filters for the two

smaller pore sizes (1.2 and 0.45 µm nominal pore sizes, 47 mm diameter; Merck). A supplementary experiment was conducted to visualise the physical structure of the net and membrane filters used. A Hitachi field emission gun scanning electron microscope (SEM) was used to capture images of unused filters. Secondary electron images of unused filters were captured at 70× and 220× magnification for 20, 10, and 5 µm filters and 2000× and 5000× magnification for 1.2 and 0.45 µm filters (Figure S4.1).

To physically separate the aquatic particle size classes, triplicate 500 mL water samples were collected directly from the tank containing the largetooth sawfish using individual sterile polyethylene bottles and were sequentially filtered using filter pore sizes from largest to smallest. Specifically, the triplicate water samples were each filtered through 20 µm filters held in individual sterile filter housing units (Thomas et al., 2018; Smith-Root, Washington, United States) that were attached to a diaphragm pump (Grover Scientific, Townsville, Australia) using clear 10 mm nylon tubing. Following filtration, each water sample was collected in a new, sterile polyethylene bottle and then filtered through 10 µm filters that were housed in new filter housing units. This method was repeated for each step of the sequential filtration experiment. Diaphragm pumps and nylon tubing were reused throughout the experiment because they are downstream of the filter and so were not considered a contamination risk. The external surfaces of the pumps and tubing were cleaned by wiping with 10% bleach and reverse osmosis-purified (RO) water and gloves were changed at each step to minimise risk of contamination.

Immediately after filtration at each point of the experiment, filters were cut in half using sterile forceps and scissors. Each filter half was then placed in 1.5 mL of Longmire's buffer (Longmire et al., 1997) contained in a 2 mL LoBind® microtube (Eppendorf South Pacific Pty Ltd, New South Wales, Australia) (Lecerf & Le Goff, 2010) to minimise potential loss of low-copy DNA due to biochemical or electrostatic retention and stored at ambient room temperature

until extraction. One half was archived for future use. In addition, triplicate 500 mL water samples were filtered once through 0.45 µm nominal pore size nylon membrane filters to estimate target capture efficiency when target particles are ultimately captured by a single filtration, hereafter termed the 'single filtration samples'. A filtration negative control consisting of passing 500 mL MilliQ water through a clean 0.45 µm filter was taken before tank water sample filtration.

Whole water samples were also collected from the same tank containing the juvenile largetooth sawfish for eDNA extraction via precipitation. Five replicate 375 mL water samples were collected and decanted into a new, clean HDPE plastic bottle (700 mL capacity) containing 125 mL Longmire's preservative buffer. The final volume of each tank replicate was therefore 500 mL. Additionally, a field control consisting of decanting 375 mL laboratory-grade water into a container filled with 125 mL Longmire's buffer was included. For ease of extraction in 50 mL falcon tubes, the 500 mL sample was thoroughly mixed by inversion and then 20 mL aliquots were decanted into twenty-five 50 mL LoBind® falcon tubes (Eppendorf South Pacific Pty Ltd, New South Wales, Australia) (Figure 4.1).

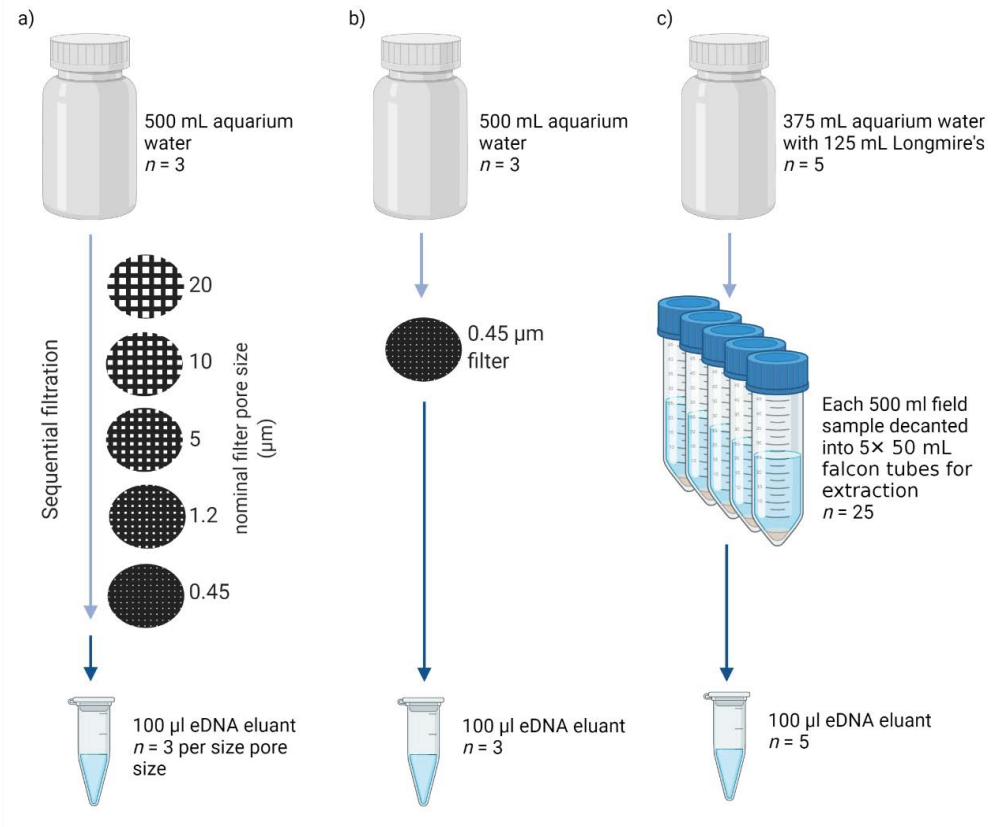

Figure 4.1. Schematic representation of the workflow for: (a) particle size fractionation; (b) 0.45 µm control and (c) whole water samples (3:1 aquarium water with Longmire's). Following filtration or precipitation, the resultant product was extracted using the same method and eDNA eluted in the same volume (100 µL). Created with BioRender.com.

4.3.3. Comparison of capture efficiency between filtration and whole water precipitation

The eDNA capture efficiency of filtration through 0.45 µm filters, 20 µm filters, and water precipitation was evaluated. Here, copy number estimated from qPCR Ct values were used for the 0.45 µm treatment from the single filtration samples; for the 20 µm treatment from the 20 µm filters used in the particle size fractionation experiment; and the whole water precipitation sample.

4.3.4. Environmental DNA extraction and quantification

Environmental DNA was extracted from the Longmire's buffer-preserved filter paper samples using the glycogen-aided precipitation extraction method described in 3.3.3.1. Extraction of eDNA from 20 mL aliquots of water in 50 mL LoBind® falcon tubes followed the protocol detailed in Villacorta-Rath et al., (2021). Briefly, eDNA was precipitated at 4°C overnight with 20 ml isopropanol, 10 µl glycogen (20 mg/mL; Merck, Victoria, Australia), and 5 ml 5 M sodium chloride 5 M. Precipitant was pelleted for 90 min at 3270 g (SX4750 rotor, Allegra X-12 R, Beckman Coulter Inc., Indianapolis, IN, USA) and 600 µL of pH 10 lysis buffer solution containing 0.8 M guanidine hydrochloride and 0.5% TritonX (Lever et al., 2015) was added with the precipitant for a freeze-thaw-lysis treatment, wherein samples were frozen overnight, thawed, vortexed, and incubated at 50°C for ≥3 h. Environmental DNA was then precipitated overnight, washed, and eluted in 100 µL UltraPure distilled water as per the protocol above.

An extraction control for each batch of extracted samples was included to account for contamination in the laboratory. Extracted eDNA from each sample was eluted in 100 µL UltraPure distilled water (ThermoFisher Scientific Pty Ltd, Victoria, Australia) in a 2 mL LoBind® microtube (Eppendorf South Pacific Pty Ltd, New South Wales, Australia) (Lecerf & Le Goff, 2010). Following extraction, each sample was briefly vortexed before 5 µL subsamples were taken for quantification of total recovered eDNA using a Quantus™ Fluorometer dsDNA System (Promega Pty Ltd, Australia). Extracted eDNA samples were stored at 4°C until qPCR screening.

4.3.5. Quantitative PCR analysis

A partial fragment of the largetooth sawfish *12S* ribosomal RNA was amplified using a QuantStudio 5 quantitative real-time PCR machine (Life Technologies, ThermoFisher Scientific Pty Ltd, Victoria, Australia) with a previously optimised primer and TaqMan probe assay (section 3.4.1). To estimate copy number, triplicate 12-point standard curves were run in adjacent wells on the same qPCR plate. For generation of standard curves, double-stranded synthetic DNA fragments (gBlocks™; Integrated DNA Technologies Pty Ltd, New South Wales, Australia) were synthesised to match the largetooth sawfish target fragment and serially diluted by 8-point $\log_{10}$ (1E+08–10 copies per assay) and 4-point $\log_2$ (5–0.65 copies per assay).

Quantitative PCR analysis was performed in six replicate 10 µL reactions run in adjacent wells on a MicroAmp™ Optical 384-well plate (Applied Biosystems, ThermoFisher Scientific, Victoria, Australia) as per condition described in section 3.4.1. Additionally, each qPCR plate included a triplicate no-template control (NTC) sample.

QuantStudio Analysis Software version 1.4.2 was used to analyse threshold cycle value (Ct) based on automatic baseline and manually determined threshold fluorescence values (0.7 ΔRn). All amplicons were sent to the Australian Genome Research Facility (AGRF Pty Ltd, Brisbane, Australia) for clean-up and bidirectional Sanger sequencing for verification that the product amplified was from the target species. Species-specificity of each sequence was confirmed using BLASTn searches against the entire NCBI nucleotide database. Detections were considered true positives and were used in subsequent analyses if amplification curves crossed the fluorescence threshold within 50 cycles, BLASTn search of sequence matched target species with ≥98% pairwise identity, and corresponding negative controls exhibited no amplification.

To test for qPCR inhibition, I used a TaqMan™ Exogenous Internal Positive Control (IPC) qPCR assay (Applied Biosystems; Hartman et al., 2005) with a custom internal probe modification (i.e., TAMRA-VIC changed to ABY-QSY) to not compromise amplification efficiency of the target, which uses a VIC-labelled reporter dye, in the same qPCR reaction. The assay was applied in duplexed reactions, as per the manufacturers' optimised conditions, with the 3 µL of eDNA in three technical replicates. Three reactions containing only IPC DNA were included as 'inhibitor-free' positive controls. To distinguish types of inhibition, I used an IPC ΔCt of 3 cycles as the threshold (Hartman et al., 2005). Specifically, IPC ΔCt of 3 or more cycles was considered partial inhibition and no amplification for the IPC was considered complete inhibition.

4.3.6. Data analyses

All statistical analyses were completed in R v 3.5.0 (R Core Team, 2017). Differences in largetooth sawfish *12S* gene copy number were assessed using linear mixed effects (lme) and generalised least squares (gls) models with a Gaussian distribution in the 'nlme' R package (Pinheiro et al., 2013), using DNA copy number (inferred from 12-point standard curves; $1\times10^{08–0.25}$ copies per reaction) as response variable. Prior to model testing, copy number data from the sequential filtration experiment were log transformed to reduce skewness and conform to normality (Figure S4.2). Given that the data were auto-correlated, I accounted for non-independence of the response variable (Figure S4.3), by including the 'AR-1' auto-correlation structure (corAR1) (Zuur et al., 2009). The explanatory variables tested in the full model for the sequential filtration experiment were filter pore size (fixed effect) and tank replicate and technical replicate (nested random effects). Models were fitted using the restricted maximum likelihood (REML) function. The best performing model was chosen based on the Akaike Information Criterion (AIC). Post-hoc multiple comparisons of means were conducted using

the general linear hypotheses function (glht) Tukey contrasts and included a Bonferroni correction in the 'multcomp' R package (Hothorn et al., 2020).

Differences in sawfish eDNA capture sensitivity (total number of DNA copies captured) and capture efficiency (relative sawfish eDNA capture per 100 mL of processed water) across different methods were assessed using a linear model (lm). This analysis used the single filtration, 20 µm filter and water precipitation samples as model methods for eDNA capture. In both cases, the response variable was *12S* copy number and the explanatory variable was eDNA capture method. Three different models were run to test; i) the effect of technical replicate as an additive factor, ii) the interaction between technical replicate and eDNA capture method, and iii) eDNA capture method as the sole explanatory variable. The unbalanced sampling design (three tank replicates used for water filtration and five tank replicates used for water precipitation) was corrected using a type II analysis of variance (ANOVA) in the 'car' R package (Fox & Weisberg, 2019). Post-hoc paired comparisons of means were performed using Tukey's HSD. Based on data normality testing, DNA copy number data were log transformed and relative copy number data were square root transformed.

Finally, the coefficients of variation (CV; standard deviation divided by the mean) for all experimental data were assessed using a one-way ANOVA to evaluate the relative stability of sawfish *12S* copy numbers across filter pore size and method.

Data used in the analyses can be found in Table S4.1 and Table S4.2.

4.4. Results

Largetooth sawfish eDNA was detected in all qPCR reactions (excluding negative and no-template controls) in the particle size distribution (n = 90) and Longmire's buffer preservation (n = 25) experiments and in the single filtration sample (n = 18). The standard

curve used to estimate *12S* copy number had a y-intercept of 38.97 cycles, slope of -3.11, efficiency of 109.29%, and R^2 of 0.98. All tested filter samples and whole water samples showed no evidence of inhibition (IPC ΔCt range: 0.18–1.09 cycles). Finally, field controls, filtration blanks, extraction negative controls, and qPCR no-template controls tested negative for largetooth sawfish eDNA and all sequenced amplicons matched to reference *P. pristis* sequence, confirming true positive detection.

4.4.1. Estimation of particle size distribution

The best performing model was the one assessing DNA copy number as a function of pore size and field and technical replicates (Table 4.1). There were significant differences in the estimate of sawfish *12S* copy numbers across different pore sizes (Table 4.2; Figure 4.2A). The mean total number of copies (± SE) captured in the experiment was 17,748.4 (± 6,037.5). Overall, the 1.2 µm filter retained the highest number of copies (10,413.7 ± 3,263.7 copies). Copy number estimates for the 1.2 µm filter were 3.1-times higher than those for the 20 µm filter (3,356.1 ± 348.6 copies) (β = 0.83, SE = 0.16, $z(90)$ = 5.1, $p < 0.001$). Copy number estimates for the 20 µm filter were on average 3.9-times higher than the subsequent 10 µm filter (851.5 ± 94.5 copies) (β = 1.37, SE = 0.18, $z(7.57)$, $p < 0.001$) and 1.7-times higher than the 5 µm filter (2,029.5 ± 324.5 copies; β = 0.58, SE = 0.18, $z(90)$ = 3.21, p = 0.01). Capture on the 5 µm filter was on average 2.4-times higher than the 10 µm filter (β = 0.79, SE = 0.16, $z(90)$ = 4.87, $p < 0.001$). Copy number estimates for the 10 and 0.45 µm filters, which retained the least number of copies, were not significantly different (851.5 ± 94.5 vs 1,097.6 ± 71.5 copies, respectively). Total dsDNA (ng/µL) values followed the same trend as sawfish copy number (Figure 4.2A). Relative variability, measured through the CV, was not significantly different and constant across filter pore sizes (Figure S4.4).

Table 4.1. Particle size fractionation model comparisons.

Model	Method	df	AIC	BIC	logLik	Test	L.Ratio	p-value
Reduced model log.sawfish 12S copy number ~ pore size	gls	6	158.52	173.17	-73.26			
Full model1 log.sawfish 12S copy number ~ pore size, random = ~1|field replicate/technical replicate	lme	8	156.81	176.35	-70.40	1 vs 2	5.71	0.058
Full model2 log.sawfish 12S copy number ~ pore size, random = ~1|field replicate/technical replicate, correlation = corAR1()	lme	9	156.03	178.02	-69.02	2 vs 3	2.78	0.096

Table 4.2. Particle size fractionation final model.

Model: lme(sawfish 12S copy number ~ pore size, random = ~1/field replicate/technical replicate, correlation = corAR1())					
Predictor	β	SE	df	t	p
Intercept	6.97	0.12	68	56.17	0.000
Pore_size1.2	1.89	0.18	68	10.75	0.000
Pore_size5	1.89	0.18	68	2.73	0.008
Pore_size10	-0.31	0.18	68	-1.76	0.082
Pore_size20	1.06	0.18	68	6.05	0.000
Observations	90				
Marginal R^2 / Conditional R^2	0.68 / 0.73				
AIC	156.03				

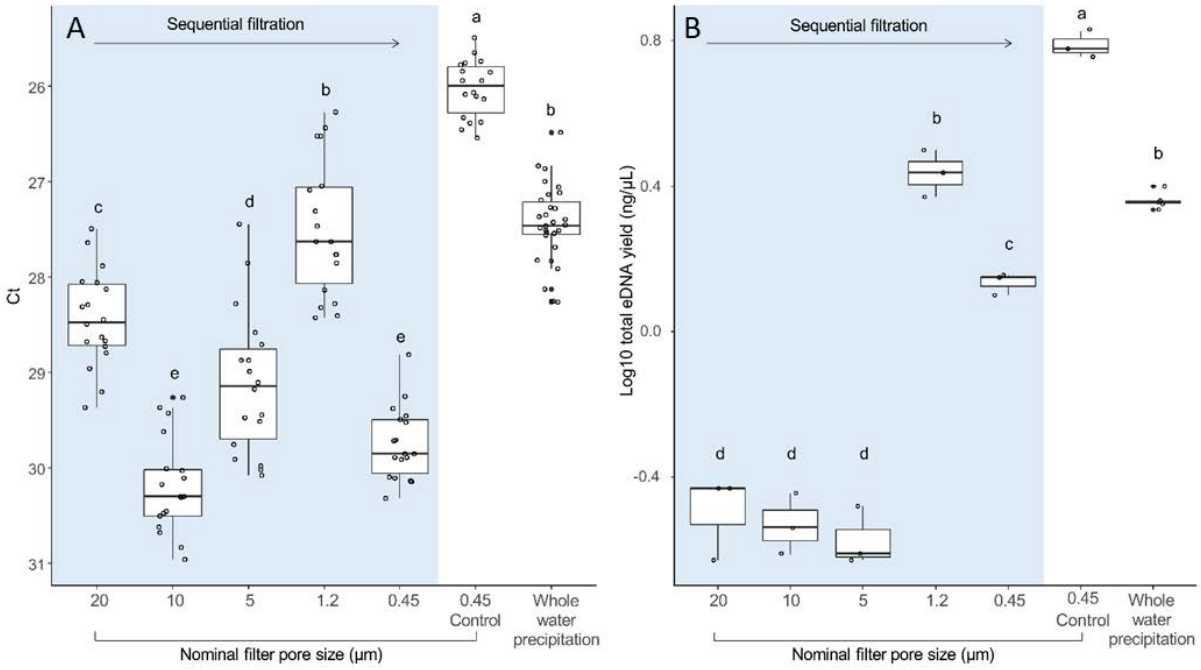

Figure 4.2. Tukey box plots with scattered points showing amount of A) largetooth sawfish (*Pristis pristis*) *12S* gene copies represented as Ct measured using qPCR and B) total eDNA represented as ng per µL measuring using Quantus™ Fluorometer dsDNA System.

4.4.2. Comparison of capture sensitivity and efficiency across methods

The best performing models to assess differences in capture sensitivity and efficiency of sawfish *12S* eDNA estimate copy number considered capture method as the sole explanatory variable (Table 4.3). Mean estimate copy numbers differed significantly across all three capture methods ($F_{(2,63)}$ = 113.24, $p < 0.001$). The single filtration method (filtering 1 L of water through a 0.45 µm filter) yielded the highest mean number of copies (23,152.2 ± 1,788.6 copies), performing better than the 20 µm filtration method (3,356.1 ± 348.6 copies; β = -2.64, SE = 0.18, $t(63)$ = -14.96, $p < 0.001$) and the precipitation method (time 0, processed immediately after collection) (1,215.3 ± 58.7 copies; β = -1.54, SE = 0.16, $t(63)$ = -9.74, $p < 0.001$) (Figure 4.2A). The precipitation method also performed better than the 20 µm filtration method (β = 1.1, SE = 0.16, $t(63)$ = 6.98, $p < 0.001$). Although relative variability (CV) values were not statistically different across capture methods, they showed a decreasing trend from

single filtration to precipitation samples (Figure S4.4). Total dsDNA (ng/µL) yield followed a similar pattern to mean sawfish copy number (Figure 4.2B).

Table 4.3. Best method model comparisons.

Method sensitivity (total sawfish copy number)						
Model	Res. df	RSS	df	Sum of Sq	F	Pr(>F)
Reduced model sawfish 12S copy number ~ method	63	9.65				
Additive model sawfish 12S copy number ~ method + technical replicate	58	9.42	5	0.23	0.25	0.94
Interaction model sawfish 12S copy number ~ method * technical replicate	48	8.89	10	0.53	0.29	0.98
Method efficiency (relative sawfish copy number)						
Model	Res. df	RSS	df	Sum of Sq	F	Pr(>F)
Reduced model relative copy number ~ method	63	8897.9				
Additive model relative copy number ~ method + technical replicate	58	8710.0	5	187.84	0.22	0.95
Interaction model relative copy number ~ method * technical replicate	48	8298.1	10	411.94	0.24	0.99

Relative estimate copy numbers also differed significantly across all three capture methods ($F_{(2,63)} = 184$, $p < 0.001$). The precipitation method, which captured 7,390.19 ± 519.19 per 100 mL, outperformed both the single filtration (2,315.22 ± 229.38 copies per 100 mL; $\beta = 1.14$, SE = 0.16, $t(63) = 9.74$, $p < 0.001$) and 20 µm filtration methods (335.61 ± 34.86 copies per 100 mL; $\beta = -0.82$, SE = 0.12, $t(63) = -6.98$, $p < 0.001$).

4.5. Discussion

Environmental DNA detectability of rare or threatened species relies on the successful capture and preservation of eDNA particles. The study of target species particle size distribution is important to help the user select the most effective sampling method to capture

existing eDNA. Additionally, how effectively eDNA particles are preserved prior to processing and analysis has important implications to avoid false negatives due to eDNA degradation. Based on the models, the particle size distribution of eDNA in marine tank water was non-linear and was most abundant at 1.2−5 and ≥20 µm size classes. Finally, when the volume of water was standardised across capture methods, precipitating sawfish eDNA from whole water samples exhibited higher capture efficiency than filtering water through either a 0.45 or 20 µm filter. Conversely, filtration was more sensitive to capture of largetooth sawfish eDNA than precipitation due to the higher volume of water that was able to be processed. Given these findings, and as to my knowledge this is the first study to investigate eDNA particle size distribution of any elasmobranch species, I discuss methodological points relevant for the capture of elasmobranch aqueous mitochondrial eDNA.

4.5.1. Particle size fractionation

The findings presented here show that the mitochondrial-derived eDNA particle size from largetooth sawfish was most predominantly in the 1.2−5 and ≥20 µm size classes and that the distribution was non-linear. The former is generally consistent with comparable studies by Sassoubre et al., (2016), Turner et al., (2014), and Wilcox et al., (2015), where fish mitochondrial eDNA particles were predominantly in the 1 µm size class. More recently, Barnes et al., (2020) multi-species analyses demonstrated that 1 µm filters captured most of the eDNA available in experimental ponds, supporting a growing body of evidence on eDNA particle size across fish species. A 20 µm pore size filter is not often used in particle size distribution studies, which precludes detailed comparison to the results presented here. Yet, Turner et al. (2014) found that common carp (*Cyprinus carpio*) mitochondrial eDNA copies in the 20 µm size class were twice as abundant than in the 10 µm size class and whole water precipitation sample. Jo, Arimoto, et al., (2019a) quantified Japanese jack mackerel (*Trachurus*

japonicus) mitochondrial eDNA particle size with greater resolution by using an additional size fraction (i.e., 3 μm) and showed that particles were most abundant in the 3–10 μm size fraction. Lastly, a recent meta-analysis by Jo & Minamoto, (2021) suggested that water temperature does not uniformly affect eDNA degradation, whereby larger particles are more susceptible to decay induced by higher water temperatures.

In this study, the 1.2 μm filter captured 58.7% of target eDNA and this was almost one order of magnitude greater than the subsequent 0.45 μm filter. This suggests that the eDNA in the study system was likely derived from within intact mitochondrial organelles, or mitochondria within cells, and not extra-cellular/organellar DNA. Eukaryote mitochondria range between 0.5–10 μm in size (mean 0.75–3 μm), but this can vary considerably depending on the cell type, physiological state, organ, and species (Miyazono et al., 2018). For aquatic organisms, the regular apoptotic shedding of epithelial cells releases apoptotic cellular bodies with intact mitochondria, which supports the hypothesis that intact mitochondria can exist and persist in the water column and are available for capture. However, the possibility of capture of some extra-cellular/organellar DNA cannot be ruled out, given that the breakdown of large eDNA particles into smaller particles is positively correlated with higher water temperature and time since deposition (Jo, Arimoto, et al., 2019b; Jo & Minamoto, 2021). This is partially owing to increased microbial growth at elevated temperature and an associated microbial-utilisation of DNA for phosphorus (Strickler et al., 2015), which is likely to be a feature for warm tropical waters (Huerlimann et al., 2020). Therefore, the filtration fractionation method used here is likely to oversimplify the reality of particle size distribution, given the above factors and the expected, but unmeasured, variation induced by the tank system and the behaviour of the inhabiting sawfish. In addition, differences in filter membrane material type can also affect eDNA yield (Hinlo et al., 2017; Turner, Miller, et al., 2014). In this study,

differences in the physical texture and uniformity of the pores across the five nylon membrane and nylon net filter types is likely to have produced differences in total eDNA yield.

The >20 µm filter size class comprised the second greatest proportion of largetooth sawfish mitochondrial eDNA, wherein capture was 3.9-times greater than the subsequent 10 µm filter. The latter trend was also observed for common carp eDNA from lake and pond environments (Turner, Barnes, et al., 2014). This suggests that eDNA may exist in greater abundance at larger size classes than is often referred to by eDNA survey users, who intuitively use the smallest possible filter pore size suitable to the conditions. Mitochondria that comprise aqueous eDNA from macrofauna originate predominantly from waste products, shed epidermal tissues and secretions, and on the occasion of birth or death, reproductive material or post-mortem debris. Depending on this biological source, mitochondria can be arranged within the shed/released cells or in large aggregates of biological material. These particles may remain suspended in stratified sea water, or transported horizontally by currents (Wotton & Malmqvist, 2001). Given that the tank water was sampled during the period that a single sawfish was present, it is possible that clumps of biological material were captured directly. However, large particles, for example faecal pellets, may rapidly settle out of shallow still water (Wotton & Malmqvist, 2001). In addition, eDNA may be associated with other large particles such as algal cells and sediments (Barnes et al., 2020). Overall, I suggest that larger pore sizes (1.2–20 µm) are effective for capture of mitochondrial-derived eDNA using filtration. To my knowledge, this is the first study to provide evidence of eDNA particle size distribution of elasmobranchs and can be applied to eDNA studies targeting other sharks and ray species. This is particularly informative for users conducting field sampling in challenging environments (e.g., turbid or highly productive environments) where the use of larger filter pore sizes will commensurately increase the viable filtrate volume and ameliorate challenges with rapid filter clogging (Goldberg et al., 2018; Hinlo et al., 2017; Kumar et al., 2021; Robson et al., 2016; Wittwer et

al., 2018). Notably, the advent of high-volume filtration methods, such as tow nets (Sepulveda et al., 2019), that use >60 µm mesh to process 1,000's of litres of water rely on the capture of eDNA in large particle sizes. These methods have already demonstrated increased detection sensitivity for rare taxa in large study areas. For example, Villacorta-Rath et al., (2021) reported high eDNA detection frequency of a Critically Endangered rainforest frog 22.8 km downstream from the population when using a large-volume filter unit that could process > 1,000 L of stream water.

4.5.2. Method sensitivity and efficiency

The performance of eDNA surveys relies on the sensitive and efficient capture and preservation of target particles. It is evident that capture of eDNA from target species that are rare or in low abundance is especially difficult, so choice of method is important. The results of this study suggest that there are trade-offs between sensitivity and efficiency for different eDNA capture methods. Overall, the model suggests that filtering 1 L of water through a single 0.45 µm filter is more sensitive to detection of target eDNA than filtering 1 L through a 20 µm filter, or precipitating 100 mL of water. This was shown as sawfish eDNA yields that were on average 6.9- and 3.1-times greater, respectively. A previous study demonstrated that aqueous eDNA in larger size fractions (i.e., intra-cellular and -organellar DNA) experience faster decay rates in higher water temperatures (>20°C) (Jo & Minamoto, 2021). This is possibly because eDNA degrades into small sizes, and therefore a large amount of the available eDNA is captured by the smallest filter pore size (0.45 µm).

Yet, when the volume of water sampled was standardised across methods, which was used as a proxy measure of method efficiency, the precipitation method outperformed both filtration methods. In this case, yields per 100 mL were on average 3.2- and 22-times greater

than 0.45 µm and 20 µm filters, respectively. Existing literature on methods comparison also provide evidence of differences in efficiency and sensitivity, supporting either precipitation (Muha et al., 2019; Raemy & Ursenbacher, 2018), or filtration (Eichmiller et al., 2016; Hinlo et al., 2017; Peixoto et al., 2020), but in context of the study system and species. It is therefore evidence that the choice of method would be dependent on the particular characteristics of the water body. For example, greater turbidity (or suspended solids) and the associated problems introduced by filter clogging may make the precipitation method more attractive.

I qualify the results presented in this study by suggesting that it is generally unlikely that users of the filtration method would filter only 100 mL using a 0.45 µm filter, and even less likely with a 20 µm filter. The volume of water than can be processed in a single filtration event can be several orders of magnitude larger than precipitation (e.g., ≥2,000 L; Sepulveda et al., (2019), but typically 0.5–5 L vs. 15–100 mL), making it a more sensitive method and therefore the more popular choice for capture of eDNA. In addition, the ease at which hundreds of filters can be stored and extracted post-collection compared to whole water samples is a major advantage (e.g., for 100 samples; 47 mm disc filters folded in 2 mL microtubes stored in a single 100-well storage box vs. 3 L of water in 100 50 mL falcon tubes as a minimum). In contrast, it is evident that the precipitation method captures both intra- and extra-cellular/organellar eDNA and, because of this, the method can be more efficient than filtration (Minamoto et al., 2015; Muha et al., 2019; Piaggio et al., 2013). Its other major advantages are the portability and low cost of field equipment, simplicity of handling, and reduced chances of contamination in the field, which allows for engaging with non-specialists for sample collection (Villacorta-Rath et al., 2020).

Yet the advantages of both methods are offset by their limitations, which are especially problematic for rare species detection. In the case of filtration, where it is recommended to maximise collection of trace eDNA through use of a small pore size (Minamoto et al., 2015;

Turner, Barnes, et al., 2014; Turner, Miller, et al., 2014) or increase filtrate volume (Sepulveda et al., 2019), highly turbid or productive environments will cause rapid filter clogging (Robson et al., 2016). Filtering water in turbid environments could be considered one of the most widespread, yet undesirable methodological challenge (Ip et al., 2021; Robson et al., 2016), but may be compensated with the use of larger pore size filters (e.g., 1.2–20 μm, as in this study; Barnes et al., (2020)), pre-filtration (Takahara et al., 2013), or multiple filter replicates (Hunter et al., 2019). The downside of these options would be the increase in cost and time for field and laboratory processing of additional replicates (Sepulveda et al., 2019). In addition, filtration can also concentrate a higher amount of qPCR inhibitors in the samples (Raemy & Ursenbacher, 2018; Sepulveda et al., 2019), therefore, inhibition testing should be routinely applied to confirm that any negative result is not due to qPCR inhibition. Conversely, Williams et al., (2017) suggest that whole water sample collection is the most optimal method for eDNA capture in turbid waters to avoid problematic filter clogging. Yet, whole water precipitation is limited by collection of smaller water volumes, as the DNA extraction step is limited by centrifuge size, which may be especially undesirable in large rivers and lakes, or the open ocean where eDNA is highly dispersed or diluted. The volume and weight of water samples may be expensive to ship via air freight regionally or internationally and whole water samples must be subsampled for extraction, which increases the cost and time of DNA extraction proportionally. Other options that were not tested here include filtration with syringe and enclosed filter (e.g., Sterivex filters; Buxton et al., 2018; Spens et al., 2016); however, I recommend pilot studies are carried out to test the suitability of the method for the study system. It is important to note that the results of the present study are based on eDNA collected from a tank housing the target species and therefore I would expect a much lower concentration of target eDNA in the wild.

4.6. Conclusion

Based on the results of the present study, water filtration using filters of pore size ranging 1.2–20 µm may be beneficial in contexts such as the detection of rare sawfishes in a large, fast-flowing river or dynamic ocean system. I note that, depending on logistical and environmental constraints, precipitation may be the more user-friendly option. However, given the opportunity to commensurately increase the filtrate volume when using pore sizes greater than 1.2 µm filtration is the preferred choice, which is strengthened by evidence in the literature to suggest improvements in detection probabilities of aquatic species when using filtration to capture eDNA.

Additionally, given the threatened status of many elasmobranch species, I consider that this study and future studies on eDNA particle size distribution and capture efficiency will improve implementation and interpretation of eDNA surveys and thereby strengthen its usefulness in providing crucial baseline information for management practitioners and researchers.

4.7. Supplementary Information

Data S4.7.1 Imaging net and membrane filters used in the particle size fractionation experiment

Clean net and membrane filters used in this experiment were imaged using a Hitachi field-emission gun scanning electron microscope housed at the Advanced Analytical Centre, James Cook University, Bebegu Yumba Campus, Townsville, Australia. Secondary electron images of unused filters were captured at 70× and 220× magnification for 20, 10, and 5 µm filters and 2000× and 5000× magnification for 1.2 and 0.45 µm filters to inspect their physical structure. Visual inspection of nylon net and membrane filters demonstrated differences in uniformity and texture (Fig. 2). There were distinct visual differences between net (20, 10, and 5 µm) and membrane (1.2 and 0.45 µm) filters, where the net filters had a more consistent overall texture and pores were more uniform in size and distribution. Membrane filters exhibited an uneven distribution of pores, which were also variable in size and shape.

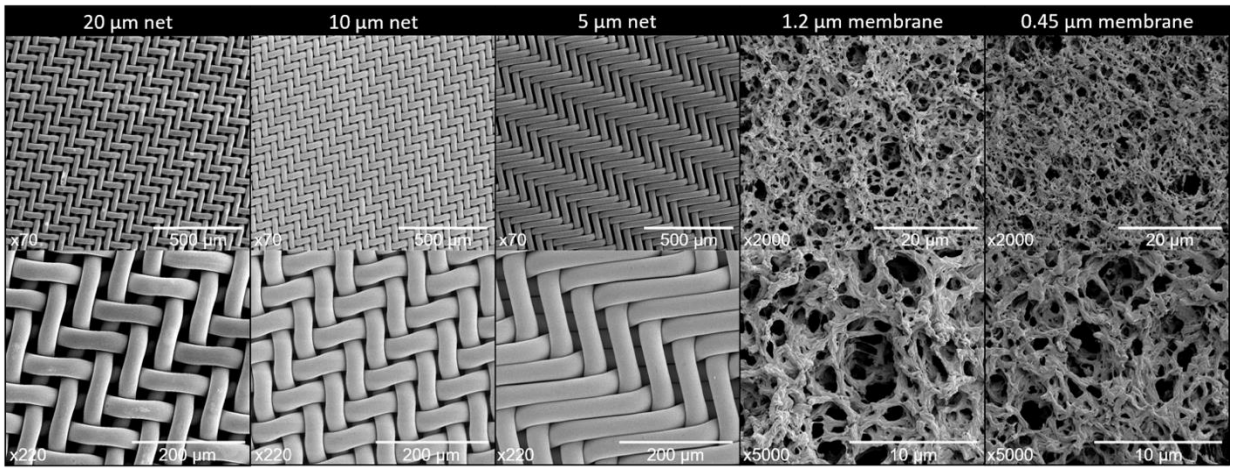

Figure S4.1. Secondary electron images of unused filters captured at 70× and 220× magnification for 20, 10, and 5 µm filters and 2000× and 5000× magnification for 1.2 and 0.45 µm filters to inspect their physical structure.

Table S4.1. Data generated for and utilised in particle size fractionation analysis.

Pore size (µm)	Field replicate	Technical replicate	Cycle (Ct)	eDNA concentration (ng/µL)
0.45	1	1	29.459	984.481
0.45	1	2	29.483	967.041
0.45	1	3	29.562	912.609
0.45	1	4	29.685	833.275
0.45	1	5	29.691	829.531
0.45	1	6	29.839	743.36
0.45	2	1	28.911	1,476.02
0.45	2	2	28.906	1,480.55
0.45	2	3	29.428	1,007.63
0.45	2	4	28.865	1,526.76
0.45	2	5	29.199	1,192.49
0.45	2	6	29.011	1,370.88
0.45	3	1	28.68	1,750.53
0.45	3	2	29.765	785.301
0.45	3	3	29.214	1,179.57
0.45	3	4	29.777	778.183
0.45	3	5	29.455	987.055
0.45	3	6	29.506	951.199
1.2	1	1	27.42	4,438.21
1.2	1	2	27.229	5,112.03
1.2	1	3	27.504	4,171.30
1.2	1	4	27.788	3,381.53
1.2	1	5	27.822	3,297.53
1.2	1	6	27.952	2,995.94
1.2	2	1	27.09	5,662.08
1.2	2	2	26.957	6,247.25
1.2	2	3	26.863	6,699.19
1.2	2	4	27.21	5,182.82
1.2	2	5	27.232	5,097.53
1.2	2	6	27.742	3,498.31
1.2	3	1	26.701	7,548.63
1.2	3	2	25.741	15,333.04
1.2	3	3	25.853	14,118.95
1.2	3	4	26.286	10,255.09
1.2	3	5	25.223	22,492.90
1.2	3	6	23.852	61,913.61
10	1	1	28.71	1,711.35
10	1	2	29.545	924.071
10	1	3	28.988	1,393.62
10	1	4	28.682	1,748.05
10	1	5	29.263	1,137.50
10	1	6	30.294	531.527
10	2	1	30.591	426.585
10	2	2	30.069	627.304
10	2	3	30.163	585.186
10	2	4	29.919	701.154
10	2	5	30.466	468.011
10	2	6	30.272	540.172
10	3	1	29.916	702.293
10	3	2	29.916	702.708
10	3	3	29.906	707.742
10	3	4	29.48	969.416
10	3	5	29.672	841.05
10	3	6	30.109	609.261
20	1	1	27.771	3,425.20

20	1	2	26.88	6,613.18
20	1	3	27.495	4,199.43
20	1	4	27.431	4,403.18
20	1	5	27.466	4,289.41
20	1	6	27.357	4,651.21
20	2	1	28.916	1,470.52
20	2	2	29.043	1,338.98
20	2	3	28.446	2,079.66
20	2	4	28.338	2,253.02
20	2	5	28.277	2,356.62
20	2	6	28.046	2,795.82
20	3	1	27.768	3,433.16
20	3	2	28.234	2,432.81
20	3	3	26.962	6,226.78
20	3	4	28.289	2,336.52
20	3	5	27.827	3,286.58
20	3	6	28.035	2,818.43
5	1	1	29.241	1,156.60
5	1	2	29.889	716.594
5	1	3	29.509	948.806
5	1	4	29.585	896.885
5	1	5	29.391	1,035.20
5	1	6	29.247	1,151.06
5	2	1	29.092	1,290.58
5	2	2	28.676	1,755.29
5	2	3	28.495	2,005.88
5	2	4	29.072	1,310.29
5	2	5	27.706	3,594.46
5	2	6	27.957	2,984.89
5	3	1	27.645	3,757.95
5	3	2	28.487	2,017.78
5	3	3	28.235	2,430.93
5	3	4	28.759	1,651.37
5	3	5	28.796	1,606.53
5	3	6	26.963	6,219.09

Table S4.2. Data generated for and utilised in method sensitivity and efficiency analysis.

ID	Method	Field replicate	Technical replicate	CT	Sawfish eDNA concentration (copy number)	Total eDNA yield (ng/µL)
0.45_Control_rep_1	Filter0.45	1	1	25.677	16,084.90	6
0.45_Control_rep_1	Filter0.45	1	2	25.585	17,213.32	6
0.45_Control_rep_1	Filter0.45	1	3	25.25	22,038.90	6
0.45_Control_rep_1	Filter0.45	1	4	24.793	30,887.35	6
0.45_Control_rep_1	Filter0.45	1	5	25.468	18,757.99	6
0.45_Control_rep_1	Filter0.45	1	6	24.155	49,478.62	6
0.45_Control_rep_2	Filter0.45	2	1	24.89	28,747.05	5.7
0.45_Control_rep_2	Filter0.45	2	2	25.257	21,930.11	5.7
0.45_Control_rep_2	Filter0.45	2	3	25.062	25,329.32	5.7
0.45_Control_rep_2	Filter0.45	2	4	25.829	14,370.37	5.7
0.45_Control_rep_2	Filter0.45	2	5	25.77	15,010.99	5.7
0.45_Control_rep_2	Filter0.45	2	6	25.971	12,936.78	5.7
0.45_Control_rep_3	Filter0.45	3	1	25.255	21,967.86	6.8
0.45_Control_rep_3	Filter0.45	3	2	25.582	17,248.28	6.8
0.45_Control_rep_3	Filter0.45	3	3	25.944	13,205.64	6.8
0.45_Control_rep_3	Filter0.45	3	4	24.423	40,609.84	6.8
0.45_Control_rep_3	Filter0.45	3	5	25.211	22,680.15	6.8
0.45_Control_rep_3	Filter0.45	3	6	24.914	28,241.74	6.8
20_rep_1	Filter20	1	1	27.771	3,425.20	0.371
20_rep_1	Filter20	1	2	26.88	6,613.18	0.371
20_rep_1	Filter20	1	3	27.495	4,199.43	0.371
20_rep_1	Filter20	1	4	27.431	4,403.18	0.371
20_rep_1	Filter20	1	5	27.466	4,289.41	0.371
20_rep_1	Filter20	1	6	27.357	4,651.21	0.371
20_rep_2	Filter20	2	1	28.916	1,470.52	0.37
20_rep_2	Filter20	2	2	29.043	1,338.98	0.37
20_rep_2	Filter20	2	3	28.446	2,079.66	0.37
20_rep_2	Filter20	2	4	28.338	2,253.02	0.37
20_rep_2	Filter20	2	5	28.277	2,356.62	0.37
20_rep_2	Filter20	2	6	28.046	2,795.82	0.37
20_rep_3	Filter20	3	1	27.768	3,433.16	0.235
20_rep_3	Filter20	3	2	28.234	2,432.81	0.235
20_rep_3	Filter20	3	3	26.962	6,226.78	0.235
20_rep_3	Filter20	3	4	28.289	2,336.52	0.235
20_rep_3	Filter20	3	5	27.827	3,286.58	0.235
20_rep_3	Filter20	3	6	28.035	2,818.43	0.235
T0_rep_1	Precipitation	1	1	26.229	10,697.12	2.49
T0_rep_1	Precipitation	1	2	26.506	8,717.86	2.49
T0_rep_1	Precipitation	1	3	25.577	17,317.30	2.49
T0_rep_1	Precipitation	1	4	26.563	8,357.04	2.49
T0_rep_1	Precipitation	1	5	26.186	11,043.09	2.49
T0_rep_1	Precipitation	1	6	26.078	11,955.52	2.49
T0_rep_2	Precipitation	2	1	26.853	6,746.01	2.44
T0_rep_2	Precipitation	2	2	27.349	4,678.29	2.44
T0_rep_2	Precipitation	2	3	27.822	3,297.19	2.44
T0_rep_2	Precipitation	2	4	27.253	5,019.23	2.44
T0_rep_2	Precipitation	2	5	26.926	6,390.72	2.44

T0_rep_2	Precipitation	2	6	27.801	3,349.73	2.44
T0_rep_3	Precipitation	3	1	26.596	8,154.94	2.42
T0_rep_3	Precipitation	3	2	26.987	6,111.51	2.42
T0_rep_3	Precipitation	3	3	26.336	9,883.30	2.42
T0_rep_3	Precipitation	3	4	26.984	6,124.40	2.42
T0_rep_3	Precipitation	3	5	26.93	6,371.51	2.42
T0_rep_3	Precipitation	3	6	26.577	8,274.05	2.42
T0_rep_4	Precipitation	4	1	26.751	7,274.34	2.29
T0_rep_4	Precipitation	4	2	26.891	6,557.65	2.29
T0_rep_4	Precipitation	4	3	26.892	6,555.72	2.29
T0_rep_4	Precipitation	4	4	26.751	7,275.76	2.29
T0_rep_4	Precipitation	4	5	26.856	6,732.48	2.29
T0_rep_4	Precipitation	4	6	26.961	6,229.56	2.29
T0_rep_5	Precipitation	5	1	27.768	3,431.29	2.24
T0_rep_5	Precipitation	5	2	26.457	9,037.80	2.24
T0_rep_5	Precipitation	5	3	26.782	7,110.64	2.24
T0_rep_5	Precipitation	5	4	26.966	6,208.30	2.24
T0_rep_5	Precipitation	5	5	27.253	5,019.90	2.24
T0_rep_5	Precipitation	5	6	26.659	7,783.52	2.24

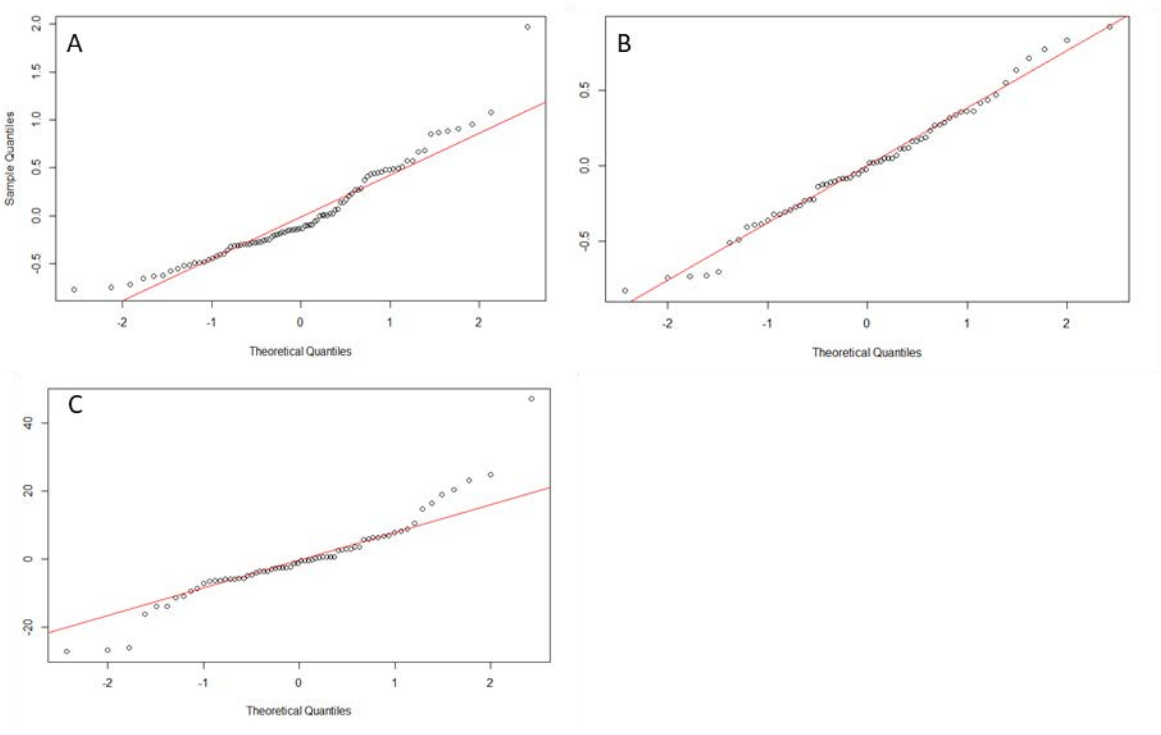

Figure S4.2. QQ-plot to demonstrate assumption of normality for A) particle size fractionation experiment following log transformation, B) capture sensitivity comparison, and C) capture efficiency comparison.

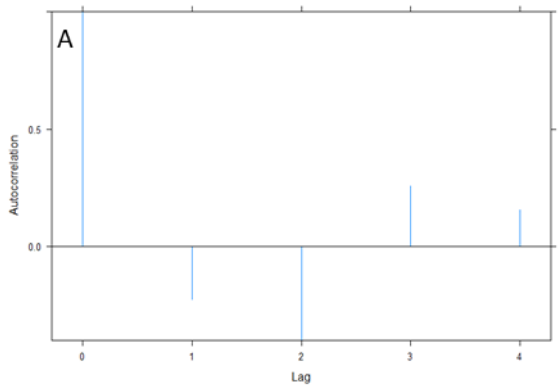

Figure S4.3. Autocorrelation plot for particle size fractionation experiment full model to validate the inclusion of autocorrelation structure 'AR-1' in the final model.

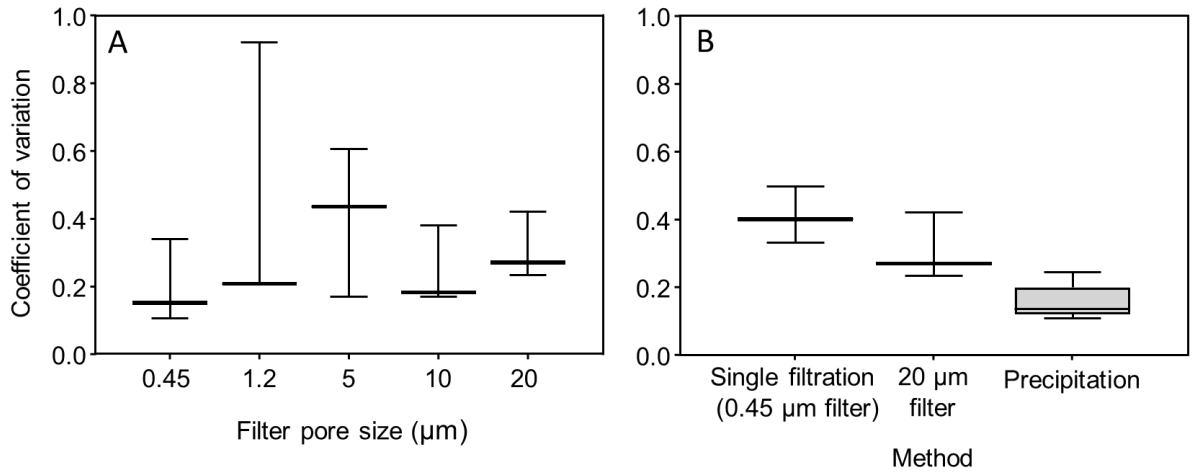

Figure S4.4. Coefficient of variation plots (Tukey boxplot) across A) filter pore sizes and B) capture methods.

4.8. References

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Chapter 5

A comparison of survey effort and detection sensitivity of scientific gillnet and environmental DNA methods for sawfish

Statement of the Contribution of Others

Nature of assistance	Contribution	Names & affiliations of co-contributors
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	Theoretical and methodological	Dr Colin Simpfendorfer, JCU Dr Dean Jerry, JCU
	Editorial	Dr Colin Simpfendorfer, JCU Dr Dean Jerry, JCU
Financial	Research Funding	Save Our Seas Foundation awarded to Dr Colin Simpfendorfer, JCU & Dr Dean Jerry, JCU
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5.1. Abstract

The Critically Endangered smalltooth sawfish (*Pristis pectinata*) represents one species of sawfish of high conservation concern due to profound population declines. Efficient detection and monitoring are essential if monitoring efforts are to be successful. As the species are easily concealed in murky, coastal habitat and the geographical range has significantly contracted, traditional monitoring methods currently in use are often inefficient, time-consuming, and are prone to detection error. Here, I investigated the efficacy of environmental DNA (eDNA) surveys as an alternative or supplementary method for surveying the southwest Florida population of smalltooth sawfish. The species occur within a core range that includes the coastal waters of Charlotte Harbour, Ten Thousand Islands National Wildlife Refuge, and Everglades National Park in southwest Florida, USA, which were surveyed in this study. I applied eDNA methods to 19 sites within these regions, as well as other potential habitat further north, and successfully detected smalltooth sawfish at six sites. One positive detection site was outside of the core range and where sawfish have not been detected in fishing surveys in 20 years, but where sawfish are known to migrate. Scientific gillnet surveys were conducted concurrently at eight of the 19 sites. Sawfish eDNA was detected in three of these sites; scientific gillnet fishing confirmed presence at only one of these sites. There was one site where eDNA failed to detect a sawfish that was captured in the gillnet. Furthermore, this study examined eDNA detection probabilities in relation to the total volume of eDNA assessed using qPCR replication and efficient removal of DNA-bound inhibitors. Results indicate that increases in detection sensitivity from 50 to 90% can be achieved with increases in qPCR technical replication. When applied correctly, the eDNA survey required less in person-hours and provided equal or greater detection sensitivity, though both methods were prone to false negative detection. This study confirms that eDNA monitoring is a valid supplement to

traditional monitoring methods currently applied to monitor rare and threatened elasmobranchs.

5.2. Introduction

Reliable and robust detection remains a critical and often difficult component for conservation planning and monitoring of aquatic (Margules & Pressey, 2000; McCauley et al., 2015; Webb & Mindel, 2015). Often, presence-only data are the best or only data available to discern species distribution thanks to the fast development of new technologies (Curnick et al., 2022; Waddell et al., 2021), citizen science programs (Biggs et al., 2015; Granroth-Wilding et al., 2017), and the opportunity to collate images and information from social (Kroetz et al., 2021). A range of methods are frequently used for aquatic species detection including, more recently, the use of environmental DNA (Huerlimann et al., 2020; Taberlet et al., 2018; Thomsen & Willerslev, 2015; Yao et al., 2022). Environmental DNA (eDNA) provides a genetic fingerprint of the biota that live in the surveyed environment (Taberlet et al., 2012). The expansion of eDNA methodologies into the ecology toolkit has been important for improving our ability to detect imperilled species that are scarce or have reduced geographical ranges (Boussarie et al., 2018; Budd et al., 2021; Lehman et al., 2022; Seymour, 2019; Vörös et al., 2017). When applied correctly and reliably, eDNA surveys can be an important detection tool in conservation and management programs that require rapid assessment of a species' geographic distribution (Sigsgaard et al., 2015; Thomas et al., 2020; van Rooyen et al., 2021). However, little is known about the efficacy of the eDNA approach compared to conventional scientific fishing approaches for exceptionally rare, or highly threatened species, where the cost of false negative detection is in context of failure to correctly conserve or manage the species (Gu & Swihart, 2004).

Environmental DNA promises higher detection rates, is non-invasive, and requires less time and labour in the field (Deiner et al., 2017; Dougherty et al., 2016; Jerde et al., 2011; Smart et al., 2015; Thomsen & Willerslev, 2015; Valentini et al., 2016). In comparison, conventional scientific fishing methods, such as nets, traps, and lines, are often limited by high

field costs and low detection rates, and their methods are time-intensive and have the potential to be invasive and ecologically destructive (Dougherty et al., 2016; Iknayan et al., 2014; Jerde et al., 2011; Smart et al., 2015). Yet, the biological metadata garnered from fishing is still a major drawcard (e.g., merisitic data, morphological data, body condition). Moreover, complementary research methods such as blood and tissue sampling for analysis of isotope chemistry or genetics (Johri et al., 2019), and tagging for analysis of movements and behaviour (Graham et al., 2021; Hays et al., 2019), advocate for their use. On the contrary, eDNA is primarily used for presence-only detection. This is in the absence of established methodologies for population genetics from eDNA or eRNA, which hold promise for understanding functional and biological components of species and populations (Jensen et al., 2021; Pochon et al., 2017; Tsuri et al., 2021). It is for this reason that eDNA is largely promoted as a complementary tool to traditional single-species monitoring programs rather than a replacement (Hänfling et al., 2016; Nevers et al., 2018).

As the strength of the eDNA method for aquatic species detection is generally accepted and becomes widely utilised (Huerlimann et al., 2020; Thomsen & Willerslev, 2015; Yao et al., 2022), there is an increasing need for rigorous, standardised, and reliable estimates of sensitivity that allow for comparison among methodologies and studies (Doi et al., 2021a; Klymus et al., 2019; Yao et al., 2022). Direct comparisons between eDNA and conventional fishing approaches are limited for marine species (Dougherty et al., 2016; Gehri et al., 2021; Hallam et al., 2021; Jerde et al., 2011; McColl-Gausden et al., 2021; Smart et al., 2015; Valentini et al., 2016), so further research that compares the detection sensitivity of eDNA to traditional marine survey methods via comparative studies are useful for scientists, managers, and conservation practitioners that are charged with employing monitoring programs. Furthermore, it is important to identify the circumstances in which false negatives occur (Shaw et al., 2016). That is, a species may be present but undetected, leading to ambiguous zeros that

are treated as species absences. False negative detection is well known to occur in traditional fishing methods and often gets short or little discussion in the literature (though see Bayley & Peterson, 2001). Conversely, the rate and cause of false negative detection in eDNA surveys has been heavily scrutinised in the literature (Hansen et al., 2018; Takahara et al., 2015; Tyre et al., 2003). In comparison to conventional fishing methods, where sources of error are well-understood, eDNA methods have more sources of underlying error that could give rise to false negatives (Jerde, 2021). Sources of negative results include the natural variability of eDNA within the spatiotemporal scales studied and the eDNA survey method itself (Burian et al., 2021; Mathieu et al., 2020). For the latter, understanding of the levels of variability in the technical aspects of the eDNA method is critical for determining the appropriate workflow to reduce the inherent variability.

In general, variability of the eDNA survey method is the combined results of bias and errors of the field and laboratory components (Furlan et al., 2016; Goldberg et al., 2016; Turner, Miller, et al., 2014). More specifically, this can be expressed as the probability that a field sample will contain target DNA given the species is present at a survey site and the probability that the target DNA will be amplified in a PCR replicate taken from a sample that contained target DNA (Furlan et al., 2015). Detection of eDNA under natural conditions, for example dynamic coastal or riverine environments, is inherently variable due to dispersion and degradation of eDNA after it is released (Deutschmann et al., 2019; Jo et al., 2019; Kelly et al., 2018; Sansom & Sassoubre, 2017). The amount of eDNA captured in field collection protocols is therefore highly variable and some individual samples may contain very little (i.e., picograms; Lehman et al., 2020), or no eDNA at all (Furlan et al., 2015). In the laboratory, detection is also affected by the occurrence of PCR inhibitors and the number of repeated measures (replication) to obtain an accurate result (Doi et al., 2015; Piggott, 2016; Williams et al., 2017). DNA-bound PCR inhibitors, such as polyphenolic compounds (e.g., humic acids),

inhibit PCR reactions through sequence specific-binding to DNA, limiting the amount of available template, and thus producing an observable impact on amplification efficiency (Matheson et al., 2010; Opel et al., 2010). Using an inhibitor-resistant DNA polymerase (Schrader et al., 2012) or the addition of bovine serum albumin (BSA) to PCR reactions (Sidstedt et al., 2017), can often relieve inhibition. Sample dilution is also a useful technique (McKee et al., 2015a) but will further reduce template concentration (Schrader et al., 2012). More frequently, the use of inhibitor removal kits has aided in improving PCR amplification of target species eDNA (Budd et al., 2021; Deiner et al., 2018; Djurhuus et al., 2017; Kumar et al., 2021; Villacorta-Rath et al., 2020). Samples are also subjected to stochastic processes during extraction and PCR (Ficetola et al., 2015). A strategy to mitigate stochasticity and other sources of experimental error and the impact on detection probability is through the use of replicates at the extraction and PCR step. The use of replication (i.e., the repeat analyses of the same sample) is a method to yield accurate and reliable data that permeates almost all scientific disciplines. Yet, replication is often used without understanding stochasticity and bias on the overall result. In order to evaluate the outcome of any eDNA-based survey method, each of these aspects need to be understood.

To evaluate the performance of eDNA approaches over traditional survey methods for detecting rare species, this study examined catch per unit effort for eDNA and scientific gillnet surveys and examined the influence of replication and inhibitor-treatments on eDNA detection probabilities. The data presented in this case study are the presence of smalltooth sawfish *Pristis pectinata* in the coastal waters of southwest Florida, U.S. The principal aims were to identify some of the technical factors that influence detection probability of this species in environmental DNA samples and examine the similarities or differences in detection results between using eDNA and scientific gillnet surveys for documenting species occurrence.

5.3. Methods

5.3.1. Study species and region

The smalltooth sawfish (*P. pectinata*) is a large (up to 5.5 m) shark-like batoid. It was once a dominant species throughout the Atlantic (Dulvy et al., 2016), but the species has undergone significant declines across its former range and is currently found in less than 20% of its former distribution (Dulvy et al., 2016). Once widespread in the tropical and subtropical waters of the Atlantic Ocean, *P. pectinata* are only now reliably found in the U.S. along both coasts of Florida and in the western Bahamas (Brame et al., 2019). The species is listed as Critically Endangered on the IUCN Red List of Threatened Species (Carlson et al., 2013) and is considered one of the most imperilled elasmobranch species, along with the four other sawfish species (family Pristidae) (Dulvy et al., 2016) and their relatives, the wedgefishes (family Rhinidae) and giant guitarfishes (family Glaucostegidae) (Kyne et al., 2020; Moore, 2017). *Pristis pectinata* was afforded protection in the U.S. under the Endangered Species Act in 2003, which also mandated that a Species Recovery Plan be developed to promote recovery and long-term viability of the species in U.S. waters. A lifeboat population of *P. pectinata* inhabit coastal southwest Florida, U.S., including Charlotte Harbour, Ten Thousand Islands National Wildlife Refuge, Everglades National Park and the Florida Keys; these regions are designated Critical Habitat for juvenile *P. pectinata* (Norton et al., 2012). Habitat use of juvenile *P. pectinata* in these regions is well-understood, with small juveniles exhibiting ontogenetic habitat shifts and high habitat fidelity to shallow, mangrove-lined estuaries (Hollensead et al., 2016; Norton et al., 2012; Poulakis et al., 2011; Wiley & Simpfendorfer, 2010) and larger juveniles moving to deeper water initially to feed and eventually to move beyond the estuaries into deeper marine waters (Simpfendorfer et al., 2010; Waters et al., 2014). Since the species' protection and implementation of a Species Recovery Plan in 2008,

sightings of individuals outside of these core areas have been recorded on public encounter databases, indicating that the species may be moving northward (Brame et al., 2019).

5.3.2. Field methods

5.3.2.1. Gillnetting survey To monitor the southwest Florida *P. pectinata* population and their movement, NOAA National Marine Fisheries Service conducts fishing surveys to tag large juvenile and adult *P. pectinata* with acoustic tracking devices (Graham et al., 2021). During the last four days of the eDNA survey conducted for this study (31 March and 3 April 2018), sawfish were caught using routine scientific gillnet procedures. The gillnets were 60.96 m (200 ft) and 30.48 m (100 ft) long, 1.5 m deep, and consisting of 10.2 cm (4 inch) stretch mesh and were set perpendicular to the coastline from a 21 ft boat (Tran Cat, 150 HP Evinrude). A single gillnet was deployed per site, where it was soaked for a minimum of 1 h (unless an animal was captured), continuously monitored, and completely checked every 0.5 h. Upon capture of sawfish, physiological data was recorded, acoustic transmitters were deployed, and then all individuals safely released, in accordance with ESA permit 17787. Gillnet work and sawfish handling was conducted by crew members who were not participating in eDNA sample collection. Gillnets were deployed from, and the associated equipment were handled, at the bow of the boat.

5.3.2.2. Environmental DNA survey Environmental DNA samples were collected between 27 March and 3 April 2018, from 19 sites within the following regions 1) Tampa Bay; 2) greater Charlotte Harbour, 3) Ten Thousand Islands National Wildlife Refuge (NWR); and 4) Everglades National Park (NP), Florida, U.S. (Figure 5.1). Sampling sites in the northern-most region, Tampa Bay, were selected based on public reports of *P. pectinata* and highly

suitable habitat (Wiley & Simpfendorfer, 2010); elsewhere, sampling sites were within regions with scientific evidence of long-term habitat use by the species (i.e., ESA critical habitat: Norton et al., 2012; Simpfendorfer et al., 2010; Wiley & Simpfendorfer, 2010). Sampling sites in Tampa Bay and Charlotte Harbour regions were travelled to by car and accessed by foot (i.e., sample collection at the waters' edge or from a wharf or jetty). Sampling sites in Ten Thousand Islands NWR and Everglades NP were accessed by boat (21 ft Tran Cat, 150 HP Evinrude), except for sites 18 and 19, which were accessed from the waters' edge. Use of the boat for eDNA sampling coincided with sawfish gillnet surveys. eDNA sampling was conducted, and the associated equipment stored, at the stern. Water samples were collected upstream of the gillnet when it was in the water. If a sawfish was handled alongside the boat, eDNA sample collection ceased for the day due to the risk of residual target species eDNA on the boat. Survey location and environmental data can be found in Table S5.3.

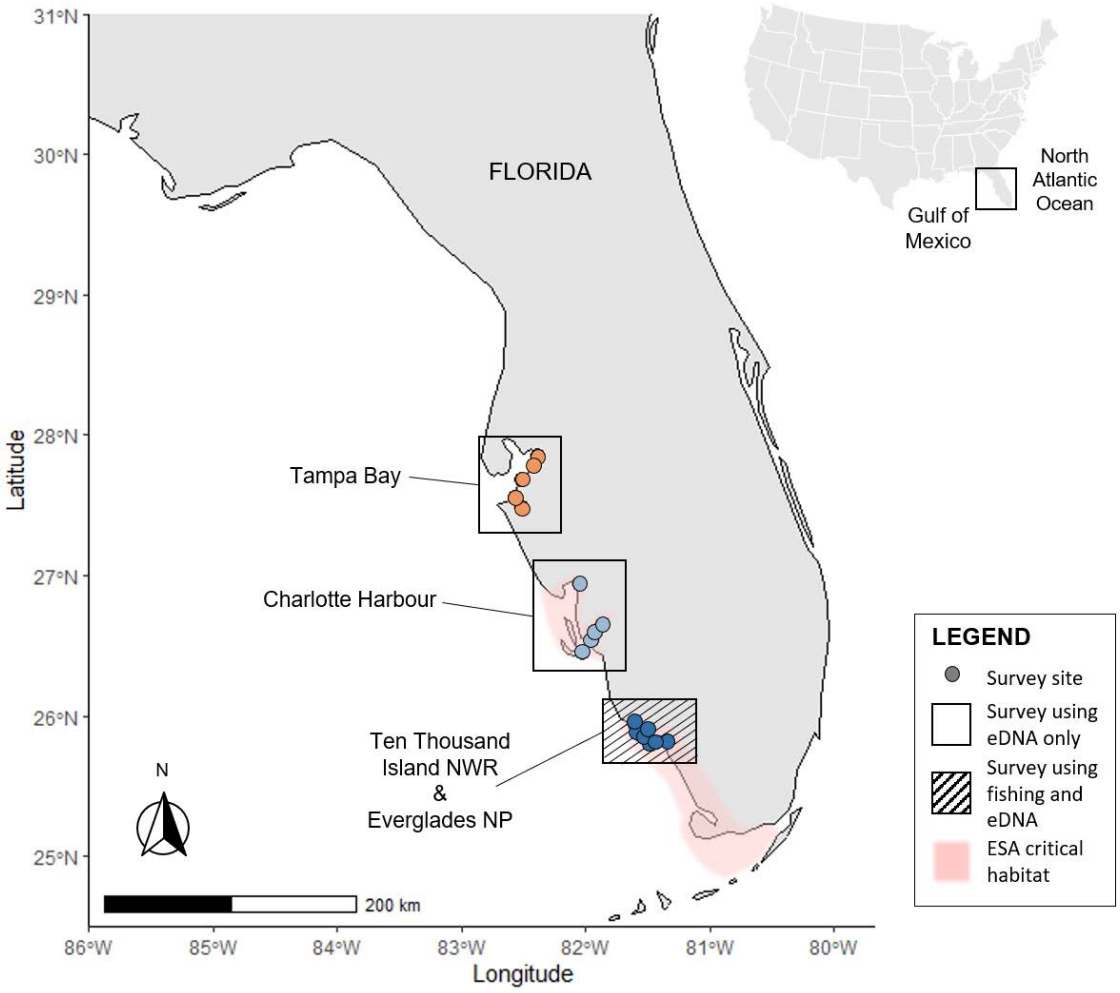


Figure 5.1. Overview of the study sites in southwest Florida, U.S. The monitoring methods used in each region are indicated in the legend. Red shading represents the two designated Endangered Species Act (ESA) critical habitat areas for smalltooth sawfish (*Pristis pectinata*) (Norton et al., 2012).

To collect each eDNA sample ($n = 5$ replicates per site), surface water was immediately filtered using a portable diaphragm pump (Grover® Scientific, Queensland, Australia, www.groverscientific.com.au) by submerging a filter housing unit (Smith-Root; Washington, US) containing a 5 µm nominal pore size filter paper (Nylon net, 45 mm diameter; Merck) below the surface with a gloved hand, following the protocol outlined in Chapter 2. Filtered water (2 L) was collected in a 10 L graduated bucket to measure sampled water volume and was returned to the water on completion of filtering. At sites where the use of a 5 µm filter was not possible due to rapid filter clogging, a 10 or 20 µm nominal pore size filter paper (Nylon

net, 45 mm diameter; Merck) was used to filter at least 4 L of surface water. Opportunistic samples were collected *ad hoc* at some sites using 1.2 µm nylon membrane filters and filtering up to 1 L (Table S5.4). These samples were used to assess differences in inhibitor removal kit efficacy, as they were expected to have concentrated higher amounts of DNA-bound inhibitors (see section 5.3.5).

Prior to sampling at each site, a field blank was collected by filtering 500 mL of bottled spring water through a filter paper, in the same manner as a field sample. Following filtration, filters were rolled and cut in half using sterile forceps and scissors, and each half was placed in screw-top microcentrifuge tubes (2 mL; Sarstedt, Germany) containing 1.5 mL of 96% non-denatured ethanol and kept at ambient temperature until short-term storage at 4 °C in the laboratory. To minimise cross-contamination, gloves were changed between samples and one pre-sterilised filter housing unit and one set of pre-sterilized forceps and scissors were used per site (see decontamination procedures in section 3.3.2.3). All surfaces and other equipment were cleaned with 10% *w/v* sodium dichloroisocyanurate (bleach tablets dissolved in bottled spring water) between sites.

Extractions were completed using a previously optimised protocol for high eDNA yield (section 3.4.2), including a tested modification for use with filters preserved in ethanol (data not shown). Briefly, the 96% non-denatured ethanol was transferred from each 2 mL field tube to a DNA-free 15 mL LoBind® (Eppendorf South Pacific Pty Ltd, New South Wales, Australia; Spens, et al., 2016) conical tube, diluted up to 5 mL with Longmire's solution (100 mM Tris pH 8, 100 mM EDTA pH 8, 10 mM NaCl, 0.5 % SDS; Longmire et al., 1997), and eDNA precipitated at 4 °C overnight in 7 mL isopropanol, 3.5 µL glycogen (20 mg/mL; Merck, Victoria, Australia), and 1.7 mL 5M sodium chloride. Precipitant was pelleted for 90 min at 3,270 g (SX4750 rotor, Allegra X-12 R, Beckman Coulter Inc., Indianapolis, US) and 600 µL of pH 10 lysis buffer solution containing 0.8 M guanidine hydrochloride and 0.5% TritonX

was added with the precipitant to the original field tube containing filter paper half for freeze-thaw-lysis treatment and subsequent incubation at 50 °C for ≥ 3 h. The solution was then transferred into one 2 mL LoBind® microtube eDNA and then precipitated at 4 °C overnight in two volumes polyethylene glycol (PEG) precipitation solution (1.6M sodium chloride, 30% PEG) and 5 µL glycogen (20 mg/mL) followed by 14,000 g for 30 min (Centrifuge 5430R, Eppendorf South Pacific Pty Ltd, New South Wales, Australia). Extracted eDNA from each sample was eluted in 100 µL UltraPure distilled water (ThermoFisher Scientific Pty Ltd, Victoria, Australia). Co-extracted inhibitors were removed using the Zymo OneStep™ PCR Inhibitor Removal Kit (Zymo Research Corp, California, USA) following the manufacturer's protocol and purified DNA stored in new 2 mL LoBind® tubes (Lecerf & Le Goff, 2010; Spens et al., 2016). Following purification, each sample was briefly vortexed before a 5 µL sub-sample taken for quantification of total recovered eDNA using the Quantus™ Fluorometer dsDNA System (Promega Pty Ltd Australia). Extracted and purified eDNA samples were kept at 4 °C for short-term storage until qPCR. For quality control measures, an extraction blank using extraction reagents only was included in each set of extractions to monitor for contamination.

All laboratory procedures were completed in dedicated, physically separated rooms for low-copy DNA extraction and pre-PCR, and post-PCR processes, within the Molecular Ecology and Evolutionary Laboratory (MEEL), at James Cook University, Townsville. Lab benches in both rooms were decontaminated prior to use by wiping with 10% *v/v* bleach, and the bleach was removed by wiping with reverse osmosis (RO) water, and finally with ≥ 70% ethanol. All procedures included the use of filter tips and *ad libitum* use of gloves to minimise cross-contamination.

5.3.3. Quantitative PCR

An *in silico* and *in vitro* optimized TaqMan™ qPCR assay was used to detect *P. pectinata* eDNA extracted from filter samples (section 3.3.1.3). The assay amplifies a 119 bp fragment of the smalltooth sawfish mitochondrial *12S* rRNA gene (5 copies per reaction 95% limit of detection). Assays were performed in 10 µL reactions following the master mix set-up and thermocycling profile described in section 3.3.1.3, and using a QuantStudio 5 quantitative real-time PCR machine (Life Technologies, ThermoFisher Scientific Pty Ltd, Victoria, Australia). Each reaction contained 3 µL of total eluted eDNA. Reactions were run on MircoAmp™ Optical 384-well plates (Applied Biosystems, ThermoFisher Scientific, Victoria, Australia) and contained triplicate no-template controls and low-copy synthetic DNA (gBlocks™ Gene Fragments, IDT) standard (10, 5, 2.5, or 1.25 copies per reaction). Field and extraction blanks were run adjacent to eDNA samples with an equivalent level of replication (see section 5.3.4). A standard curve was generated by 8-point $\log_{10}$ (10^8 to 10 copies per assay) and 3-point $\log_2$ (5, 2.5, and 1.25 copies per assay) serial dilution of gBlocks™ synthetic DNA that was 100% match to *P. pectinata* target sequence and was used to determine amplification efficiency and estimate *P. pectinata 12S* rRNA copy number. The standard curve was run in triplicate on the initial 384-well qPCR plate adjacent to eDNA samples. All plates were analysed using QuantStudio™ Design and Analysis Software, with manually adjusted threshold (0.8 ΔCt) and automatic baseline as described in section 3.3.1.3 for *in vitro* validations.

Putative positive amplicons, considered as technical replicates that showed amplification that crossed the fluorescence threshold within 50 cycles, were re-amplified using end-point PCR (see section 3.3.3.2) and the product visualized on 1.5% agarose gel to confirm a single amplicon of the correct size. Amplicons were then bi-directionally Sanger sequenced at the Australian Genome Research Facility (AGRF Pty Ltd, Brisbane, Australia) and sequence

identity was verified in Geneious (version 10.2.6; http://www.geneious.com) using BLASTn searches against the entire NCBI nucleotide database. Finally, detections were considered true positives if the above criteria were met and corresponding field and extraction blanks and NTCs exhibited no amplification.

5.3.4. eDNA sample qPCR screening percentage

A paired experiment that utilised each half of the collected filter samples was conducted to assess the impact of increased screening percentage on detection sensitivity, which is predicted to have a positive effect (Biggs et al., 2015). To increase screening percentage per qPCR replicate, sample replicates can be pooled per site prior to PCR analysis. Pooling sample replicates can also reduce laboratory costs during extraction and/or PCR (Davis et al., 2018). Moreover, eDNA in a sample of low concentration may be distributed nonrandomly among samples, or PCR subsamples, owing to the heterogenous clumping of eDNA in the water body (Furlan et al., 2016) and/or pipetting variation (McKee et al., 2015b), and therefore some samples may not contain any target species eDNA. For a threatened or endangered species, if only a small portion of the eluted eDNA from individual samples is screened, e.g., by limitation of cost or time or protocol design fault, the species may go undetected (i.e., false-negative detection). In this study, the proportion of eDNA that could be screened per site was five-times greater for pooled replicates than for individually extracted replicates (i.e., where $n = 5$). However, the trade-off of sample pooling was the potential for five-fold concentration of co-extracted inhibitors, which can be detrimental to detection sensitivity. Therefore, the effect of qPCR inhibition on detection sensitivity was tested, in addition to screening percentage.

Detection sensitivity results for filter sample halves that were extracted as their individual replicates for each site were compared to the opposing filter sample halves that were

pooled per site prior to the final precipitation step during extraction (n = 5 vs. n = 1 per site). Briefly, for extraction of pooled samples, the initial eDNA precipitant was added with 100 µL of pH 10 lysis buffer solution (instead of 600 µL) to the original field tube containing the filter paper half for the freeze-thaw-lysis treatment and subsequent 50 °C incubation. From there the solution from all five site replicates was pooled into one 2 mL LoBind® microtube eDNA for final PEG-glycogen precipitation and inhibitor removal as described in section 5.3.2.2.

To estimate detection sensitivity, 90% of the eDNA from both approaches was screened using qPCR conditions described in section 5.3.3. Of the remaining 10%, 5 µL was utilised for dsDNA quantification and the other 5 µL was used as spare volume during pipetting and transferring.

5.3.5. Assessment of qPCR inhibitors

To test for qPCR inhibition in the pooled samples, a TaqMan™ Exogenous Internal Positive Control (IPC) qPCR assay (Applied Biosystems; Hartman et al., 2005) was utilised following the manufacturers' protocol. The IPC assay was applied in duplexed reactions (see validation experiment in 5.7), with the 3 µL of eDNA in three technical replicates of the pooled samples. Three reactions containing only IPC DNA were included on each plate as 'inhibitor-free' positive controls or standards (IPC STD). To distinguish types of inhibition, an IPC ΔCt ($Ct_{positive\ control} - Ct_{sample}$) of 3 cycles as the threshold was used (Hartman et al., 2005). Specifically, IPC ΔCt of 3 or more cycles was considered partial inhibition and no amplification for the IPC was considered complete inhibition.

In addition, since the efficacy of the commonly used Zymo OneStep™ PCR Inhibitor Removal Kit (Zymo Research Corp, California, USA) compared to other commercially available inhibitor removal kits is unknown, I compared this approach with a recently revised

commercial inhibitor removal kit: QIAGEN DNeasy® PowerClean® Pro Cleanup Kit (formerly MO BIO; ThermoFisher Scientific, MA, USA) (Minegishi et al., 2019; Villacorta-Rath et al., 2020). The eDNA from the opportunistically collected 1.2 µm filter membrane samples were extracted individually, eluted in 200 µL UltraPure distilled water, and then halved for the paired comparison. Paired 100 µL eDNA subsamples were purified either using the commonly used Zymo OneStep™ PCR Inhibitor Removal Kit, or QIAGEN DNeasy® PowerClean® Pro Cleanup Kit following the manufacturers' instruction, respectively, and the final 100 µL of eluted eDNA stored in new 2 mL LoBind® tubes.

To examine the efficacy of inhibitor removal and the impact of the two inhibitor removal kits on detection sensitivity, 90% of the eDNA from both approaches was screened during qPCR, as described above and included the IPC. I also tested the addition of 1 µL of 20 mg/mL bovine serum albumin (BSA) in the qPCR reaction mixture for 50% of technical replicates to assess its impact on the reduction of inhibitors in qPCR reactions. McKee et al., (2015) indicated that addition of 0.5 µg/µL BSA is generally sufficient for reducing inhibition. Samples that remained inhibited were diluted 1:5 or 1:10 in UltraPure distilled water.

5.3.6. Comparison of survey effort

For each survey method (eDNA vs gill netting), the total invested efforts measured as person-hours was estimated. The estimate of survey effort for gillnetting was based on a three-person crew, two crew handling the net and a skipper to operate the boat during the procedure, and included the time used to set, soak and haul the net. This resulted in a conservative estimate that does not include time for travel, preparation, and supplementary fieldwork. For the eDNA method, the survey effort estimate included person-hours for a single person to conduct sample collection and laboratory processing, and does not include time for travel, preparation, and

supplementary fieldwork. Detection (1,0; 1 = detection, 0 = non-detection) per person-hour effort for each survey were then compared.

5.3.7. Statistical analysis

A binary logistic regression model was used to assess the effect of qPCR screening percentage on the probability of detection. The model was constructed using 'glm' with a quasi-binomial family structure to account for over-dispersion and logit link function in R (Gelman and Hill, 2006). In the final model, the response variable was cumulative detection rate (1 = detection, 0 = non-detection), the predictor variable was technical replicate, and site was included as a random factor. Goodness-of-fit was assessed using a Hosmer and Lemeshow test. A two-way analysis of variance (ANOVA) type-II test was used to assess the efficacy of the two inhibitor removal kits and the addition of BSA at reducing the impact of PCR inhibitors. The response variable, Ct, was $\log_2$ transformed to reduce skewness and conform to model assumptions. Post-hoc paired comparisons of means were done using the 'TukeyHSD' function in base R. A one-way ANOVA was used to assess differences in total DNA yield between the two inhibitor removal kits. The response variable, total yield (ng/μL), was $\log_{10}$ transformed. A Welch t-test was used to test for differences in person-hours between the two gillnet sizes. All statistical analyses were completed in R v 1.3.1 (R Core Team, 2017).

5.4. Results

5.4.1. *Pristis pectinata* detection

Pristis pectinata eDNA was detected in eDNA samples at six of 19 sampling sites. One site that was sampled twice recovered positive detections in both samples (Chokoloskee Island; eDNA sites 18 and 19) (Figure 5.2; Table S5.4). In the Tampa Bay region, *P. pectinata* eDNA

was detected in water sampled at the waters' edge of Apollo Beach (site 2). The positive detection was recovered from one replicate filter sample and consisted of one positive qPCR amplification curve (Ct 41.05). In the Charlotte Harbour region, *P.pectinata* eDNA was detected in water sampled from the pier at Cape Coral Yacht Club, Caloosahatchee River (Site 7). The positive detections included six qPCR amplification curves in two replicate filter samples (Ct mean 34.71) and three qPCR amplifications in the pooled sample (Ct mean 40.4).

In the Ten Thousand Island NWR and Everglades NP region, where both survey types were used, presence of *P. pectinata* was visually confirmed by capture of individuals in six of 22 gillnet surveys (Figure 5.2; Table S5.2). Environmental samples were collected concurrently at eight of the 22 gillnet survey sites. *Pristis pectinata* eDNA was detected at three of the eight sites, including Indian Key Pass (16), Panther Key (17) and Chokoloskee Island sites (18 and 19). At the first two sites (16 and 17), *P. pectinata* were not captured in gillnets. Positive detections at these sites were in the opportunistically collected 1.2 µm filter samples and consisted of one (Ct 36.65) and two positive qPCR amplification curves (Ct mean 32.34), respectively. In samples collected at Chokoloskee Island, *P. pectinata* was detected in all filter replicates and exhibited 95-100% qPCR amplification curve success per technical replicate (Ct mean 30.78), except for the site 18 pooled sample (see section 5.4.2). The presence of dozens of neonate *P. pectinata* adjacent to this sampling site was documented visually and due to their age were not subject to gillnet surveys. At one other site (Grocery Creek; 13) a sawfish was captured in the gillnet and not detected by eDNA. Surveys at the remaining sites did not detect the presence of *P. pectinata* in either method.

Pristis pectinata eDNA was not detected in any of the blanks and negative controls, including field blanks, extractions blanks, and qPCR NTCs. All putative positive qPCR amplifications for *P. pectinata* were confirmed as true positive via Sanger sequencing. The

standard curve used to assess assay efficiency had a y-intercept of 33.58 cycles, slope of -2.83, efficiency of 125.51%, and R^2 of 0.99.

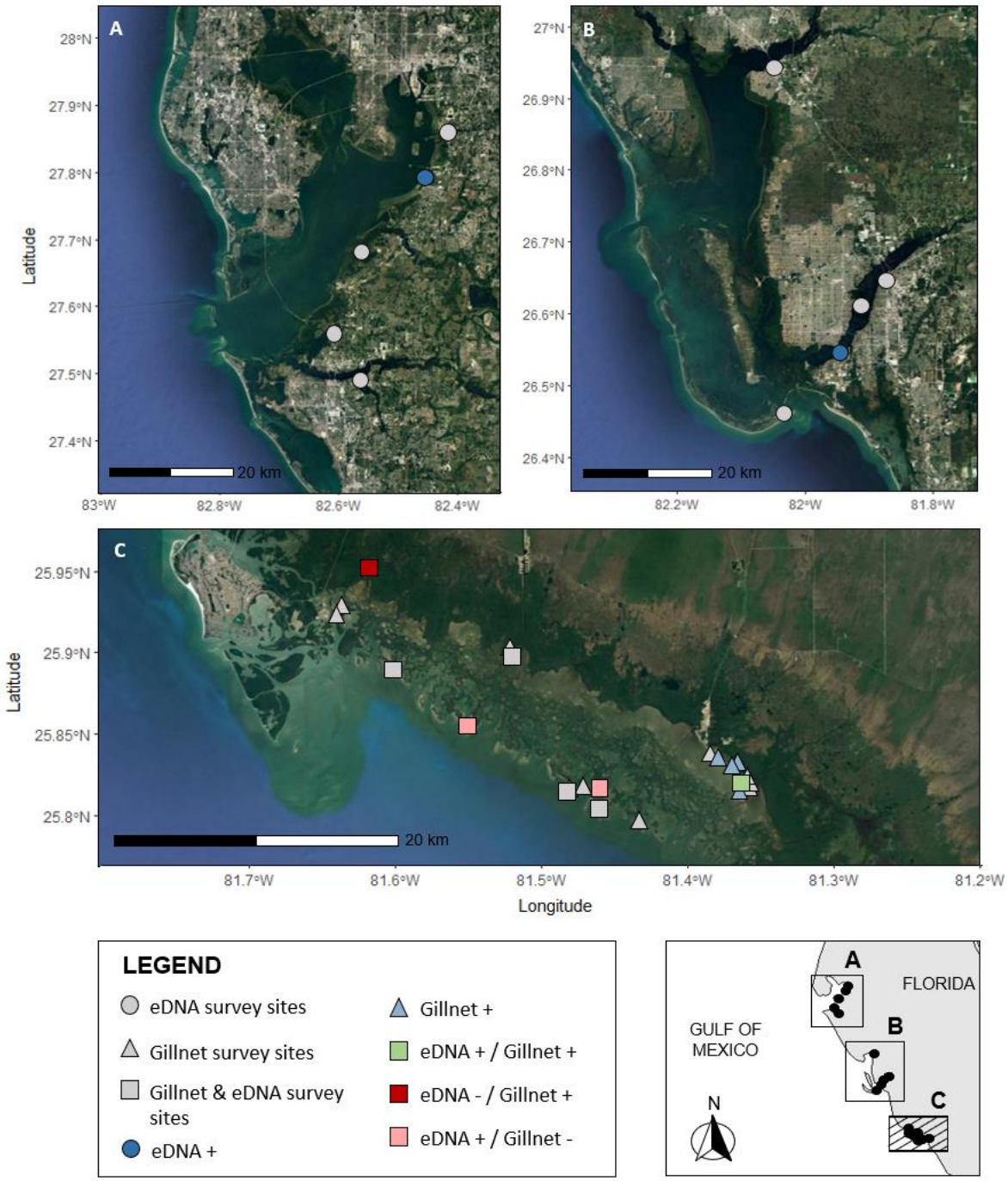


Figure 5.2. Map of regions in southwest Florida, U.S.A, where smalltooth sawfish (*Pristis pectinata*) were studied using environmental DNA (eDNA) and scientific gillnet surveys in March and April 2018. Study regions were A) Tampa Bay, B) Caloosahatchee River, and C) Ten Thousand Islands National Wildlife Refuge and Everglades National Park. The survey method used at each site are indicated in the legend. Presence of smalltooth sawfish (*Pristis pectinata*) eDNA or in scientific gillnets is indicated by the legend. Sites correspond with survey data in Table S5.2. Map C excludes one gillnet survey site (13), in which no sawfish were detected, that was located 12.8 km S from the south-eastern most sample sites shown here.

5.4.2. Effect of eDNA sample screening level on detection sensitivity

There was an observable difference in detectability between the two strategies (pooled vs individual replicates). Firstly, when field replicates where pooled into a single sample for qPCR screening, there was a loss of detection observed at one site (Apollo Beach, site 2; Table 5.1). Of the remaining three sites, where positive detections were recorded in both strategies, there were differences in the level of replication required to achieve positive detection. Specifically, at Caloosahatchee River (site 7; Table 5.1) detection probability was low in the five individual replicates; 96% of the total eDNA (i.e., 432 µl of the total 450 µl) needed to be analysed for a positive result. Detection probability was observed to be higher in the pooled sample, where the overall volume and therefore number of qPCR replicates was five-time less (i.e., total volume decreased from 450 µl to 90 µl) and a positive result was achieved after screening 83% of the pooled sample; though, the mean Ct (amplification cycle threshold) of positive pooled samples was 5.69 cycles higher than that of the positive individual replicates, representing a 17-fold difference (decrease) in template copy number. Of the two remaining sites at Chokoloskee Island (sites 18 and 19), detection probability in both strategies was high (0.96–1;), except for the site 18 pooled sample where 89% of eDNA was analysed for a positive result. Overall, the loss in detection sensitivity and potential indication of PCR inhibition in the pooled replicates indicated that the replicate pooling strategy was less optimal.

Table 5.1. Summary of smalltooth sawfish (*Pristis pectinata*) eDNA detections recovered from individual and pooled sample replicates. In each approach, 90% of the final eDNA was screened using qPCR to estimate differences in detection sensitivity (i.e., 450 µl of individual replicate eDNA vs. 90 µl of pooled eDNA). The required screening percentage to achieve a positive detection was determined as the inverse of the proportion of technical replicates that were positive. The required screening percentage represents the stochasticity across PCR reactions.

Site	Site No.	Region	Individual Replicates			Pooled Replicates		
			Detection	Mean Ct	Observed detection probability	Detection	Mean Ct	Observed detection probability
Apollo Beach	2	Tampa Bay	1	41.05	0.01	0	-	-
Caloosahatchee River (1)	7	Charlotte Harbour	1	34.71	0.04	1	40.40	0.17
Chokoloskee Island (1)	18	Everglades NP	1	28.86	0.96	1	29.02	0.11
Chokoloskee Island (2)	19	Everglades NP	1	33.27	0.97	1	33.75	1

A binary logistic regression model using the unpooled, individual replicate data indicated that there was a significant positive relationship between technical replicate and detection probability (Figure 5.3; Table S5.5). The model excluded sites with ≥90 % detection rate (i.e., Chokoloskee Island) and included positive amplification data from the opportunistically collected 1.2 µm filter samples. The results indicate that a 50% or greater probability of detection required at least 40% of eDNA to be screened (Figure 5.3). In this study, where 3 µl of eDNA is screened per reaction (i.e., per technical replicate), the minimum replication level to achieve 50% probability of detection, was 14 qPCR technical replicates per sample. The model suggests that a 90% probability of detection was achieved at 19 technical replicates (57% screened) and that improvements in detection probability diminish with each additional technical replicate thereafter (i.e., plateau phase of sigmoidal curve).

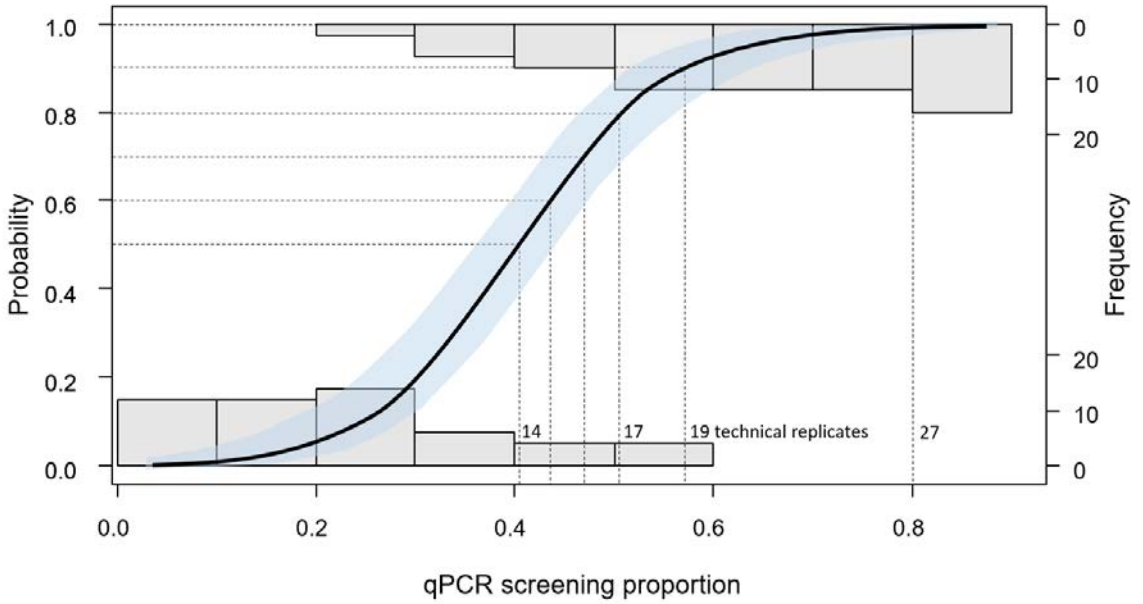


Figure 5.3. Smalltooth sawfish (*Pristis pectinata*) detection probability across qPCR technical replicates generated from a binary distributed response variable (cumulative presence of target eDNA in qPCR). Boxes are frequency of response data points, which are generated from qPCR technical replicates. Sites used were those that had less than 90% detection rate across biological replicates (i.e., Chokoloskee Island, Everglades NP). Blue ribbon is 95% confidence interval. Dotted lines plot step-wise (0.1) increases in detection sensitivity from 50-100% with the corresponding screening proportion. The number of technical replicates in this study that correspond with the detection probability intervals 50, 80, 90 and 100% are shown next the dotted lines adjacent to the x-axis.

5.4.3. qPCR inhibitor removal

Smalltooth sawfish eDNA was detected in pooled eDNA samples treated with both inhibitor removal kits (Zymo and Qiagen) and in both PCR strategies (BSA or no BSA). Analysis of variance of positive amplification value (Ct) data indicated that there was a significant effect of inhibitor removal kit (two-way analysis of variance, $F_{1,142} = 10.64$, $p = 0.001$) and the addition of BSA ($F_{1,142} = 80.09$, $p < 0.00$), but not their interaction on PCR inhibition. Paired comparisons of the main effects indicated that the mean Ct in both approaches were not significantly different to the IPC STD mean Ct (23.14 ± 0.18 SE), indicating that both Qiagen and Zymo kits perform equally on the removal of inhibitors. However, the mean Ct value of the IPC in Qiagen (mean ± SD; 23.43 ± 0.76) treated samples

was significantly lower than Zymo (23.93 ± 1.28) (Tukey HSD; $p < 0.000$) treated samples. The mean Ct value of the IPC in samples containing BSA (23.03 ± 0.68) and without BSA (24.29 ± 1.03) was significantly different (Tukey HSD; $p = 0.00$), but these were also not significantly different to the IPC STD (23.14 ± 0.81). An alternative analysis, using the inhibition threshold of ΔCt of 3 cycles (Hartman et al., 2005), wherein ΔCt (mean) = $Ct_{IPC\ control} - Ct_{sample}$ equated to -0.29 and -0.79 cycles for Qiagen and Zymo, respectively, also indicated no difference in inhibitor removal efficiency. Analysis of ΔCt, which was 0.11 and -1.15 for BSA and without BSA, respectively, did not meet the ΔCt 3 inhibition threshold. Lastly, there was no significant positive linear relationship between total dsDNA yield and inhibition ($R = 0.05$, $p = 0.76$) (Figure 5.4B).

The above analysis considered only positive amplification data (Ct), yet most samples in the paired experiment showed both positive and negative amplification. For site 18, two samples (18.1 and 18.8) treated with Zymo and without the addition of BSA exhibited negative detection in 14 of 18 qPCR replicates. For the sites 16 and 17 in Ten Thousand Island NP, positive detections were recovered from only one and two qPCR replicates, respectively. For site 16, the single positive replicate was from a Zymo treated sample without BSA addition. For site 17, the two positive replicates were in Qiagen treated samples both with and without BSA.

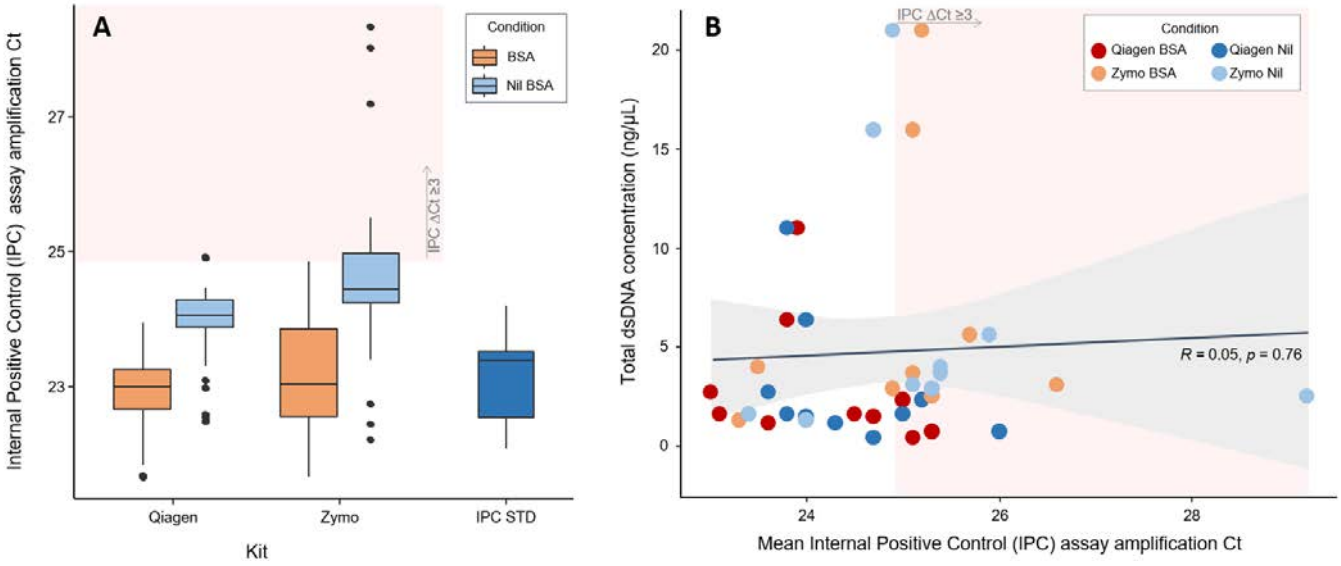
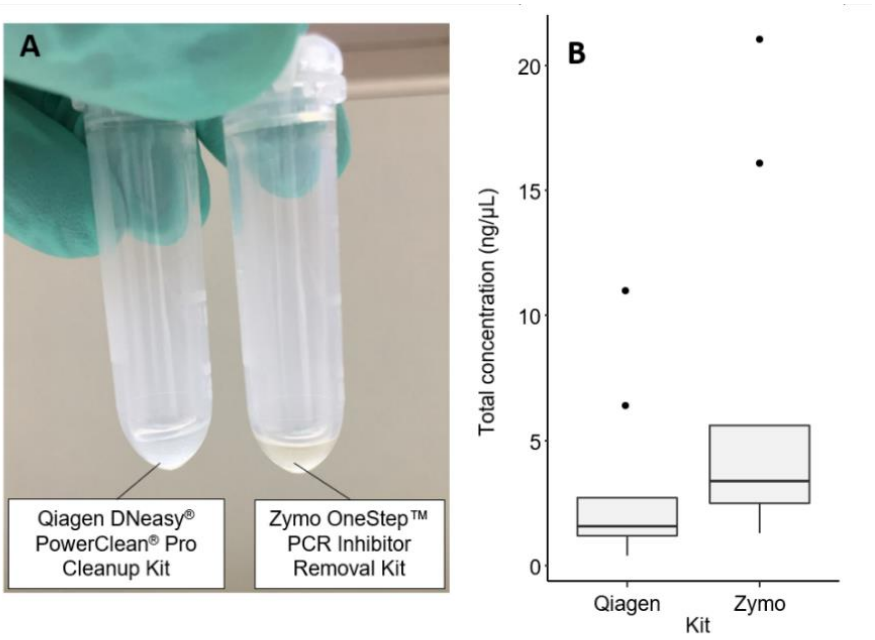


Figure 5.4. Environmental DNA inhibitor removal results presented as internal positive control (IPC) amplification Ct and double stranded DNA (dsDNA) concentration in ng/µL, which were measured using quantitative PCR (qPCR) and fluorescent dsDNA binding dye, respectively. A) Environmental DNA samples treated with either QIAGEN DNeasy® PowerClean® Pro Cleanup Kit or Zymo OneStep™ PCR Inhibitor Removal Kit. Their qPCR reaction mixtures contained or did not contain bovine serum albumin (BSA), represented by orange and light blue shading. A ΔCt of 3 or more cycles was used as a theoretical inhibition threshold (indicated by red shading). B) Correlation plot for mean IPC Ct value and total dsDNA concentrations for the same eDNA samples. There was no positive linear relationship between total dsDNA concentration and qPCR inhibition inferred from increased IPC Ct.

Samples treated with Qiagen had significantly lower total dsDNA (mean ± SE; 2.94 ± 0.91 ng/µL) than Zymo (5.83 ± 1.81 ng/µL) (one-way analysis of variance, $F_{1,38} = 7.24$, $p = 0.01$) (Figure 5.5B). An observation of paired eDNA samples extracts post-treatment with both inhibitor removal kits showed a yellow-brown stain in the eDNA extract treated with the Zymo kit (Figure 5.5A). Despite the absence of a stain in the Qiagen eDNA extract, mean 260/280 and 260/230 values for both treatments were lower than the expected ratios for pure DNA (Figure 5.5C), indicating that samples were potentially contaminated with organic polymers (i.e., compounds that are co-isolated with eDNA in water samples), or chemical residues from the extraction protocol. However, the very low concentrations of nucleic acids measured (i.e., < 10 ng/µL) had the potential to cause inaccurate ratios (ThermoScientific, 2012).



C	Qiagen (Mean ± SE)	Zymo (Mean ± SE)	Expected ratios
260/280	1.65 ± 0.18	1.67 ± 0.09	1.80
260/230	0.77 ± 0.15	0.51 ± 0.04	2-2.2

Figure 5.5. Difference in A) sample colour and B) total dsDNA concentration (ng/µL) following clean-up using two commercial inhibitor removal kits. C) eDNA purity, measured using the NanoDrop 2000 Spectrophotometer, for opportunistic samples using different nominal filter pore size, collected at some sites in addition to the routine samples in Table S5.4. Extracted eDNA from second half of each filter paper was divided and used in a paired experiment testing two commercial inhibitor removal kits.

5.4.4. Survey effort comparison

For the entire eDNA survey period, an average of 00:14:45 ± 00:01:16 (hh:mm:ss ± SE) person-hours were spent filtering five independent replicate samples per site. It is estimated based on data from the entire project that an average of 3:00:00 person-hours of laboratory work were spent per site. This includes the working time that is split over multiple days for conducting extractions, inhibitor removal, and qPCR preparation and does not include breaks in workflow or overnight incubation steps. Therefore, the average total effort invested to complete an eDNA survey for a single site was 03:14:45 ± 00:01:16 person-hours. During the

gillnet survey period, the average total effort invested to deploy, monitor and retrieve a gillnet per site was 04:00:25 ± 00:14:35 person-hours. There was no significant difference in time for the two different net lengths (Welch two-sample t-test, t = -1.63, df = 13.92, p = 0.125).

At sites where both eDNA and gillnetting were conducted, gillnet surveys took an average of 00:42:14 person-hours longer than eDNA surveys per site. When accounting for only the field component of the eDNA surveys (i.e., excluding laboratory-based work), gillnet surveys took an average of 3:42:14 person-hours longer. At sites where smalltooth sawfish were captured, gillnets were hauled upon notice of capture and therefore in advance of the required 1-hour soak time to minimise capture stress of the animal/s. At these sites, gillnet surveys took an average of 2:27:34 ± 00:11:26 person-hours, which was 1:14:40 person-hours more than eDNA.

5.5. Discussion

Survey methods based on environmental DNA (eDNA) analysis from water samples and scientific gillnetting were used in this study to detect the Critically Endangered smalltooth sawfish (*Pristis pectinata*) in southwest Florida. The results presented here support growing evidence that eDNA surveys can detect the presence of a rare species with equal or greater sensitivity and time-efficiency than conventional fishing methods (Wilcox et al., 2016). Within the species' global hotspot and ESA critical habitat, the Ten Thousand Islands NWR and Everglades NP region, smalltooth sawfish eDNA was detected at three of the eight sites with concomitant survey types. At two of these sites with eDNA detections, sawfish were not captured in gillnets. At the third site, smalltooth sawfish were detected in both surveys. Interestingly, the presence of dozens of neonate smalltooth sawfish was documented in the shallow water adjacent to the sampling site, which resulted in high detection probability of the

species eDNA in filtered water samples. At one other site a sawfish was captured in the gillnet and not detected by eDNA. These results highlight the susceptibility of both methods to false negative detection. In the Charlotte Harbour and Tampa Bay regions, where only eDNA surveys were conducted, positive eDNA detections were sparse and the model reported here demonstrated that increased eDNA screening depth (qPCR technical replication) is important to mitigate false negative detection. The use of an inhibitor removal kit and inclusion of BSA in qPCR mastermix are also likely to be important consideration for eDNA-based surveys. However, due to the small sample size in this study, analyses were inconclusive and these suggestions are based on observation.

Uncertainty associated with interpretation of negative results may impede the use of eDNA methods as a management tool, as false negatives can certainly occur at any point in the eDNA workflow. In this study, I screened samples using a TaqMan-probe quantitative PCR (qPCR) assay to reduce the rate of false negatives relative to conventional PCR (ref) and double stranded DNA-binding dye (e.g., SYBR Green) qPCR assays (Sidstedt et al. 2015). At the qPCR step, I identified that false negative detection can be mitigated through increased technical replication (i.e., increased PCR replicates). The typical replication strategy of eDNA studies is 3 PCR replicates (reviewed by Doi et al., 2021). In this study, the rate of false negative detection using only 3 replicates was high, where four of the six positive sites would have been considered negative for smalltooth sawfish presence. I suggest that 3 qPCR replicates is a poor strategy for rare species detection using eDNA. The model presented here suggests detection probabilities from 0.5 to 0.9 require 40 to 60% of eDNA to be screened (i.e., 14 to 19 replicates). The results here are consistent with other eDNA studies that highlight the importance of PCR replication (e.g., n = 8; Ficetola et al., 2015; Tucker et al., 2016) and supports the idea that the concentration of rare target species eDNA is low and patchily distributed across replicate filter samples and within purified eDNA extracts, and undoubtedly

within the environment (Barnes et al., 2020; Turner, Barnes, et al., 2014). Greater survey effort (consistently large or more intensively sampled areas) could compensate also for lower target species eDNA concentration in the environment and inherent heterogeneity within samples and help reach a satisfactory detection probability. However, an argument can be made for the poor feasibility and practicality of a survey effort approach, where maintaining a level of effort for consistent detectability of a rare species will result in a large number of required samples.

Pooling sample replicates has also been suggested as a strategy to increase detection probability in single-species studies (Brunner, 2020; Sabino-Pinto et al., 2019). For instance, the intrasample variability or stochastic loss of rare target species eDNA during PCR could be reduced when a greater proportion of the final pooled eDNA sample can be screened during PCR versus individual replicates. This strategy also decreases cost and labour of the eDNA workflow (Sabino-Pinto et al., 2019). In this study, the observed difference between pooled and individual eDNA sample replicates suggests that the pooling strategy likely resulted in a decrease in detection sensitivity, despite a five-fold reduction in the number of PCR replicates, and therefore, time and cost required to screen the total volume of eDNA. At sampling sites 18 and 19, the posteriori probability of detection for smalltooth sawfish was 1, wherein the presence of dozens of juvenile smalltooth sawfish at the time of sampling was visually confirmed. However, the observed detection probability for site 18 pooled samples was 0.11 (versus 0.96 for individual samples). Detection probability at site 19 was high for both pooled and individual replicates (0.97 and 1, respectively). Considering that site 18 and 19 were adjacent, the observed inter-sample variability that may be a result of the heterogenous dispersal of eDNA in the water column could be accounted for in future studies through increased spatial replication. Yet, the mean concentration of smalltooth sawfish eDNA in positive qPCR replicates for sites 18 and 19 was >10 copies per µL, which indicates that the method successfully collected and isolated target species eDNA. The result raises concern for

the potential impact of co-extracted PCR-inhibitors in the pooled sample (see below). For example, the yellow stain colour of the eDNA sample (Figure 5.5) is indicative of organic compounds such a tannic or humic acids. At sites 2 and 7, where sawfish eDNA was detected in very low concentration (Ct >36, or less than 1 copy per µL based on the standard curve), amplification is stochastic and small sample size means it is difficult to discern the impact of pooling on detection probability. Observed differences here may be a result of the heterogenous distribution of target eDNA captured on the filter paper (i.e., both halves of the filter paper are not representative of one another). Therefore, in order to confidently obtain all eDNA from a sample, extraction should be complete on whole filter papers instead of half. This may be a more appropriate strategy than pooling sample replicates and should minimise stochasticity due to template eDNA heterogeneity.

False negatives are also likely to occur if co-extracted DNA-bound PCR inhibitors are inadequately removed from the eDNA sample. The impact of PCR inhibition on eDNA amplification can be determined through effect on amplification efficiency. Differences in relative amplification efficiency can be seen in changes in the slope of the exponential amplification curve compared to a non-inhibited control sample (in this study, an exogenous internal positive control). While the results here are not statistically significant, due to small sample size and high inter-sample variability of positive replicates, the potential impact of inhibitor removal kits and inclusion of BSA in qPCR reaction mixture on mitigating false negative detection is documented elsewhere and these published procedures are recommended for sensitive, inhibitor-free eDNA surveys. It is also plausible that pooling sample replicates may proportionately increase the amount of co-extracted DNA-bound inhibitors, though I did not find a positive relationship between total dsDNA yield and shift in IPC amplification curves. The inability of the Zymo Inhibitor Removal Kit to completely remove phenolic acids,

seen as a yellow or brown stain in the eDNA extract, indicates that it may not be the most effective method.

Finally, in Charlotte Harbour and Tampa Bay regions, where only eDNA surveys were conducted, the positive eDNA detections are consistent with the patterns of occurrence and distribution inferred from acoustic tagging studies of smalltooth sawfish along the Gulf Coast of Florida (Graham et al., 2021) and were verified by reports from recreational fishers (Figure S5.1). Soon after the eDNA sample had been collected, documented reports were made by recreational fishermen of the capture of juvenile smalltooth sawfish in the days and months prior to eDNA sample collection at the Caloosahatchee River location on the Sawfish Conservation Society Facebook webpage (Figure S5.1), adding support to the positive eDNA result. In the northern-most region of Tampa Bay surveyed using eDNA, the occurrence of smalltooth sawfish is not well-understood. Sawfish have not been captured at this location using scientific fishing methods for the past 20 years since research conducted by Simpfendorfer (2001) (Figure S5.2), though it is noted that in the year since this study was conducted collaborator T. Wiley captured and acoustically tagged two juvenile *P. pectinata* (https://sevenseasmedia.org/public-reported-sightings-led-to-first-two-sawfish-tagged-in-tampa-bay-by-tonya-wiley-havenworth-coastal-conservation/). Additionally, monitoring data that employed the vast network of acoustic arrays along the Gulf Coast of Florida and the large scientific effort to deploy internal acoustic transmitters in smalltooth sawfish indicates that sawfish are infrequently migrating north via the Tampa Bay region and on to Apalachee Bay (Graham et al., 2021). There are also infrequent citizen science reports via the Sawfish Conservation Society Facebook webpage of smalltooth sawfish within Tampa Bay. Growing reports of sawfish outside of their core range are positive signs for the recovery of the species and expansion of the population across historically occupied regions in U.S. waters. Given potential conservation consequences of smalltooth sawfish residing outside of the ESA critical

habitat, targeted eDNA surveys should be considered by management authorities to assist with documenting the recovery and expansion of the species geographic range.

5.6. Conclusion

The detection of smalltooth sawfish in filtered seawater samples was significantly influenced by screening depth, indicating that eDNA molecules are patchily distributed across replicate filter samples and within purified eDNA extracts. As a consequence, the proportion of the total eDNA extract that is analysed per site is critical to maximise the detection sensitivity and decrease PCR stochasticity. Specifically, these results demonstrate that the potential of this survey tool can be maximised if greater attention is applied to replication strategy. While the results here are not statistically significant, the potential impact of inhibitor removal kit and inclusion of BSA in qPCR reaction mixture on mitigating false negative detection is documented elsewhere and it is recommended that these procedures are considered for sensitive, inhibitor-free eDNA surveys. Lastly, the key demonstration here is that the two methods (eDNA and scientific gillnetting) have different detection probabilities and are susceptible to false negatives, and therefore one method will detect the species before the other, which often justifies the potential need for a combined approach.

5.7. Supplementary information

Data S5.7.1 IPC duplex reaction optimisation and validation.

To validate the use of a TaqMan™ Exogenous Internal Positive Control (IPC) qPCR assay (Applied Biosystems) for this study, I tested the IPC in duplex reactions with a synthetic low-copy DNA standard (5, 2.5, and 1.25 copies per reaction; i.e., expected working range for eDNA). To do this, IPC DNA and low-copy synthetic DNA standards (STD) were run in singleplex and duplexed reactions on a MicroAmp Optical 96-well qPCR plate (Applied Biosystems, ThermoFisher Scientific, Victoria, Australia) according to the IPC manufacturer's instructions and using the reaction master mix and thermocycling profile described in section 5.3.3 on the QuantStudio 5 quantitative real-time PCR machine (Life Technologies, ThermoFisher Scientific Pty Ltd, Victoria, Australia). Single and duplex reactions were repeated three times and the mean Ct was used to assess if the duplex reaction reduced the efficiency of the qPCR reaction at detecting low copy numbers. A $\Delta Ct_{singleplex-duplex}$ threshold was set at 3 cycles to account for the natural variability between replicates and because 3 or less cycles is unlikely to equate to a loss in detection sensitivity. The $\Delta Ct_{singleplex-duplex}$ values (mean ± SD) for both the *P. pectinata* synthetic DNA standard (-0.61 ± 0.6) and IPC DNA standard (0.36 ± 0.11) did not meet the 3 ΔCt threshold and therefore I determined that detection sensitivity was not compromised by the use of a duplex reaction.

Table S5.1. Result of qPCR experiment to validate the use of a TaqMan™ Exogenous Internal Positive Control (IPC) qPCR assay (Applied Biosystems) in a duplex reaction with low-copy DNA. Low-copy smalltooth sawfish (*Pristis pectinata*) synthetic 12S rRNA DNA standards (STD) of 5, 25., and 1.25 copies per reaction were used to replicate the expected working range of smalltooth sawfish eDNA. Quantitative-PCR reactions were in triplicate, excluding those marked with an asterisk (*) due to a plate-loading error. A $\Delta Ct_{\,singleplex-duplex}$ threshold of 3 or more cycles indicated that the sensitivity of the qPCR assays was reduced in a duplexed reaction.

Target	Reaction Type	Sample	Ct Mean	Ct SD	$\Delta Ct_{singleplex-duplex}$	ΔCt Mean	ΔCt SD
P. pectinata	Singleplex	5 copies STD	28.98	0.02			
		2.5 copies STD	30.18	0.05			
		1.25 copies STD	31.29	0.10			
	Duplex	5 copies STD × IPC 1X	29.05	0.82	-0.02		
		2.5 copies STD × IPC 1X*	31.44	-	-1.21	-0.61	0.60
		1.25 copies STD × IPC 1X*	31.79	-	-0.59		
IPC	Singleplex	IPC DNA 1X	29.41	0.16			
	Duplex	5 copies STD × IPC 1X	28.59	0.62	0.25		
		2.5 copies STD × IPC 1X*	28.79	-	0.47	0.36	0.11
		1.25 copies STD × IPC 1X*	28.90	-	0.35		

Table S5.2. Gillnet survey data with summary of paired eDNA sample results. Regions include TTINWR; Ten Thousand Islands National Wildlife Refuge and ENP; Everglades National Park. Bold font indicates sample sites where sawfish were detected using either method.

Region	Site	No.	Latitude (N)	Longitude (W)	Sawfish Presence/ Absence	No. Sawfish per set	Paired eDNA sample	eDNA site No.	eDNA Presence/ Absence	Date	Mesh (in)	Length (ft)	Gear Set Time (mm:ss)	Gear Haul Time (mm:ss)	Soak Time (h:mm)
TTINWR	Goodland Outer Isl.	1	25.88822	-81.59753	0	0	Y	11	0	31/3/2018	4	200	08:45	09:45	1:00
	Stop Keys	2	25.81307	-81.47900	0	0	Y	12	0	31/3/2018	4	200	10:15	11:20	1:04
	Stop Keys	3	25.81760	-81.46987	0	0	N	-	-	31/3/2018	4	200	11:30	12:30	1:00
	Grocery Creek	4	25.95108	-81.61483	**1**	**1**	**Y**	13	**0**	31/3/2018	4	100	13:30	14:10	0:40
	Keys_Goodland	5	25.93348	-81.63460	0	0	N		-	31/3/2018	4	100	15:55	16:55	1:00
	Keys_Goodland	6	25.92440	-81.64145	0	0	N	-	-	31/3/2018	4	100	17:10	18:15	1:04
	Faka Union Bay	7	25.89888	-81.51763	0	0	Y	14	0	1/4/2018	4	200	10:15	11:15	1:00
	Kingston Key	8	25.80322	-81.45647	0	0	Y	15	0	1/4/2018	4	200	12:05	13:15	1:10
	Keys_TTINWR	9	25.79750	-81.43438	0	0	N	-	-	1/4/2018	4	200	13:25	14:30	1:04
	Indian Key Pass	10	25.81503	-81.47678	**0**	**0**	**Y**	16	**1**	1/4/2018	4	200	14:45	15:25	0:40
	Panther Key	11	25.85415	-81.54740	**0**	**0**	**Y**	17	**1**	1/4/2018	4	200	15:50	17:00	1:10
	Faka Union Bay	12	25.90005	-81.51758	0	0	N	-	-	1/4/2018	4	200	17:20	18:20	1:00
ENP	Keys_NENP	13	25.70273	-81.33995	0	0	N	-	-	2/4/2018	4	200	10:15	11:25	1:10
	Chokoloskee Island	14	25.81815	-81.35950	**1**	**4**	N	-	-	2/4/2018	4	100	14:00	14:20	0:19
	Chokoloskee Island	15	25.81860	-81.359	0	0	N	-	-	2/4/2018	4	100	15:50	17:25	1:35
	Chokoloskee Island	16	25.82748	-81.36375	**1**	**3**	**Y**	18,19	**1**	3/4/2018	4	100	08:35	09:00	0:25
	Chokoloskee Island	17	25.82818	-81.36390	**1**	**3**	N	-	-	3/4/2018	4	100	09:30	09:55	0:25
	Chokoloskee Island	18	25.83567	-81.37612	**1**	**2**	N	-	-	3/4/2018	4	100	10:30	10:55	0:25
	Chokoloskee Island	19	25.81887	-81.35908	0	0	N	-	-	3/4/2018	4	100	11:10	12:10	1:00
	Chokoloskee Island	20	25.81995	-81.35955	**1**	**3**	N	-	-	3/4/2018	4	100	12:30	12:50	0:19
	Chokoloskee Island	21	25.82150	-81.35917	0	0	N	-	-	3/4/2018	4	100	13:20	14:30	1:10
	Chokoloskee Island	22	25.84103	-81.384	0	0	N	-	-	3/4/2018	4	100	14:35	15:30	0:55

Table S5.3. eDNA survey location and environmental data. Regions include Tampa Bay (TB), Charlotte Harbour (CH), Ten Thousand Islands National Wildlife Refuge (TTINWR), and Everglades National Park (ENP). Depth measurements marked with an asterisk were recovered from tampabay.wateratlas.org.

Region	Site	Site No.	Collection Date	Coordinates (DD)		Habitat Type	Salinity	Tide	Depth (m)	Temp (°C)	Turbidity (cm)
				Latitude (N)	Longitude (W)						
TB	Alafia River	1	27/03/2018	27.86025	-82.38554	Sand	Brackish	In	0.15*	-	0.15
	Apollo Beach	2	27/03/2018	27.79249	-82.41884	Sand	Brackish	In	0.2*	-	-
	Cockroach Bay	3	27/03/2018	27.68689	-82.52037	Mud	Brackish	In	0.2*	-	-
	Braden River	4	27/03/2018	27.49615	-82.52552	Mud	Brackish	Out	0.2*	-	-
	Terra Ceia Bay	5	27/03/2018	27.56100	-82.57137	Sand	Brackish	Out	-	-	-
CH	Sanibel Island	6	28/03/2018	26.45365	-82.03566	Sand	Marine	In	-	-	-
	Caloosahatchee River (1)	7	28/03/2018	26.54255	-81.95244	Sand	Marine	In	-	-	-
	Caloosahatchee River (2)	8	28/03/2018	26.60756	-81.91343	Sand/mud	Brackish	Out	-	-	-
	Caloosahatchee River (3)	9	28/03/2018	26.64635	-81.87257	Sand/mud	Brackish	Out	-	-	-
	Peace River	10	28/03/2018	26.94116	-82.05000	Mud/oyster	Brackish	-	-	-	-
TTINWR	Goodland Outer Island	11	31/03/2018	25.88822	-81.59753	Mud/oyster	Marine	In	0.4	24.6	60
	Stop Keys	12	31/03/2018	25.81307	-81.47900	Sand/mud	Marine	In	0.65	24.7	100
	Grocery Creek	13	31/03/2018	25.95108	-81.61483	Mud	Marine	In	0.55	25.8	45
	Faka Union Bay	14	1/04/2018	25.89863	-81.51713	Mud/oyster	Marine	Out	0.7	24.9	80
	Kingston Key	15	1/04/2018	25.80322	-81.45647	Sand	Marine	In	0.6	28.7	70
	Indian Key Pass	16	1/04/2018	25.81497	-81.45647	Sand	Marine	Out	0.9	26.6	110
	Panther Key	17	1/04/2018	25.85417	-81.54740	Sand/shell	Marine	Out	1.1	26.6	110
ENP	Chokoloskee Island (1)	18	3/04/2018	25.82748	-81.36375	Mud	Marine	In	0.4	25.9	20
	Chokoloskee Island (2)	19	3/04/2018	25.82748	-81.36375	Mud	Marine	In	0.4	25.9	20

Table S5.4. *Pristis pectinata* eDNA detection data for filter samples extracted either individually or pooled per site. Regions include Tampa Bay (TB), Charlotte Harbour (CH), Ten Thousand Islands National Wildlife Refuge (TTINWR), and Everglades National Park (ENP). Positive qPCR amplification and corresponding Ct Mean data are in bold.

Region	Site	Site No.	Pore size (µm)	Filtrate volume (L)	Filtering time (min:sec)	Individual replicates					Pooled replicates			
						Extraction Date	Replicate No.	Conc. (ng/µl)	qPCR amp (0, 1)	Ct Mean	Extraction Date	Conc. (ng/µl)	qPCR amp (0, 1)	Ct Mean
TB	Alafia River	1	5	2	2	19/10/2018	1	0.661	0	-	18/02/2019	9	0	-
			10	2	2		2	0.648	0	-				
			10	1	2		3	5.5	0	-				
			10	1	1		4	4.77	0	-				
			10	1	1		5	3.34	0	-				
	Apollo Beach	2	5	2	2	19/10/2018	1	4.37	0	-	18/02/2019	17	0	-
			5	2	1		2	6.6	0	-				
			5	2	1		3	3.5	0	-				
			5	2	1		4	5.5	**1**	**41.04**				
			5	2	1		5	4.24	0	-				
	Cockroach Bay	3	5	2	3	19/10/2018	1	2.5	0	-	18/02/2019	13	0	-
			5	1.5	2		2	2.37	0	-				
			5	2	2		3	4.28	0	-				
			5	2	2		4	2.82	0	-				
			5	2	2		5	2.82	0	-				
	Braden River	4	5	1	3	29/10/2018	1	3.11	0	-	18/02/2019	12	0	-
			5	2	1		2	1.22	0	-				
			5	2	1		3	1.26	0	-				
			5	2	1		4	0.845	0	-				
			5	2	2		5	1.11	0	-				
	Terra Ceia Bay	5	5	2	3	29/10/2018	1	1.84	0	-	18/02/2019	12	0	-
			5	1	3		2	1.12	0	-				
			5	1.5	3		3	1.74	0	-				
			5	1	2		4	0.987	0	-				
			5	1.25	2		5	1.16	0	-				
	Sanibel Island	6	5	2	3	29/10/2018	1	4.49	0	-	18/02/2019	19	0	-
			5	1.5	5		2	1.8	0	-				
			5	2	4		3	4.1	0	-				
			5	2	3		4	3.3	0	-				

			5	2	3		5	2.47	0	-				
CH	Caloosahatchee River (1)	7	5	2	3	14/11/2018	1	2.1	0	-	18/02/2019	10	**1**	**40.396**
			5	2	4		2	1.28	**1**	**37.48**				
			5	1.75	3		3	1.52	0	-				
			5	2	1		4	1.3	**1**	**36.95**				
			5	2	2		5	2	0	-				
	Caloosahatchee River (2)	8	5	1.75	3	14/11/2018	1	1.27	0	-	18/02/2019	8.5	0	-
			5	1.75	3		2	1.65	0	-				
			5	2	3		3	1.53	0	-				
			5	2	3		4	2.76	0	-				
			5	1.75	3		5	2.17	0	-				
	Caloosahatchee River (3)	9	5	2	4	14/11/2018	1	2.39	0	-	18/02/2019	15	0	-
			5	2	3		2	2.45	0	-				
			5	1.5	2		3	1.6	0	-				
			5	2	2		4	1.53	0	-				
			5	2	1		5	5.6	0	-				
	Peace River	10	5	1.25	4	14/11/2018	1	2.09	0	-	18/02/2019	10	0	-
			5	0.5	1		2	0.978	0	-				
			5	0.5	1		3	1.66	0	-				
			10	1.2	3		4	4.89	0	-				
			10	1	2		5	5.5	0	-				
TTINWR	Goodland Outer Island	11	20	5	2:30	14/11/2018	1	9.2	0	-	18/02/2019	41	0	-
			20	5	2:30		2	13	0	-				
			20	5	2:30		3	6.1	0	-				
			20	5	2:30		4	9	0	-				
			20	5	2:30		5	9.8	0	-				
	Stop Keys	12	10	5	3	14/11/2018	1	5.3	0	-	18/02/2019	30	0	-
			10	5	4		2	5.8	0	-				
			10	5	6		3	4.79	0	-				
			10	5	4		4	5.9	0	-				
			10	5	4		5	5.9	0	-				
	Grocery Creek	13	20	5	5	12/12/2018	1	4.34	0	-	18/02/2019	13	0	-
			20	5	5		2	3.85	0	-				
			20	5	5		3	3.71	0	-				
			20	5	4		4	3.47	0	-				
			20	5	6		5	4.23	0	-				
	Faka Union Bay	14	10	4	5	12/12/2018	1	3.99	0	-	18/02/2019	8	0	-
			10	4	5		2	3.43	0	-				
			10	4	5		3	4.35	0	-				

			10	4	5		4	3.88	0	-				
			10	4	5		5	4.57	0	-				
			1.2	1	5		6	9.6	0	-	-	-	-	-
	Kingston Key	15	10	5	3	12/12/2018	1	3.14	0	-	18/02/2019	26	0	-
			10	5	3		2	4.44	0	-				
			10	5	3		3	10	0	-				
			10	5	4		4	4.07	0	-				
			10	5	4		5	4.96	0	-				
			5	3	4		6	1.84	0	-	-	-	-	-
			1.2	1	4		7	2.8	0	-	-	-	-	-
	Indian Key Pass	16	10	5	3	12/12/2018	1	4.19	0	-	18/02/2019	19	0	-
			10	5	3		2	5.4	0	-				
			10	5	3		3	2.62	0	-				
			10	5	3		4	4.41	0	-				
			10	5	2		5	3.99	0	-				
			1.2	0.5	5		6	9.4	**1**	**36.65**	-	-	-	-
	Panther Key	17	10	5	3	12/12/2018	1	9.9	0	-	18/02/2019	16	0	-
			10	5	2		2	8.2	0	-				
			10	5	2		3	3.72	0	-				
			10	5	2		4	4.73	0	-				
			10	5	2		5	4.78	0	-				
			1.2	1	2		6	7.9	**1**	**32.34**	-	-	-	-
ENP	Chokoloskee Island (1)	18	10	2	4	12/12/2018	1	3.38	**1**	**29.6**	18/02/2019	7.3	**1**	**29.02**
			10	2	4		2	3.53	**1**	**29.1**				
			10	2	4		3	4.26	**1**	**27.25**				
			10	1.5	4		4	4.46	**1**	**28.1**				
			10	1.5	4		5	4.64	**1**	**30.26**				
			5	0.5	5		6	2.1	**1**	**32.18**	-	-	-	-
			1.2	1			7	8.5	**1**	**27**	-	-	-	-
			20	2	4		8	5.7	**1**	**32.15**	-	-	-	-
	Chokoloskee Island (2)	19	10	1.5	4	12/12/2018	1	3.77	**1**	**32.04**	18/02/2019	21	**1**	**33.75**
			10	3	3		2	5.9	**1**	**32**				
			10	3	3		3	11	**1**	**32.83**				
			10	3	3		4	7.8	**1**	**33.35**				
			10	4			5	10	**1**	**36.12**				
			1.2	1			6	7	**1**	**34.13**	-	-	-	-

Table S5.5. Quasibinomial model fit using 'glm' function in R and goodness-of-fit test using Hoslem test in R.

	Estimate	S.E.	95% CI	Odds ratio	t value	Pr(>|t|)
Intercept	-5.27	0.79	0.001 – 0.024	0.01	-6.68	<0.000
Technical replicate	0.39	0.06	1.34 – 1.65	1.48	7.06	<0.000
Null deviance:	164.22	df	119			
Residual deviance:	66.22	df	118			
Goodness of Fit (Hosmer-Lemeshow):						
X-squared 3.85	df 8		p-value 0.87			

Figure S5.1. Juvenile smalltooth sawfish (*Pristis pectinata*) captured off cape Coral Yacht Club, Caloosahatchee River in the days and months prior to eDNA sampling at the location in 2018. Dates of capture include A) January 2 B) March 14 C) March 27. Photos were retrieved from the Sawfish Conservation Society Facebook webpage, 9 March 2021.

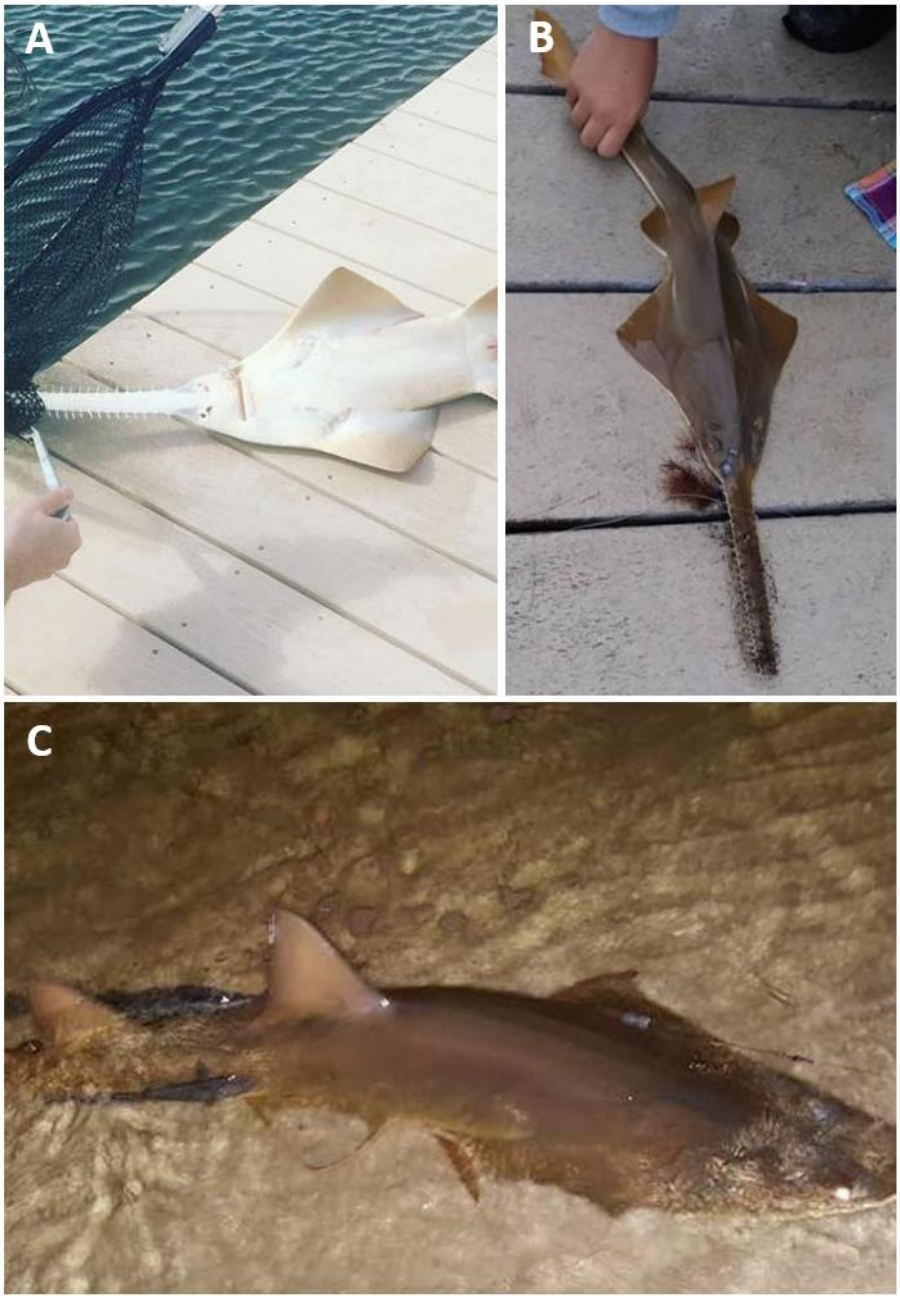


Figure S5.2. Historical capture of smalltooth sawfish (*Pristis pectinata*) in Apollo Bay by Mote Marine Laboratory, ca. 2001 (Simpfendorfer, 2001). Photo credit: Colin Simpfendorfer.

5.8. References

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Chapter 6

Environmental DNA survey confirms extant populations of sawfishes within their historical range

Statement of the Contribution of Others

Nature of assistance	Contribution	Names & affiliations of co-contributors
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6.1. Abstract

Decades of fisheries overexploitation have devastated sawfish populations, leaving considerable doubt as to their contemporary global distribution. Much of what is known about sawfishes has been inferred from commercial and recreation catch records and knowledge surveys, but in many countries that comprise the historical distribution of sawfishes their contemporary presence remains uncertain. Scaling-up monitoring programs, particularly where the need is required the most, is a key attribute of environmental DNA (eDNA) monitoring that can be harnessed for assessment of the contemporary distribution of sawfishes. Here, an eDNA sampling campaign called Global Sawfish Search was deployed to assist in determining the global distribution of sawfishes. A total of 1,537 eDNA samples were collected across coastal regions in the Pacific, Indian and Atlantic Oceans over three years. Collaborators used a two-piece sampling kit, containing an in-field filtration set-up and a complete set of consumables, along with a 10-min training video on eDNA sample collection. An adaptive sampling strategy was used that included targeting regions and sites coinciding with sawfish historical or recent records and presence of preferred habitat types. The results demonstrate that sawfishes are rare and primarily absent throughout much of their historical range, observing no sawfishes in 6 of 10 nations surveyed. In the remaining nations, positive sawfish eDNA detections were only recovered in 3.83% (59/1,541) of survey sites. The results support evidence for the persistence of largetooth sawfish *Pristis pristis* in a previously identified riverine hotspot in Costa Rica and the persistence of *P. pristis* and *Anoxypristis cuspidata* in Papua New Guinea. In these nations, recovery of sawfish populations may be observed by engaging key socio-economic aspects of tropical fisheries in combination with improvements in information regarding the distribution of species and management of key habitats. The results also confirm the presence of all four Indo-West Pacific species (largetooth sawfish *P.* pristis, dwarf sawfish *P. clavata*, green sawfish *P. zijsron* and narrow sawfish *A. cuspidata*) in northern Australia and smalltooth

sawfish *P. pectinata* in Florida, U.S., which are considered lifeboat populations for sawfishes. Overall, the data support the use of eDNA analysis to detect and monitor sawfish populations that are changing in response to environmental and anthropogenic drivers in coastal tropical and subtropical areas. The combination of increased frequency and intensity of eDNA sampling and cooperation with complementary monitoring approaches is most likely to provide the best evidence for the contemporary occurrence of sawfishes where their abundance is critically low.

6.1. Introduction

Understanding changes in species distributions and population status is fundamental to guiding management decisions and conservation outcomes (Lindenmayer & Likens, 2010; Pacifici et al., 2020; Silvy, 2020; Zale et al., 2012). In the marine environment, anthropogenic impacts have resulted in decline in populations and the contraction of species' range, including notably the global decline in reef (MacNeil et al., 2020) and oceanic (Pacoureau et al., 2021) sharks and rays. These trends are driven by overexploitation of marine resources and an increase in pollution and coastal land use (Dulvy et al., 2021; McCauley et al., 2015). While there are some conservation successes (i.e., improvements in managed and monitored fisheries stocks; Hilborn et al., 2020), rare and overexploited marine species have gone unmonitored and local extinctions have occurred (Dulvy et al., 2021; Everett et al., 2015). Inferences of species decline and extinction risk are typically based on time-series population datasets that demonstrate decreases in species range and abundance (Lindenmayer & Likens, 2010); however, these data are inadequate or lacking for many marine species, particularly sharks and rays (Costello et al., 2012; Dulvy et al., 2021; Hilborn et al., 2020). For some of the rarest species, even the most basic data such as occurrence is difficult to obtain (Jorgensen et al., 2022).

Sawfishes, a family of shark-like rays (Pristidae) well known for their characteristic toothed rostra, have undergone some of the most significant declines and range contractions of any marine species (Dulvy et al., 2016; Harrison & Dulvy, 2014; Yan et al., 2021). Sawfishes are more vulnerable to population declines than many other shark and ray species due to their specific reliance on tropical and subtropical nearshore habitats that are heavily exploited (Dulvy et al., 2016, 2021). This is amplified by their predisposition to entanglement in nets by virtue of their toothed rostra and the fact that rostra often removed to untangle the animal, a process that is often lethal to the individual, or the individual is retained for the fins and meat

(Seitz & Poulakis, 2006). In the absence of adequate fishing restrictions, intensely exploited populations collapsed rapidly and range contractions observed throughout much of their historic range (Dulvy et al., 2016; Everett et al., 2015; Hossain et al., 2015; Thorson, 1982a; Yan et al., 2021). Sawfishes were historically found in the coastal waters of 90 countries, with the greatest species richness (i.e., four of five species) historically occurring in the Indo-West Pacific (Dulvy et al., 2016; Yan et al., 2021). Currently, Australia and United States are considered "lifeboat" nations for the four Indo-West Pacific species (largetooth sawfish *Pristis pristis*, dwarf sawfish *P. clavata*, green sawfish *P. zijsron*, and narrow sawfish *Anoxypristis cuspidata*) (Morgan et al., 2011, 2015) and the Western Atlantic population of smalltooth sawfish *P. pectinata* (Norton et al., 2012; Simpfendorfer, 2001), respectively. Papua New Guinea, Costa Rica, and the Bahamas are considered "beacon of hope" nations where sawfishes are present and scientists and conservationist are working to understand their status and enact protection (Grant et al., 2021b; Guttridge et al., 2015; Valerio-Vargas & Espinoza, 2019). Elsewhere in the Indo-West Pacific, Western Indian, Atlantic, and Eastern Pacific Oceans, at least one or two species are estimated to be locally extinct or the presence of some species remains uncertain (Yan et al., 2021). In total, sawfishes are presumed extinct in more than half of their historical range states ($n = 46$), wherein at least one of the five species are missing from records (Yan et al., 2021). Compliance with international and national protective measures (e.g., Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) Appendix I, Convention on Migratory Species (CMS) Appendices I & II; Australian Environment Protection and Biodiversity Conservation (EPBC) Act 1999; U.S. Endangered Species Act (ESA) 2003), ongoing research (Abrantes et al., 2021; Graham et al., 2021; Lear et al., 2019, 2021; Lehman et al., 2020, 2022; Morgan et al., 2021), and community-based awareness and conservation efforts (e.g., sightings are encouraged to be reported to Sharks and Rays Australia, International Sawfish Encounter Database, and Florida Fish and Wildlife

Conservation Commission; Kroetz et al., 2021) contribute to the ongoing persistence of remnant populations in northern Australia and south-east United States. However, the rebuilding or persistence of global sawfish populations cannot rely on these isolated populations alone.

Given the increasing awareness of the plight of sawfishes, efforts to establish the range of their contemporary presence have grown. A number of recent studies have attempted to assess and estimate the contemporary status of sawfishes throughout the world's oceans. The various approaches include predictive modelling (Yan et al., 2021), interviews with fishers (Grant et al., 2021; Leeney & Downing, 2016; Leeney, 2016; Leeney et al., 2018; Leeney & Poncelet, 2015; Valerio-Vargas & Espinoza, 2019), landing site surveys (Haque et al., 2020; Leeney & Downing, 2016; López-Angarita et al., 2021; Rodrigues Filho et al., 2020), as well as compiling sawfish encounter reports (Cabanillas-Torpoco et al., 2020; Hossain et al., 2015; Kroetz et al., 2021; López-Angarita et al., 2021; Wiley & Simpfendorfer, 2010). These studies highlight that sawfish populations have drastically declined and sawfishes are rare and considered locally extinct in many nations outside of the United States and Australia. Although, a handful of studies have recorded evidence of sawfish presence in remote or understudied regions, the scarcity of data precludes the use of those data as conservation baselines. While the forementioned conventional monitoring tools provide critical information on species and populations, they are often constrained in scale, cost, and effort. To scale-up monitoring programs, particularly where the need is most required (i.e., for critically endangered species), the labour and financial investment for the spatial and temporal coverage is likely unattainable and thus impedes timely application. A comprehensive monitoring program needs to be delivered efficiently and at scale in order to meet the data requirements needed to valuably contribute to the conservation and management of sawfish populations.

Recent advancements in highly sensitive molecular detection tools and the discovery that extra-organismal DNA of a target species can be isolated non-invasively from its environment has revolutionised our ability to study and monitor marine species (Deiner et al., 2017; Huerlimann et al., 2020; Le Port et al., 2018; Taberlet et al., 2018). Specifically, by harnessing the high sensitivity of molecular methods it is possible to infer species' presence by detection of its genetic material left behind in the environment that it lives (Lehman et al., 2020; Simpfendorfer et al., 2016). The analysis of eDNA can yield similar or higher detection rates than conventional survey types (Akre et al., 2019; Hunter et al., 2015; Smart et al., 2015; Thomsen et al., 2016; and see Chapter 5), is relatively fast to conduct, non-invasive, and can be scaled temporally and spatially with relative ease (Bálint et al., 2018; West et al., 2021; Yamahara et al., 2019), which is particularly important for monitoring rare species that are of conservation concern (Hoffmann et al., 2016; Thomsen & Willerslev, 2015). Evidently, eDNA monitoring offers a number of key attributes that assist in overcoming the limitations of detecting rare, high conservation value marine species (Huerlimann et al., 2020; Le Port et al., 2018; Lehman et al., 2020; West et al., 2021). Specifically, for this study, the simplicity of seawater filtration and immediate preservation of eDNA at ambient temperatures in the field combined with the ability to leverage a motivated, connected community of biologists, and specifically those that are sawfish experts, made a global eDNA survey of sawfishes a possibility. The aim of this study was to harness the utility of eDNA detection methods to survey the contemporary occurrence and distribution of sawfishes in key coastal and riverine locations through the global tropics and subtropics, thereby producing data to fill the gap in spatial data that is crucial for management and conservation of sawfish populations. Environmental DNA sample collection was facilitated by in-country partners in 10 nations and samples were shipped with the necessary permits to a central laboratory for processing and analysis using optimised workflows for the detection of species-specific sawfish eDNA (see

Chapter 3). Positive detection data was analysed to provide an insight to the presence and distribution of each sawfish species throughout parts of their historic range. The results are expected to assist future targeted conservation initiatives and inform decisions at the international policy and management level.

6.2. Methods

6.2.1. Study areas

6.2.1.1. Indo-West Pacific The Indo-West Pacific historically had the greatest sawfish species richness, where all four species were thought to have occurred throughout much of the region (Dulvy et al., 2016). The predicted extinction risk of sawfish is low to very low throughout much of the Indo-West Pacific (Yan et al., 2021), yet presently their occurrence varies and sightings are sparse in many historical range states. There are some nations bordering the central and northern extent of the sawfish range in the western Pacific Ocean (e.g., China, Singapore, Vietnam, Cambodia, Thailand) and the Persian Gulf where extinction risk is very high and local extinctions have been declared (Yan et al., 2021).

The tropical and subtropical coasts of the Indo-West Pacific, including the Persian Gulf, South and Southeast Asia, Papua New Guinea, and northern Australia, comprises some of the world's major river deltas and mangrove ecosystems, which were historically and in some cases are likely still critical habitat for sawfishes (namely, largetooth sawfish *Pristis pristis*, dwarf sawfish *P. clavata*, green sawfish *P. zijsron*, and narrow sawfish *Anoxypristis cuspidata*). The key Indo-West Pacific rivers of interest, and particularly their coastal extents, include the Indus, Ganges-Brahmaputra, Ayeyarwady, Mekong, Fly, and Sepik, and the multiple rivers that drain into the Gulf of Carpentaria. These rivers are characterised by seasonal monsoonal activity during the 'wet' season, discharging a huge amount of freshwater, sediment, and

carbon into the Indo-West Pacific (De Deckker, 2016). The adjacent coastlines are (or were) predominantly mangrove habitat (i.e., the Indo-West Pacific comprises more than a third of the world's mangroves) and possess networks of estuaries and shallow embayments, key habitat for sawfishes (Wiley & Simpfendorfer, 2010). For example, the Sundarban Forest of Bangladesh, is the world's largest connected mangrove forest, comprising of the abundant mangrove tree species, the sunder or sundari (*Heritiera fomes*). Considerable mangrove forests are also found throughout Papua New Guinea and Indonesia (Osland et al., 2017). It is within these shallow, mangrove-lined estuarine habitats that particular species of sawfishes (largetooth sawfish, dwarf sawfish, and green sawfish) have been documented to show high levels of restricted site fidelity (Peverell & Pillans, 2004; Simpfendorfer et al., 2010; Stevens et al., 2008). Sawfish sightings in general throughout the Indo-West Pacific are sparse (outside of northern Australia). Pupping grounds have been documented in northern Australia, due to the presence of numerous pups that are visible in shallow riverine pools and coastal embayments (Lear et al., 2019; Morgan et al., 2015; Whitty et al., 2009), and have not been documented elsewhere.

6.2.1.1. Western Atlantic & Eastern Pacific The tropical and subtropical coastlines of North, Central, and South American nations bordering the Central Western Atlantic and Eastern Pacific are not characterised by significant river deltas, unlike much of the Indo-West Pacific, with the exception of the Amazon River, Brazil, Mississippi River, U.S., and the San Juan-Colorado River, Costa Rica. These three rivers, which drain into the Western Atlantic, were formally considered to host populations of largetooth and smalltooth sawfish. The adjacent tropical and subtropical coastal habitats of the Gulf of Mexico, Caribbean Sea, Western Atlantic Ocean, and Eastern Pacific are largely comprised of coral reefs, mangroves, and seagrasses. In particular, the Everglades National Park in Florida is home to the largest

mangrove forest in the United States and the largest refuge for smalltooth sawfish *(Pristis pectinata*) from which their population recovery is occurring (Carlson & Simpfendorfer, 2015). Large mangrove forests are found along the Caribbean and Pacific coastlines of Central America and span a large extent of Brazil's coastline (Osland et al., 2017).

6.2.2. Site selection

The following criteria were used to select regions (i) and sites (ii-iv) for eDNA surveys: (i) regions that the species are known to occur but records are sparse, very likely to occur but contemporary records do not exist, or no longer known to occur (identified in Dulvy et al., 2016); (ii) sites that had recent sawfish anecdotal or confirmed reports of captures or sightings; (iii) sites where sawfish historically occurred; and (iv) sites with no contemporary sawfish records, but that may offer suitable habitats for the species, such as mud-sandflats, mangrove-lined estuaries, shallow (<10 m) bays and rivers. Based on these criteria, a total of 10 nations were surveyed, including surveys completed in Australia and United States (data from Chapters 3 and 5) for completeness and to test eDNA would detect sawfish in areas of known occurrence (Figure 6.1). Using satellite images of selected location, an array of sampling sites were identified following a systematic stratified design, in which sites were equidistantly spaced within the location and the number of sites per location was at least 5 in order to representatively survey. Once in the field, the specifics of sampling sites were refined and altered, if necessary, based on traditional ecological knowledge, visual examination of potential suitable habitat, and logistics associated with accessibility and timing.

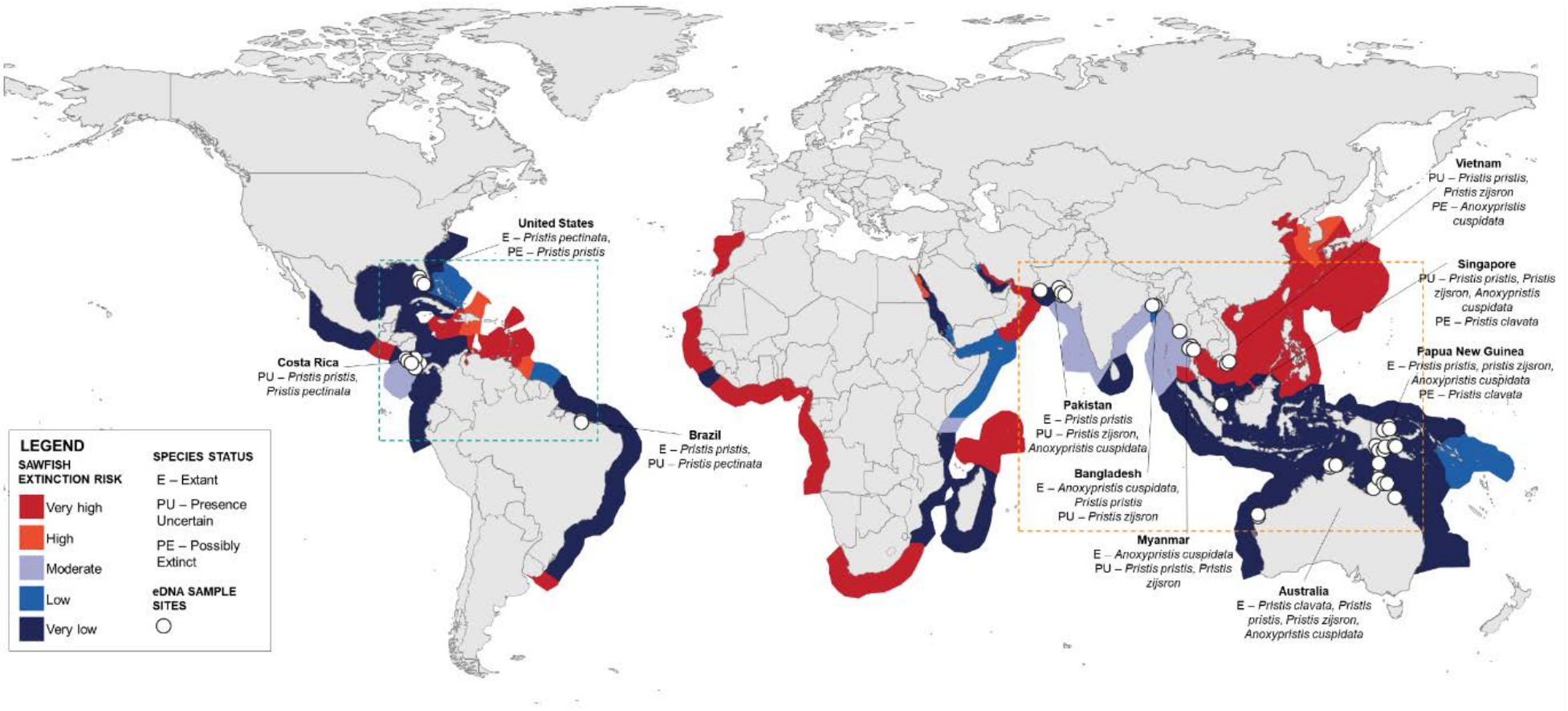

Figure 6.1. World map depicting the nations where the Global Sawfish Search eDNA sampling campaign was conducted. Circles represent sampling locations in each nation. Sawfish extinction risk in each nation is depicted by block colours in the respective economic exclusion zone (adapted from Yan et al., 2021). Green dotted line indicates region where eDNA samples were tested for presence of Western Atlantic & Eastern Pacific sawfishes (Smalltooth sawfish *Pristis pectinata* and largetooth sawfish *Pristis pristis*). Orange dotted line indicates region where eDNA samples were tested for presence of Indo-West Pacific sawfishes (largetooth sawfish *Pristis pristis*, green sawfish *Pristis zijsron*, dwarf sawfish *Pristis clavata,* and narrow sawfish *Anoxypristis cuspidata*).

6.2.3. Sample collection

Environmental DNA sampling was conducted between May 2017 and August 2020. Sampling took place primarily during the dry season of the respective country/region due to the limitation of monsoonal wet seasons on field work and the potential implications that large freshwater flow in riverine systems may have on eDNA persistence. Each sampling effort consisted of a single visit to each of the sites. Two sites, Boca San Carlos and Boca Cureñita, Costa Rica, were revisited at a second time point where accessibility and planning permitted. Field sampling metadata, including dates and locations, are included in Appendix A Table A1. Field sample collection manual and training video used by in-country collaborators are included in Appendix A.

Sample filtration and preservation procedures were carried out as per the optimised workflow presented in Chapter 3 and utilised in Chapter 5. Where site water was too turbid to complete filtration using the 10 μm pore size filter, a 20 μm pore size filter was employed. In all cases where the water was too turbid to complete filtration of the desired 5 L, filtration ceased at the point that the filter was blocked, and the total filtrate volume recorded. Where time and turbidity constraints allowed, three additional samples were collected using a 1.2 μm filter membrane (Appendix A Table A1). Preserved samples were shipped at ambient temperature from the partner institution or organisation to Australian Institute of Marine Science, Townsville, or James Cook University, Townsville, under permit (DAWR Import Permit No 0003495882 & 0002451380, respectively) for extraction in approved facilities (Q0047 and Q2138, respectively) and preparation for species detection using qPCR in a dedicated environmental DNA laboratory (TropWater eDNA Laboratory, James Cook University).

6.2.4. Laboratory analyses

The extraction and purification of total eDNA from filter membranes followed a glycogen-aided precipitation extraction method (Chapter 3) and using QIAGEN DNeasy® PowerClean® Pro Cleanup Kit (Chapter 5). Extracted eDNA from each sample was eluted in 100 µL UltraPure distilled water (ThermoFisher Scientific Pty Ltd, Victoria, Australia) in a 2 mL LoBind® microtube. Presence-only detection was conducted using quantitative PCR (qPCR) on Applied Biosystems QuantStudio 5 Real-Time PCR System (Life Technologies, ThermoFisher Scientific Pty Ltd, Victoria, Australia) and the optimised species-specific primer and TaqMan probe assays (Chapter 3). qPCR analysis was performed in twelve replicate (as determined in Chapter 5) 10 µL reactions run in adjacent wells on a MircoAmp™ Optical 384-well plate (Applied Biosystems, ThermoFisher Scientific, Victoria, Australia).

To test for qPCR inhibition, a TaqMan™ Exogenous Internal Positive Control (IPC) qPCR assay (Applied Biosystems; Hartman, Coyne, & Norwood, 2005) with a custom internal probe modification (i.e., ABY-QSY) was used. The custom modification permitted multiplexing, wherein the spectral wavelengths of the FAM, VIC, and ABY fluorophores (520 nm, 552 nm, and 583 nm, respectively) do not overlap and positive fluorescence of either or all assays can therefore be distinguished by the qPCR instrument for accurate measurement. The assay was applied in multiplex reactions, as per the manufacturers' PCR conditions in three technical replicates of the eluted DNA from each field replicate. Three replicates containing only IPC DNA were also included on the same qPCR plate as 'inhibitor-free' positive controls. To distinguish types of inhibition, we used an IPC ΔCt of 3 cycles as the threshold (Hartman et al., 2005). Specifically, if amplification of the test IPC (mean Ct of three replicates) was 3 or more cycles after the positive control IPC (mean Ct of three replicates) this was considered partial inhibition, and if there was no amplification of the test IPC this was considered complete inhibition. Samples with partial or complete inhibition were identified by

examining and applying the IPC ΔCt ≥3 threshold to amplification curves using the QuantStudio 5 qPCR System Software. Inhibited samples were diluted 1:2 and 1:10 sequentially using UltraPure distilled water and qPCR analysis repeated until inhibition was resolved.

Putative-positive amplicons were visualized on 1.5% agarose gel and compared with a DNA electrophoresis ladder to confirm correct amplicon size. Matching amplicons were sent to Australian Genome Research Facility (AGRF Pty Ltd, Brisbane, Australia) for clean-up and bidirectional Sanger sequencing. Sample detection metadata and sequence information are included in Appendix A Table A3.

6.2.4.1. Validating multiplex qPCR reactions Multiplexing a qPCR is the simultaneous amplification of two or more targets in a single well. Its key benefits are increase reliability via offsetting pipetting error and maximise experimental efficiency by increasing sample throughput, preserving limited samples, and saving reagent costs. The compatibility and efficiency of multiplexing the designed sawfish assays was assessed, such that more than one species can be detected in a single reaction. Species-specific TaqMan qPCR assays (designed in Chapter 3) that were intended to be multiplexed were assigned complementary 5' fluorophores (FAM and VIC) and were multiplexed based on overlap in species' current extent of occurrence (Figure 6.1; Dulvy et al., 2016). Multiplex tests also included the ABY-labelled exogenous internal positive control to verify that this does not cause loss in sensitivity of the target species assays. The distinct spectral wavelengths of FAM, VIC, and ABY fluorescent dyes (520 nm, 552 nm, and 583 nm, respectively) align with three distinguishable filter channels on the QuantStudio 5 quantitative real-time PCR machine (Life Technologies, ThermoFisher Scientific Pty Ltd, Victoria, Australia). Applied Biosystems TaqPath™

ProAmp™ Multiplex Master Mix was used to offset the effect of competition for PCR reagents and therefore allow simultaneous amplification of the three targets in multiplexed reactions. TaqPath™ contains the passive reference dye Mustang Purple (654 nm) providing internal reference for inter-run normalisation.

Multiplex reactions were compared to singleplex reactions to evaluate the compatibility and efficiency of the multiplex reactions following the TaqMan probe manufacturers' guide (Applied Biosystems, 2014). Each assay was optimised and verified for singleplex reactions in Chapter 3. The criteria prior to multiplex testing were to verify that the Tm of primers used in reactions were within 1−2°C of each other, the amplicons are similar sizes, and the primers and probes do not form dimers. To evaluate that there was no difference between the results from single and multiplex reactions under the selected conditions, the ΔCt, reaction efficiency, and R^2 values were measured and compared between reaction types.

Since either target species may be low abundance, serial dilutions of double-stranded synthetic DNA fragments (gBlocks™; Integrated DNA Technologies Pty Ltd, New South Wales, Australia) covering the dynamic range of potential eDNA concentrations were used. The sDNA was serially diluted by 8-point $\log_{10}$ (1E+08–10 copies per assay) and then 4-point $\log_2$ (5–0.65 copies per reaction). To check that the high amplification of one target does not impede the low amplification of the other in multiplex reactions, serial dilutions were run in a matrix across the plate such that each dilution point for one target was combined with every dilution point of the other target. Multiplex reactions were run in parallel with singleplex reactions and no-template controls following the PCR conditions in section 6.2.4. All reactions were run in triplicate and Ct values were averaged across replicates.

Serial dilutions were prepared with UltraPure distilled water (ThermoFisher Scientific Pty Ltd, Victoria, Australia) and stored at -80°C before use. Preparation of synthetic DNA

standards was completed in a laboratory physically separated from the low-copy DNA laboratory to eliminate the risk of cross-contamination.

6.2.5. Data interpretation

A sample was considered positive for the target species if its respective assay amplified eDNA in at least one qPCR replicate of the sample. Samples that showed positive qPCR amplification and subsequent positive match of Sanger sequencing amplicons to reference mitogenome was translated to presence (1) of the respective sawfish species. Samples the showed negative qPCR amplification were translated to absence (0).

6.3. Results

6.3.1. Multiplex assay validation

The T_m of primers used in multiplexed reactions were within 1−2°C of each other (Table 6.1), the amplicon lengths were within 1 and 68 nucleotide base pairs (Table 6.1), and few primer and probe combinations were predicted to form dimers (Table S6.1).

Table 6.1. Summary of multiplex assay validation criteria, including mean difference in forward and reverse primer melting temperature (T_m) and the nucleotide base pair (bp) difference in amplicon length in each multiplexed combination.

Region	Target		Fluorophore	T_m (°C)	Mean °C difference	Amplicon length (bp)	bp difference
Indo-west Pacific	Narrow sawfish	*Anoxypristis cuspidata*	VIC	61.9/58.1	1.85	114	-1
	Green sawfish	*Pristis zijsron*	FAM	58.3/58		115	
Indo-west Pacific	Largetooth sawfish	*Pristis pristis*	VIC	60.6/59.1	-0.5	179	68
	Dwarf sawfish	*Pristis clavata*	FAM	59.4/60.8		111	
Atlantic & Eastern Pacific	Largetooth sawfish	*Pristis pristis*	VIC	60.6/59.1	0.9	179	60
	Smalltooth sawfish	*Pristis pectinata*	FAM	59/58.9		119	

The data indicated an average Ct difference of -0.585 (range: -1.018–0.036; Table S6.2; excluding *P. pristis* multiplex with *P. clavata*) at each serial dilution point for singleplex and

multiplex across all multiplex pairs (Figure 6.2). There was also a high correlation of singleplex and multiplex Ct across all assays (R^2 = 0.9888–0.998; Figure 6.2) and no difference in PCR reaction efficiency (< +/-10%; Table 6.2), indicating that the performance of multiplex reactions for combinations of high and low concentration samples was similar to singleplex assays. With the exception of the *P. pristis* assay when combined with *P. clavata* that had an average Ct difference of -14.246 (Figure 6.2; Table S6.2), R^2 of 0.9663 (Figure 6.2), and PCR reaction efficiency of 72.36% (Table 6.2). The outcome of the multiplex reaction for this combination resulted in a Ct shift of the entire standard curve and did not impede amplification across the dynamic range.

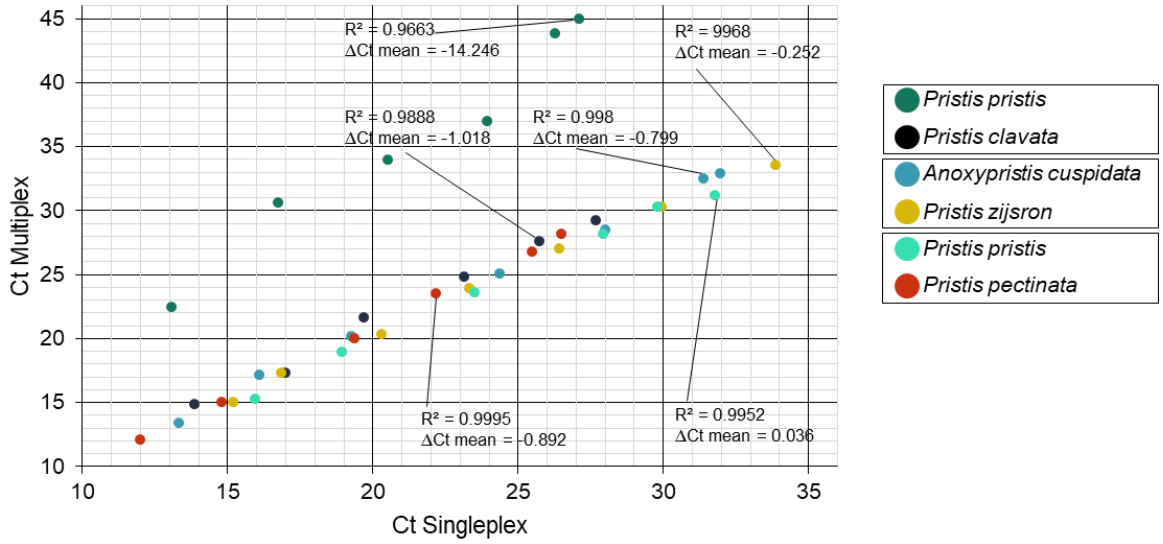

Figure 6.2. Correlation plot comparing mean Ct of singleplex and multiplex reactions across seven dilution points (1.00E+08–100 copies per reaction).

Table 6.2. Summary of PCR reaction efficiency and coefficient of correlation for each assay in both singleplex and multiplex reactions.

Assay target	PCR efficiency (%)		Coefficient of correlation (R^2)	
	Singleplex	Multiplex	Singleplex	Multiplex
Anoxypristis cuspidata	96.75	95.16	0.98	0.98
Pristis zijsron	107.44	107.89	0.99	0.99
Pristis pristis	104.99	100.29	0.89	0.92
Pristis pectinata	111.68	96.56	0.97	0.98
Pristis pristis	120.05	72.36	0.97	0.91
Pristis clavata	108.56	99.15	0.99	0.99

6.3.2. Global environmental DNA survey for sawfishes

6.3.2.1. Indo-West Pacific Ocean A total of 802 eDNA samples (excluding controls) were collected at 142 study sites in 7 nations within the Indo-West Pacific (Australia *n* = 296; Papua New Guinea *n* = 114; Bangladesh *n* = 68; Myanmar *n* = 144; Pakistan *n* = 62; Singapore *n* = 56; Vietnam *n* = 72; Appendicx A Table A1). Sampling effort was not consistent across all countries due to the availability of skilled personnel and time, and accessibility to suitable sampling locations. Sampling was conducted at a single time-point in Pakistan (February–March 2019), Bangladesh (March 2019), Myanmar (May 2019), Singapore (May 2019), and Vietnam (May 2019). Sampling was conducted over several trips in Australia, including seven trips throughout May 2017 to August 2020, and in Papua New Guinea, including three trips throughout September–December 2017 and February 2019.

Samples were analysed for the four Indo-West Pacific sawfish species, *P. pristis*, *P. clavata*, *P. zijsron*, and *A. cuspidata*. Overall, sawfish eDNA was detected in 26 (3.24%) samples from 15 different sites (Appendix A Table A2). For the 26 positive samples, the detectability of sawfish eDNA was 5 positive technical replicates from 12 total technical replicates (mean; range = 1–12), though samples tested prior to 2018 were only assessed with 6 technical replicates (Appendix A Table A2).

The data confirm the presence of *P. pristis*, *P. clavata*, *P. zijsron*, and *A. cuspidata* in northern Australia within their extant range (Figure 6.3). This nation had the greatest number

of overall detections in the Indo-West Pacific and globally (n = 17; Appendix A Table A1) and the only detections of *P. clavata* and *P. zijsron*. Specifically, *P. pristis* was detected at eight sites located on riverine systems draining into the Gulf of Carpentaria, Queensland (Figure S6.1D), *P. clavata* at three coastal sites in Darwin Harbour, West Alligator River, and South Alligator River, Northern Territory (Figure S6.1B), *P. zijsron* at two coastal sites in Exmouth Gulf, Western Australia (Figure S6.1A), and *A. cuspidata* at one beach site in Townsville region, Coral Sea, Queensland (Figure S6.1E). False positive detection of three species, *P. pristis*, *P. clavata*, and *P. zijsron*, in samples from the Skardon and Ducie Rivers, Gulf of Carpentaria, Queensland, was a result of cross-contamination from individuals of these species captured in gillnet surveys that occurred concurrently with eDNA sampling (Figure S6.1C).

Pristis pristis and *A. cuspidata* presence was confirmed in Papua New Guinea within their extant range (n = 9; Figure 6.3; Appendix A Table A2). *P. pristis* was detected at one site upstream in the freshwater extent of the Fly River, Western Province (Figure S6.2B) and *A. cuspidata* at two coastal sites in the East Sepik Province and at the mouth of Ramu River (Figure S6.2A).

No sawfish eDNA was detected in samples from Ganges River, Sundarban National Park Bangladesh (Figure S6.3), Indus River and adjacent coastal bays and lagoons, Pakistan (Figure S6.4), Ayeyarwady River and Mergui Archipelago, Myanmar (Figure S6.5), Mekong River, Vietnam (Figure S6.6), or coastal wetlands of Singapore (Figure S6.7).

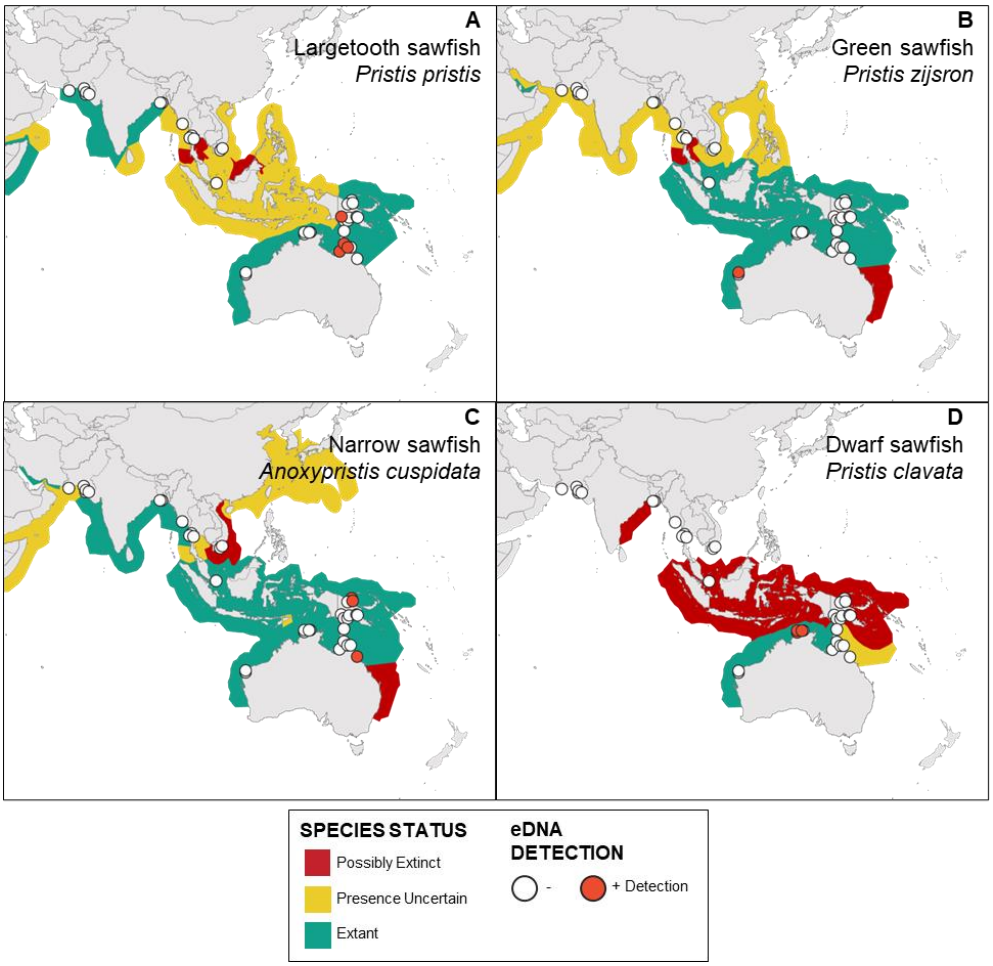

Figure 6.3. Summary of eDNA detection data for Indo-West Pacific sawfishes, A) largetooth sawfish *Pristis pristis*, B) green sawfish *Pristis zijsron*, C) narrow sawfish *Anoxypristis cuspidata*, and D) dwarf sawfish *Pristis clavata*. Circle icons are sample sites. Maps depicting species range and status is adapted from Dulvy et al. (2016). Metadata associated with each sampling point is in Appendix A Table A1.

6.3.2.1. Western Atlantic & Eastern Pacific Ocean A total of 739 eDNA samples (excluding controls) were collected at 131 study sites in 3 nations of the Western Atlantic-Eastern Pacific region (United States n = 104; Costa Rica n = 486; Brazil n = 149; Appendix A Table A1). Sampling effort was not consistent across all countries due to the availability of skilled personnel and time, and accessibility to suitable sampling locations. Sampling was

conducted a single time-point in United States (March–April 2018) and Brazil (October 2019). Sampling in Costa Rica was conducted over several trips throughout January–August 2019.

Samples from United States were analysed for the only extant species of sawfish in this region, *P. pectinata*. Samples from Costa Rica and Brazil were analysed for both *P. pectinata* and *P. pristis*. Overall, sawfish eDNA was detected in 33 (4.47%) samples from 15 sites (Appendix A Table A2). The detectability of sawfish eDNA was 2 positive technical replicates from 12 total technical replicates (mean; range = 1–5), excluding samples from United States that were assessed using 30 replicates in experiments conducted in Chapter 5 (Appendix A Table A2).

The data confirm the presence of *P. pristis* in Costa Rica, a region where the species status was considered Presence Uncertain (Figure 6.4). This nation has the only detections for *P. pristis* in the Western Atlantic-Eastern Pacific region (n = 15; Appendix A Table A2). Specifically, *P. pristis* was detected only in the Colorado River, both in the non-tidal, freshwater extent of the Northern Plains Province and in the coastal, estuarine extent where the river drains into the Caribbean Sea (Figure S6.8E). In the freshwater extent, *P. pristis* was detected in samples collected at the same sites, Boca San Carlos and Boca Cureñita, at two separate time points (March 2019 and May 2019). The genetic sequences for *P. pristis* from the Colorado River showed a single base-pair transition (T to C) of the mitochondrial *12S* gene fragment (Appendix A Table A3), which appears to be characteristic of samples from this study, as it is absent from other *P. pristis* sequences obtained from tissue samples (Chapter 3) and eDNA samples (this Chapter; Appendix A Table A3)

The data also confirm the presence of *P. pectinata* in United States within their extant range (Figure 6.4). This nation had the only detections for *P. pectinata* in the Western Atlantic-Eastern Pacific region (n = 18; Appendix A Table A2). *Pristis pectinata* was detected at one

beach site in Tampa Bay (Figure S6.9A), one estuarine site in the Caloosahatchee River (Figure S6.9B), and three sites within the Everglades National Park region (Figure S6.9C), as detailed in Chapter 5.

No sawfish eDNA was detected in samples collected from the estuarine and coastal tributaries of the Mearim River, Maranhão, Brazil (Figure S6.10; Appendix A Table A1).

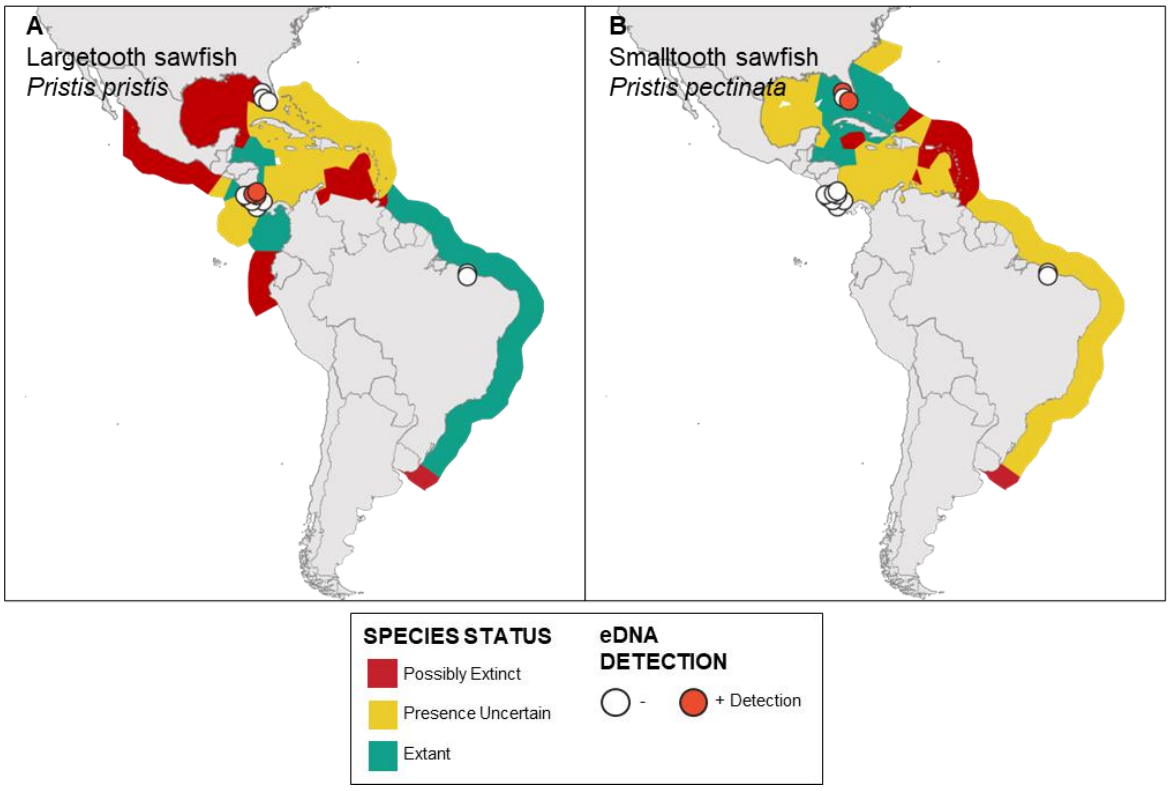

Figure 6.4. Summary of eDNA detection data for the Western Atlantic and Eastern Pacific Ocean sawfishes, A) largetooth sawfish *Pristis pristis* and B) smalltooth sawfish *Pristis pectinata*. Circle icons are sample sites. Maps depicting species range and status is adapted from Dulvy et al. (2016). Metadata associated with each sampling point is in Appendix A Table A1.

6.4. Discussion

Environmental DNA holds promise as a non-invasive biomonitoring tool that can be included in the toolkit for monitoring and managing rare and threatened species (Hansen et al., 2018; Hoffmann et al., 2016; Taberlet et al., 2018). The popularisation of the method is seen

in the rapid increase in the number of aquatic eDNA-based surveys globally (Huerlimann et al., 2020; Yao et al., 2022), but rarely are eDNA surveys applied at scale, harnessing its full potential. The 3-year program, Global Sawfish Search, is arguably one of the largest eDNA-based surveys studies to date, covering 271 unique sampling sites across 10 nations in the global tropics and subtropics, totaling 1,541 eDNA samples. The study is the largest to investigate the contemporary occurrence and distribution of sawfishes using a single methodology. The data confirmed the persistence of sawfishes in nations that are considered "lifeboat" and "beacon of hope" locations. Specifically, the eDNA data presented here provides evidence in support of recent confirmation of largetooth sawfish *P. pristis* in the San Juan-Colorado River, Costa Rica (Valerio-Vargas & Espinoza, 2019), a historically important habitat for the species but their contemporary occurrence was uncertain (Dulvy et al., 2016; Thorson, 1976, 1982a). Further, the detection of narrow sawfish *A. cuspidata* and largetooth sawfish *P. pristis* in East Sepik, Ramu River, and Fly River, Papua New Guinea, supports evidence that the region is a refuge for the Indo-West Pacific sawfishes (Grant et al., 2021; Leeney et al., 2018), second to Australia (Dulvy et al., 2016). The data also supports evidence of the persistence of all four species of Indo-West Pacific sawfishes, *P. pristis*, *P. clavata*, *P. zijsron*, and *A. cuspidata*, throughout northern Australia and *P. pectinata* in their global hotspot on the western coastline of Florida, United States, further confirming the utility of eDNA as a monitoring tool. Despite these positive findings, there are a substantial number of negative detections throughout the Indo-West Pacific and on the Western Atlantic coastline that should be interpreted with caution. The opportunity to compare these data to anecdotal reports was afforded by the increasing popularity of the Sawfish Conservation Society Facebook group, wherein fishermen, hobbyists, and scientists alike post information and images on recent and historical sightings of sawfish. The eDNA approach has advantages and drawbacks that must be considered for effective implementation of a large-scale survey or monitoring program.

Moreover, findings related to the occurrence of sawfishes are the first step in the road to species' persistence and recovery. Understanding of these findings within the geopolitical and cultural context provides clues to the potential for continued research, conservation efforts, and enforced protections.

6.4.1. Indo-West Pacific Ocean

Singapore and Vietnam are two nations where sawfishes were not detected in this study. This finding coincides with a recent analysis that predicted that sawfishes have a very high likelihood of extinction, if not locally extinct in these countries and neighbouring regions of the central west Pacific Ocean (Yan et al., 2021). Rapid coastal development, land reclamation, and urbanisation of the entire country has led to substantial declines in potential habitat for sawfishes (Hilton & Manning, 1995; Tay et al., 2018) and it is predicted that sawfish are locally extinct in Singapore waters (Yan et al., 2021). One of the last verifiable records of a sawfish (*P. pristis*) in Singaporean waters was in January 2001 (Cooke, 2001). Sampling in Singapore for this study was limited and only conducted in seven sites in the north, east, and south of the island, where patches of remnant or repatriated mangrove forests exist. In Vietnam, and specifically the Ben Tre and Soc Trang coastal districts of the Mekong Delta where eDNA sampling for this study was conducted, more than 50% of mangrove forests have been removed for aquaculture (Dang et al., 2021; Veettil et al., 2019). It is suggested that Vietnam could be within the extant range of *P. pristis* and *P. zijsron*, and that *A. cuspidata* are possibly locally extinct (Dulvy et al., 2016). The likelihood of sawfish occurrence here is very low given the predominance of trawl net fishing and high habitat degradation (Yan et al., 2021). Fishing pressure is considerably high in the Mekong and adjacent coastal region, providing a livelihood for local fishers (Tran et al., 2020). Land-sourced pollution and the accumulation of heavy

metals is considered a significant threat to marine species and fisher livelihoods in the region (Tran et al., 2020).

In the northern Indian Ocean, sawfishes were not detected in eDNA samples from Pakistan, Bangladesh, or Myanmar, but it is likely that sawfishes are present in very low abundance (Dulvy et al., 2016; Haque et al., 2021; Yan et al., 2021). These nations are within the historic range for *P. pristis*, *P. zijsron*, and *A. cuspidata* (Dulvy et al., 2016) and the predicted extinction risk of sawfishes in this region is low to very low (Yan et al., 2021). Presently, *P. pristis* and *A. cuspidata* are considered extant in Bangladesh, where they are occasionally caught as non-discarded bycatch by both artisanal and industrial fisheries, but they are considered rare and very low in abundance given the decrease in fisheries encounters (Haque et al., 2020; Haque et al., 2022; Hossain et al., 2015). Despite the lack of detections of sawfish eDNA in the Sundarbans, Bangladesh, the region is still considered a strong candidate for sawfish persistence given the low predicted probability of extinction (Yan et al., 2021). There is little information regarding the historic and contemporary occurrence of sawfishes, and marine biodiversity and conservation in general, in Myanmar given a history of geopolitical isolation, military rule, and civil unrest (Holmes et al., 2014; Howard et al., 2015; MacKeracher et al., 2021). Myanmar is considered a historic range state for *P. pristis*, *P. zijsron,* and *A. cuspidata*, but there is no formal evidence of their contemporary occurrence. During sample collection for this study in the Mergui Archipelago, a local fisherman who was facilitating sampling for this study reported an unconfirmed capture of a juvenile sawfish (≈ 1.5 m) in 2015. The fisherman suggested that the specimen was sent to a Thailand market. *Pristis zijsron* are sparsely documented in the northern Indian Ocean; however, capture records indicate that the species may be the most frequently occurring sawfish species in Pakistan, Persian Gulf, and Red Sea (Elhassan, 2018). There is one recent record of *P. zijsron* at a landing site in Bangladesh (Haque & Das, 2019), though it is likely that the *P. zijsron* are exceptionally

rare throughout the northern Indian Ocean. *Pristis clavata* were not historically found widespread in the northern Indian Ocean, with the exception of the east Indian coastline. In Pakistan, Bangladesh, and Myanmar, shark fishing is illegal or sawfishes are nationally protected; however, unregulated and non-compliant catch and trade of elasmobranchs represents a significant threat to sawfish populations (Haque et al., 2022; MacKeracher et al., 2021; Moore, 2015). Abundance of sawfish in these nations is likely relatively low and populations are likely still declining as the threats remain.

This study confirmed the presence of *A. cuspidata* at two coastal locations in the northern province of Papua New Guinea: Murik Lakes, east of the mouth of the Sepik River, and at the mouth of Ramu River. This region is considered an important habitat for *A. cuspidata* and *P. pristis*, with reports of *A. cuspidata* at the mouth and along the adjacent coastline of the Sepik and Ramu Rivers and *P. pristis* further upstream in the freshwater reaches (Grant et al., 2021a; Leeney et al., 2018). The contemporary occurrence of *P. zijsron* and *P. clavata* is not evident in the Sepik or Ramu rivers or coastal waters of the northern provinces in Papua New Guinea (Grant et al., 2021a; White et al., 2017). It is noted that sawfish populations in the Sepik and Ramu River are in decline owing to intensive, small-scale shark fisheries that operate in the river mouths targeting the harvest of shark fin (Grant et al., 2021a; Leeney et al., 2018). Additionally, individuals that are caught by artisanal and subsistence fishers are retained as sustenance for families that largely rely on fish meat as a primary protein source (Grant et al., 2021a; Leeney et al., 2018). The southern coastline of Papua New Guinea has the potential to be a significant refuge for all four species of Indo-West Pacific sawfishes, given the expanse of suitable habitat (Grant et al., 2021a, 2021b; White et al., 2017). This study, which showed the presence of *P. pristis* ≈300 km upstream in the Fly River, is in support of evidence that the freshwater environments of the deltaic rivers of the Gulf of Papua are important habitat for *P. pristis* (Grant et al., 2021a, 2021b; White et al., 2017). Specifically, White et al., (2017)

reported that the 'Middle' Fly River was likely a historic pupping ground due to the presence of neonates. Though it is noted that *P. pristis* are less frequently observed here and further upstream likely due to pollution from the Ok Tedi mine site and increase pressure on fish populations due to gillnetting (White et al., 2017). *Anoxypristis cuspidata* are considered to be the most common sawfish species in the coastal and riverine delta environments of the southern region (Grant et al., 2021a), but the species was not detected in samples collected for this study. *Pristis zijsron* and *P. clavata*, which were also not detected in this study, are very rarely encountered in the southern region (Grant et al., 2021a).

Finally, the detection of *P. pristis*, *P. zijsron*, *P. clavata*, and *A. cuspidata* in several location throughout northern Australia was expected. In some locations, the detection of one species and not others where sampling was conducted within the known distribution of the species, is likely attributable to difference in habitat use, seasonality, and abundance and the impact on detection probability (discussed in Chapter 3). The detection of *P. zijsron* at two locations in the Exmouth Gulf, Western Australia, is consistent with the understanding that this region is an important stronghold for the species following the discovery of a pupping site ≈60 km north at the estuary of the Ashburton River (Morgan et al., 2015). *Pristis zijsron* is the most frequently encountered species in this region of Western Australia. Pupping sites and nursery grounds for *P. pristis* and *P. clavata* are found a thousand kilometres further north in the Kings Sound and Fitzroy River, which was out of the range of sampling for this study (Lear et al., 2019; Morgan et al., 2021; Thorburn et al., 2007). *Pristis clavata* was detected in samples collected from estuarine environments on the Northern Territory coastline, which is within the known range and habitat use for the species (Field et al., 2013; Stevens et al., 2008; Thorburn et al., 2003), but it is unknown whether this is a nursery area or significant population centre for the species. *Pristis pristis* was detected in samples collected from the Norman and Mitchell Rivers, which drain into the east of the Gulf of Carpentaria where they are known to occur and

interact with inshore fisheries (Peverell, 2005). *Pristis pristis* are distributed throughout the Gulf of Carpentaria predominantly occupying freshwater and riverine habitats (Field et al., 2013; Thorburn et al., 2007). The detection of only *P. pristis* in samples collected on riverine and estuarine systems of the Gulf of Carpentaria is not unexpected, though it is not unreasonable to suspect that these habitats would also be used by *P. clavata* and *P. zijsron*. Lastly, *A. cuspidata* was detected in samples collected at Toolakea Beach, Townsville, Queensland. Knowledge of the north-east Queensland population of *A. cuspidata* is primarily from fishery observer data and fisheries research (Feutry et al., 2021). *Anoxypristis cuspidata* are possibly the only species of sawfish with contemporary range extending down the eastern coastline (Wueringer, 2017).

6.4.2. Western Atlantic & Eastern Pacific Ocean

The data presented here supports evidence that a population of *P. pristis* has persisted in Costa Rica and, in particular, in the San Juan-Colorado River system. There are few recent records in Costa Rica originating from the Caribbean coast and San Juan-Colorado River system, most confirmed as *P. pristis* (Valerio-Vargas & Espinoza, 2019), at sites that were historically considered a stronghold for the species (Thorson, 1982a, 1982b). In this study, *P. pristis* was detected in samples collected in both the freshwater reaches of the Colorado River, Boca San Carlos and Boca Cureñita, as well as the estuarine extent where the river meets the Caribbean Sea. Additionally, *P. pristis* eDNA was present in samples collected on repeat visits to the same locations (Boca San Carlos and Boca Cureñita) within three months, which supports our knowledge of *P. pristis* site fidelity riverine systems (Whitty et al., 2009). Site fidelity has been documented for populations in in the San Juan-Colorado (Thorson, 1976) and in northern Australia (Whitty et al., 2009). Historically, *P. pristis* were also found on the Pacific coastline of Costa Rica. However, this eDNA data, which was generated from over 400 samples

collected comprehensively across the nations' suitable sawfish habitat, did not recover any positive detections of the species on the Pacific coast. Recent fisher interviews conducted in similar regions indicate that that the Terraba Sierpa National Wetlands in the South Pacific, where significant mangrove forests are found, may be a second hotspot for the species in Costa Rica (Valerio-Vargas & Espinoza, 2019) warranting further investigation. Together, these results warrant an update to the status of *P. pristis* in Costa Rica as 'Extant' and the push for increased conservation efforts and enforced protection.

The Maranhão Amazon Coast, Brazil, is potentially an important habitat for *P. pristis* in the south west Atlantic Ocean (Manir Feitosa et al., 2017). This study did not record the presence of *P. pristis* in samples collected within a 1,500 km^2 section of the estuarine extent of the Mearim River, Maranhão, but contemporary evidence suggest that the river may be used by juvenile *P. pristis* (Manir Feitosa et al., 2017). The likelihood of sawfish occurrence is high (Yan et al., 2021), given the expanse of suitable habitat. The adjacent coastline within the Amazon basin are also recognised as an area inhabited by *P. pristis* (Nunes et al., 2016; Reis-Filho et al., 2016; Schmid & Giarrizzo, 2017).

Verifiable contemporary records of *P. pectinata* in the Central Atlantic are restricted to southeast United States (Brame et al., 2019), Bahamas (Guttridge et al., 2015) and Cuba (Figueredo Martín et al., 2013). It is unlikely that the contemporary range of *P. pectinata* extends to the Caribbean coast of Costa Rica or to the south west Atlantic coastline of Brazil (Manir Feitosa et al., 2017; Waters et al., 2014), supporting the lack of *P. pectinata* detections in both nations. Detection of *P. pectinata* within their core range in southeast United States, particularly the Everglades National Park, Ten Thousand Islands National Wildlife Refuge, Caloosahatchee River, and Tampa Bay supports the evidence of their persistence in the shallow, coastal waters of Florida, discussed further in Chapter 5.

6.4.3. Advantages and limitations of using environmental DNA to survey sawfishes

Sampling of eDNA in most nations was restricted to single time point sampling, except for Australia and Papua New Guinea where sampling was conducted over several trips, though the same sampling sites were not revisited. The latter two are the only nations where positive detections were recovered in the Indo-West Pacific, which is in support of evidence that Australia and Papua New Guinea are refuges for sawfish and also suggests that sampling frequency or intensity is an important aspect in probability of detection. This is further supported by the findings in Costa Rica, where sampling intensity was the highest of all the nations included in this study. In nations where the occurrence of sawfishes is uncertain and sightings or captures are rare and declining, such as Bangladesh and Pakistan, increased intensity of sampling in target habitats or repeat sampling at different times of the year to capture a potential seasonal component of occurrence may yield more conclusive results. Sampling in Pakistan and Bangladesh in this study occurred in February through March, coinciding with the region dry season and the period in which captures of sawfish are historically the highest, though this may be an affirmation bias driven by the increase in fisheries operating during the dry season (Haque et al., 2021; Hossain et al., 2015). Despite this, the target locations for the study cover a vast area, and the ability to sample comprehensively can become logistically and financially constrained. For example, the region comprising the mouth of the Indus River, Pakistan, that was sampled for this study was roughly 1,500 km^2 and the region sampled in the Sundarbans, Bangladesh, was roughly 2,400 km^2.

The detection of sawfish eDNA in five or fewer technical replicates of a single field replicate (i.e., detection was five or fewer given that for every site there are 60 qPCR reactions per species that are analysed), indicating that sawfish eDNA is rare in the environment. The ability to detect low copy eDNA fragment is owing to the high sensitivity of qPCR and the

rigourous testing requirements for the design optimal qPCR assays (Budd et al., 2021; Wilcox et al., 2015; Wilcox et al., 2013). Despite the considerably high detection sensitivity of eDNA, the method is not perfect. Importantly, the absence of detections does not infer the absence of the target species. In a number of sites that were sampled but no positive detections recovered, there were anecdotal reports of sawfish captures within the years since sampling took place indicating that sawfish are present but likely in very low abundance. Information and images regarding the presence of sawfishes were noted directly from collaborators or via posts on social media (Figure S6.11). The persistence of eDNA in the water column is hours to days, meaning that detection limits are restricted to this length of time since the target species was present in the environment being surveyed. Given that sawfishes are mobile, migratory species, failure to detect them is possible for any survey method conducted at a single time point.

Some of the challenges with detectability are also related to obtaining sufficient genetic information as possible from a small amount of sample. Here, we demonstrate that a multiplex qPCR solution can alleviate some of those challenges by allowing the same sample to be queried with more than one assay at a time. The same information can be obtained without setting up multiple single-assay reactions, saving time and materials that can increase the cost associated with complex projects. Though this does not come without challenges. The primary challenge in the design and validation of multiplexed PCR primer probe sets was the potential impact of primer dimer events. The data suggest that dimerisation during the pairing of *P. pristis* and *P. clavata* qPCR assays resulted in a Ct shift of the *P. pristis* standard curve, i.e., reduced reaction efficiency, which warrants further investigation. Typically, standard curves are used to infer gene quantification and when reaction efficiency is poor accurate quantification is not possible. As the intention for the use of qPCR in this study was to produce presence-absence data based on the presence or absence of a specific gene and not the quantification of the amount of the gene present, the shift in Ct was permissible for the purpose

of this study. In general, considering the success of the other multiplex assay pairings, these data suggest that qPCR multiplexing is a feasible and efficient solution.

6.4.4. Challenges to conservation in historical sawfish range states

Increasing development of coastal regions, habitat degradation, significant fisheries bycatch, and many other anthropogenic affects are responsible for the dramatic decline of sawfishes throughout their historical range (Yan et al., 2021). Combating some of these impacts is complex in the context of varying geopolitical and macroeconomic challenges in target nations, where there is no single solution that is universally appropriate for the conservation and management of marine resources and species. In developing nations, which comprise a large portion of sawfish historic geographic range, the increasing technical capacity of small-scale fishers and their preference for gillnetting, in combination with little to no capacity to manage and enforce fisheries or trade regulations, contributes to the compromised persistence of sawfishes (Haque et al., 2022; Hossain et al., 2015; Jabado, 2018; Tanna et al., 2021). Moreover, the implementation or enforcement of the law is problematic because accidental catches are unintentional (i.e., not targeted) and unpredictable (i.e., very low encounter rate), and any mechanism to involve fisherfolks in the process of implementation and enforcement is currently lacking (Haque et al., 2020; Hossain et al., 2015).

International treaty mandates and national legal protections are one such way of improving conservation outlook for threatened species. However, many of the nations surveyed where sawfish were not recorded and presence is uncertain do not adequately enforce protections or report sawfish bycatch or trade. For example, evidence of sawfishes at fish processing plants demonstrates that some trade is undocumented with regard to the function of CITES in nations that are signatories (Haque et al., 2020; Hossain et al., 2015; Feitosa et al., 2017), indicating that regulatory agencies are unprepared to manage fisheries or enforce

CITES. Resource mobilisation, capacity building, and collaboration between regulatory agencies is required to monitor trade and investigate fisheries.

6.4.5. Conclusion

Discerning contemporary distribution of a group of species such as sawfishes that are rare and relatively infrequently encountered, or for which there is little systematic monitoring, is difficult. Moreover, tracking changes in status where there is still a significant lack of data is challenging for scientists who are tasked with completing assessments for management and conservation agencies.

The results of this chapter support the use of eDNA as an efficient survey tool for sawfishes. Compared to conventional detection methods, eDNA is relatively cost-effective and highly sensitive, which are features that make eDNA an appealing tool for wide-scale monitoring efforts. The data substantiated evidence for sawfish persistence Costa Rica and Papua New Guinea, which have the potential to be significant refuge for sawfishes outside of Australia and the United States. Given the successful deployment of the tool here and the high potential for scalability, I advocate for the use of eDNA as a valuable detection tool for sawfishes granted that key limitations are addressed. The widespread use of eDNA in systematic monitoring has the potential to strengthen future conservation efforts.

6.5. Supplementary Information

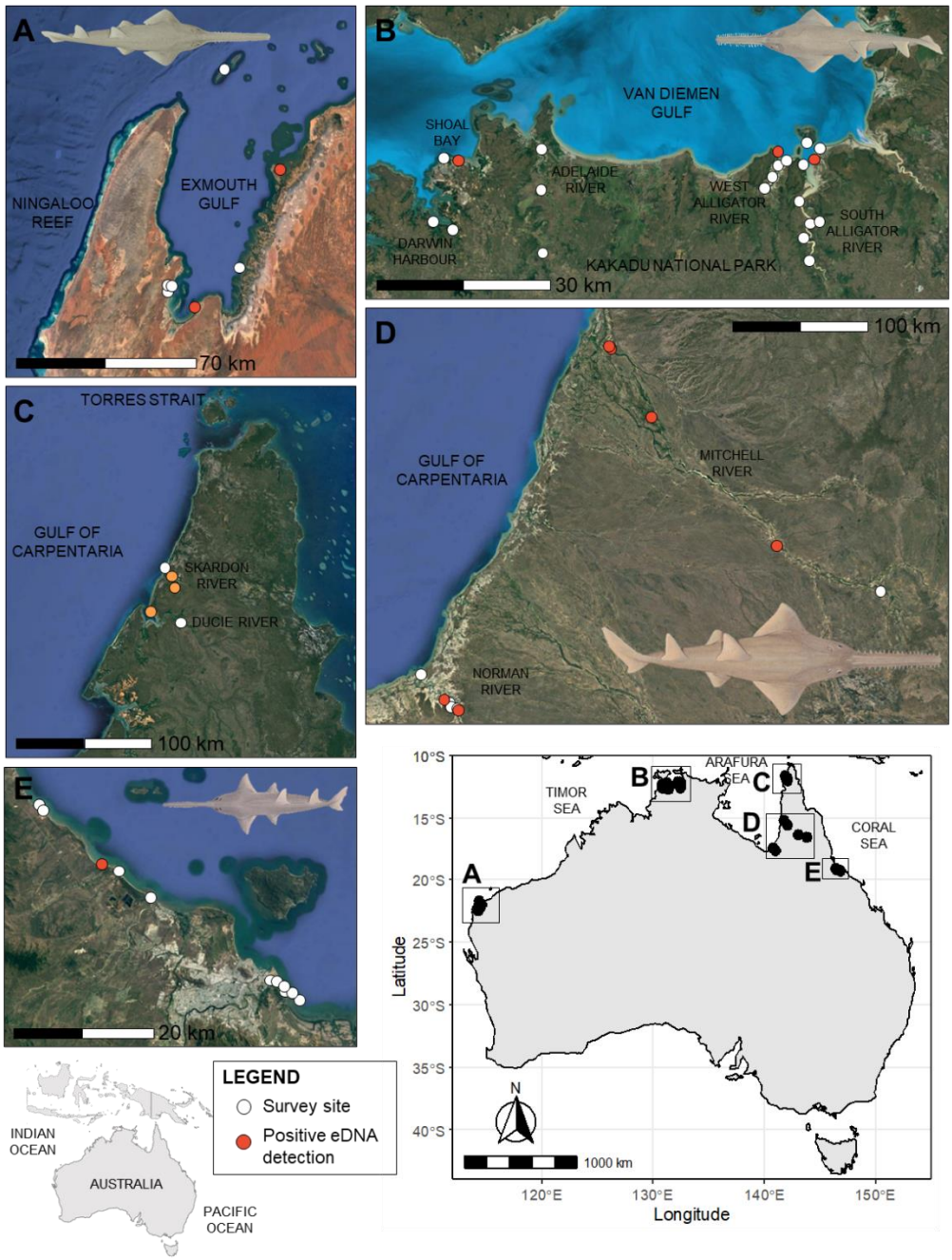

Figure S6.1. Map of environmental DNA (eDNA) survey sites (circle markers) in Australia, including A) Exmouth Gulf, B) Darwin and Kakadu National Park region, C) Cape York Peninsular region, D) Southeastern Gulf of Carpentaria region, and E) Townsville region. Red circle markers indicate sites that tested positive for (A) green sawfish *Pristis zijsron*, (B) dwarf sawfish *Pristis clavata*, and (D) largetooth sawfish *Pristis pristis* eDNA. Orange circle markers indicate sites that tested positive for largetooth sawfish, dwarf sawfish, and green sawfish eDNA, but this eDNA was also present in the field controls for these sites, rendering this eDNA result unreliable and as a result of contamination from genomic DNA originating from individuals of the same species that were captured and tagged under research permits by approved research personnel on the day of eDNA sampling. Scale bars are approximate. Sawfish drawings are from Last et al., (2016) by Lindsay Gutteridge. Images in panels A, B, C, D, and E are from Landsat Copernicus courtesy of Google earth Pro 7.3.4.8642.

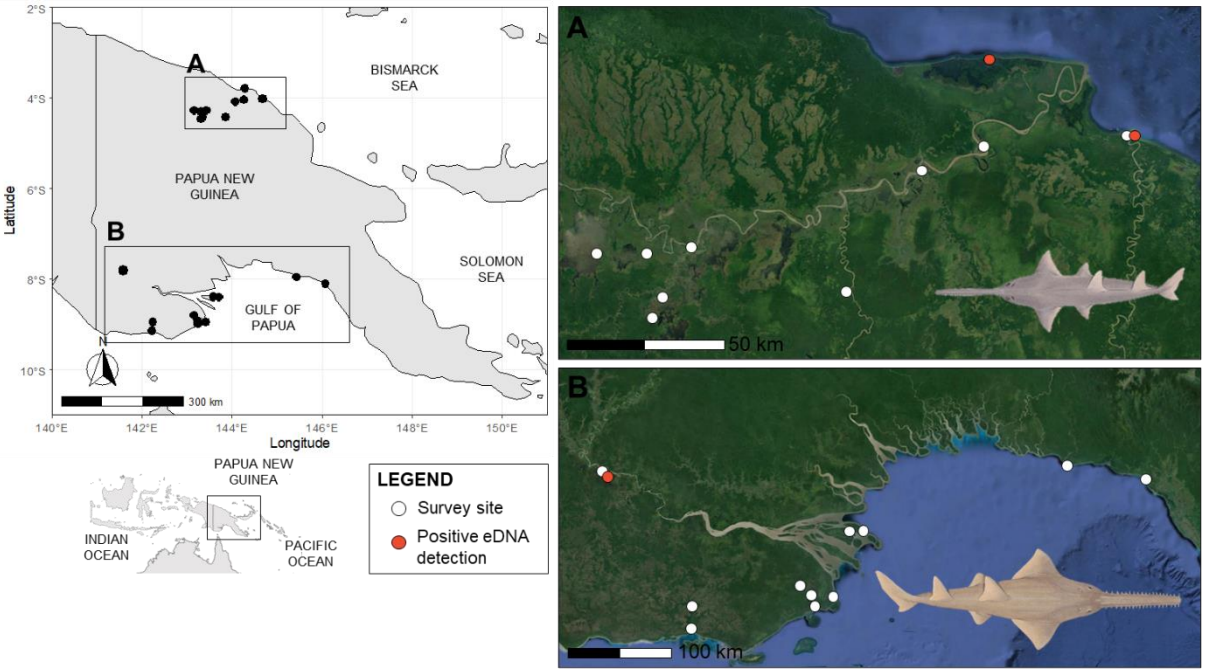

Figure S6.2. Map of environmental DNA (eDNA) survey sites (circle markers) in Papua New Guinea, including A) East Sepik and B) Fly River regions. Red circle markers indicate sites that tested positive for (A) narrow sawfish *Anoxypristis cuspidata* and (B) largetooth sawfish *Pristis pristis* eDNA. Scale bars are approximate. Sawfish drawings are from Last et al., (2016) by Lindsay Gutteridge. Images in panels A and B are from Landsat Copernicus courtesy of Google earth Pro 7.3.4.8642

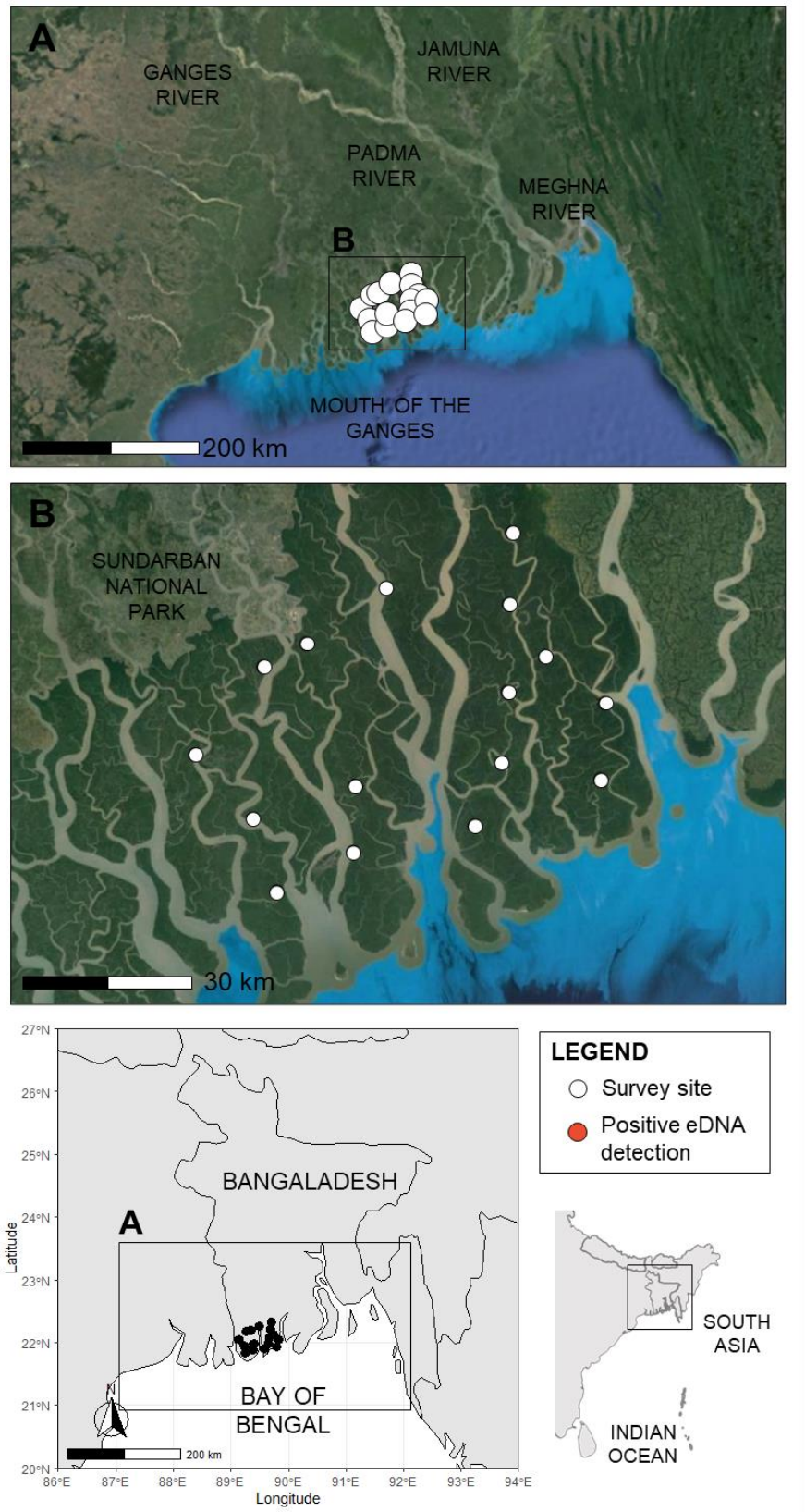

Figure S6.3. Map of environmental DNA (eDNA) survey sites (circle markers) in Bangladesh, specifically at the mouth of the Ganges River in the Sundarban National Park. Scale bars are approximate. Images in panels A and B are from Landsat Copernicus courtesy of Google Earth Pro 7.3.4.8642

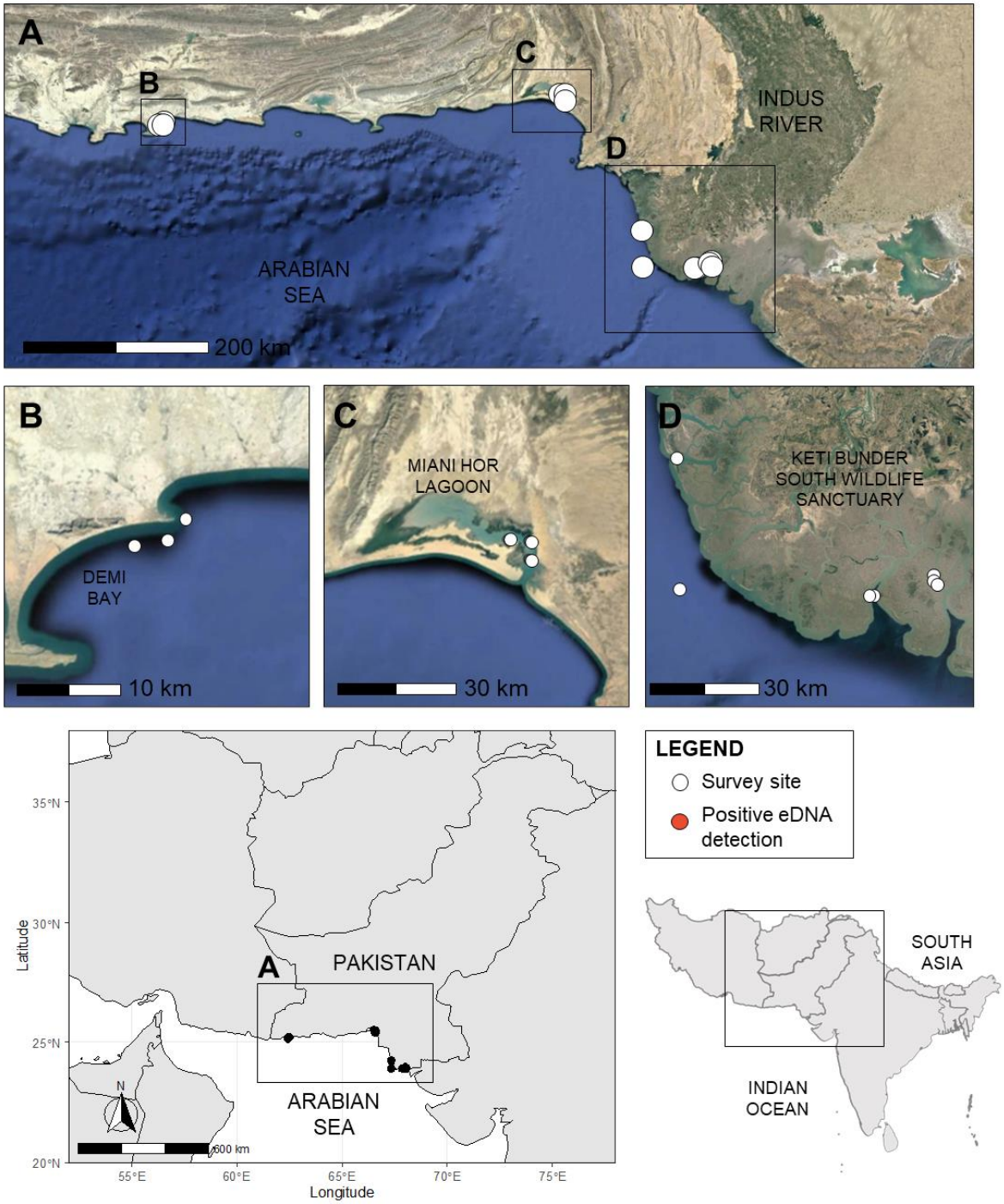

Figure S6.4. Map of environmental DNA (eDNA) survey sites (circle markers) in Pakistan, including B) Demi Bay, C) Miani Hor Lagoon, and D) Keti Bunder South Wildlife Sanctuary at the mouth of the Indus River. Scale bars are approximate. Images in panels A, B, C, and D are from Landsat Copernicus courtesy of Google Earth Pro 7.3.4.8642

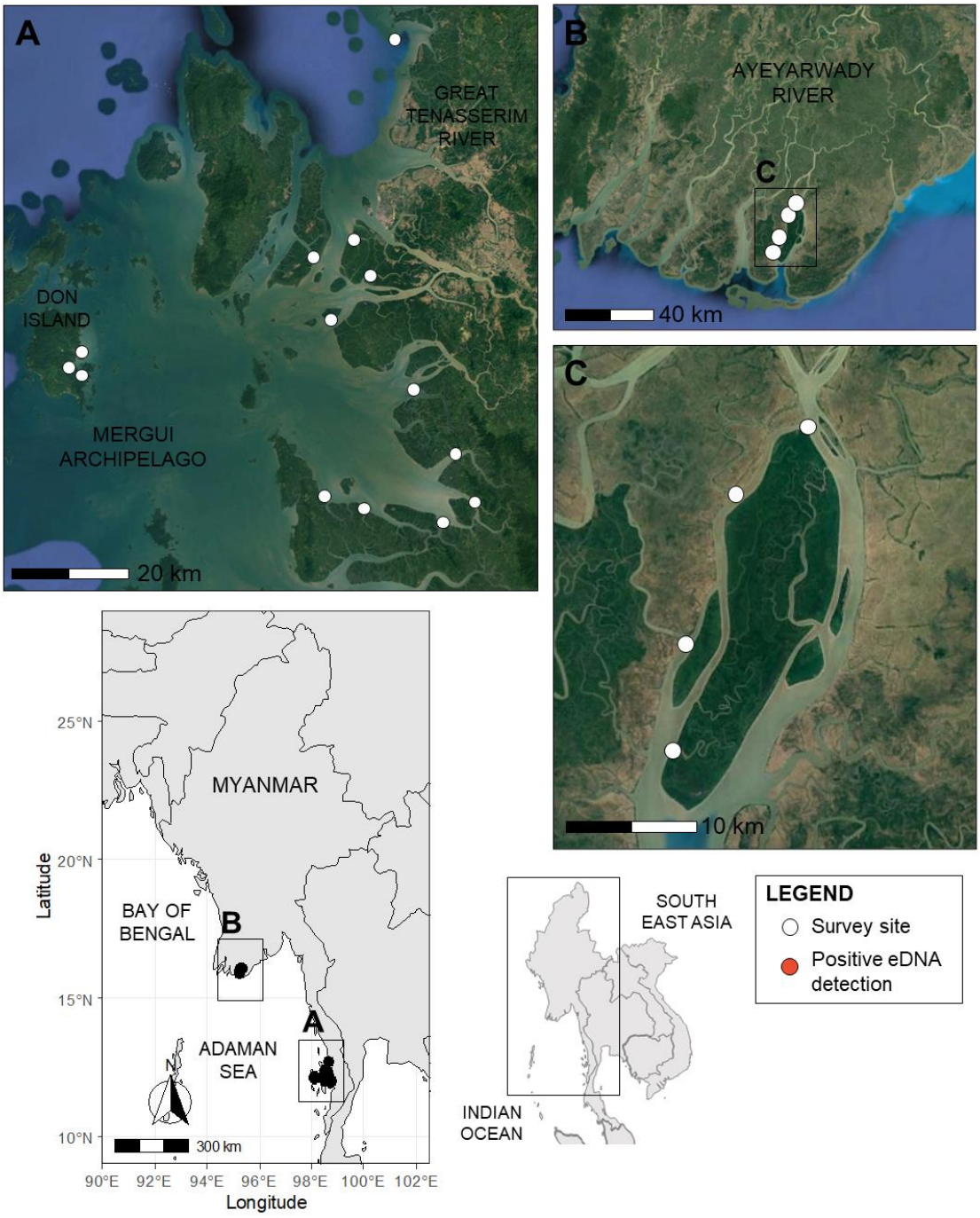

Figure S6.5. Map of environmental DNA (eDNA) survey sites (circle markers) in Myanmar, including A) Mergui Archipelago, specifically Don Island and tributaries of the Great Tenasserim River, and B, C) the Ayeyarwady River region. Scale bars are approximate. Images in panels A, B, and C are from Landsat Copernicus courtesy of Google Earth Pro 7.3.4.8642.

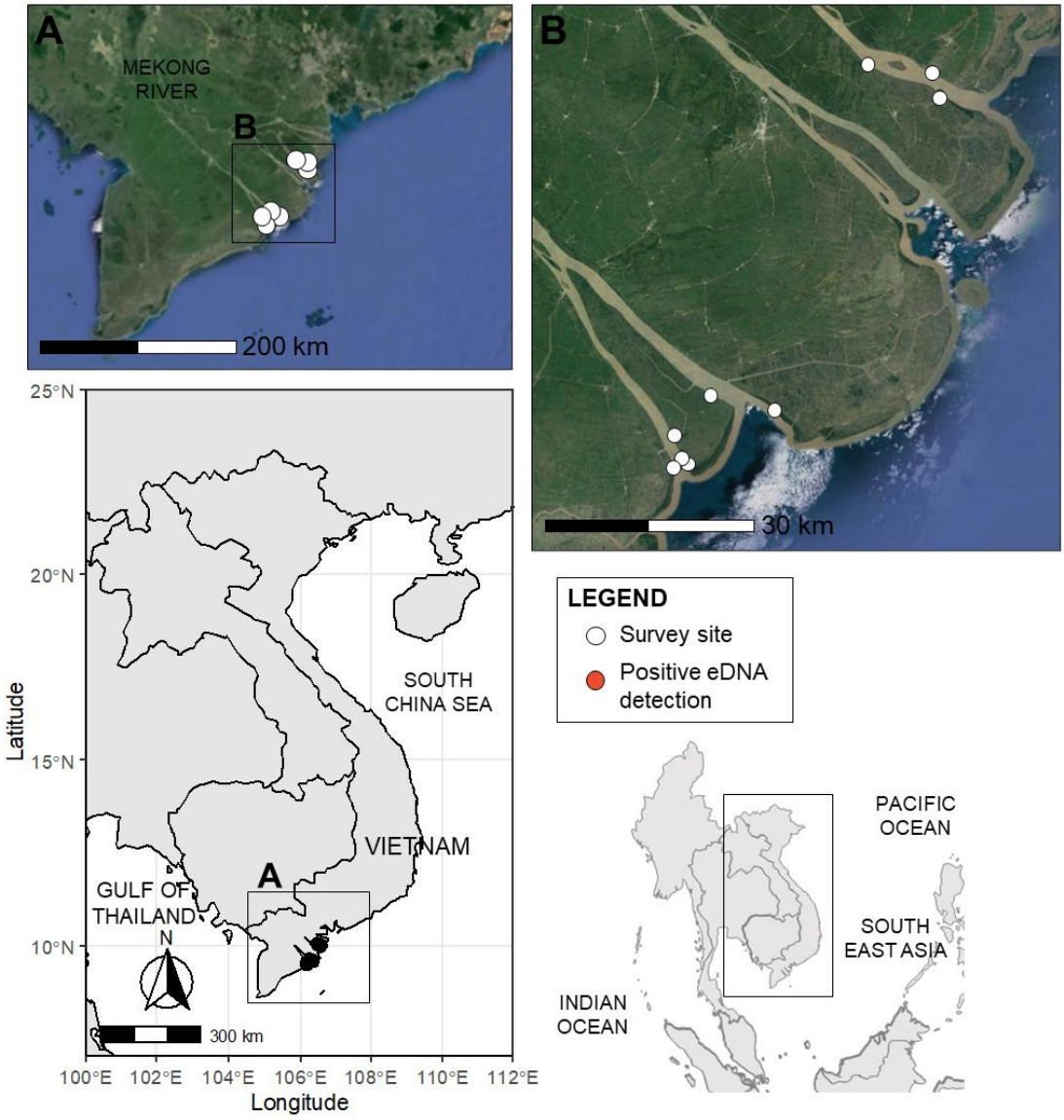

Figure S6.6. Map of environmental DNA (eDNA) survey sites (circle markers) in Vietnam, specifically at the mouth of the Mekong River (A, B). Scale bars are approximate. Images in panels A and B are from Landsat Copernicus courtesy of Google Earth Pro 7.3.4.8642.

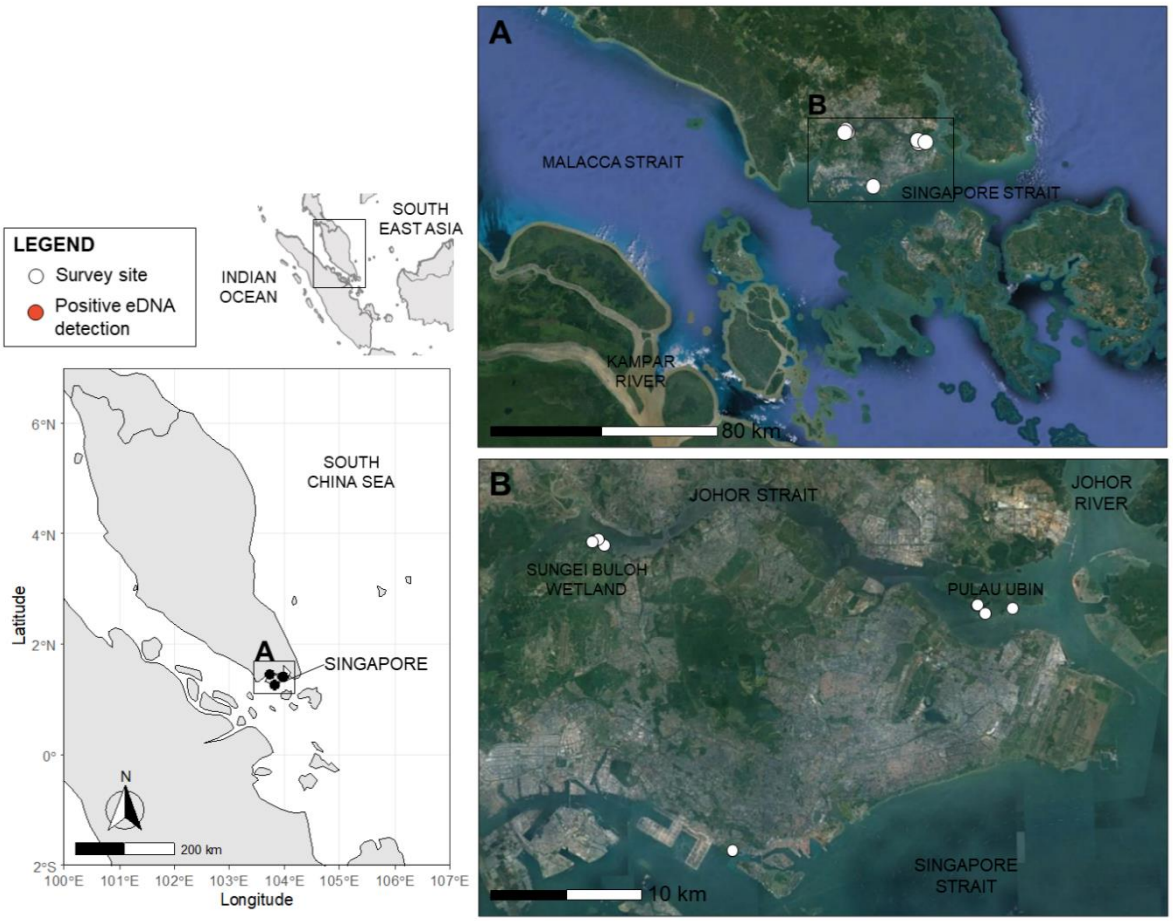

Figure S6.7. Map of environmental DNA (eDNA) survey sites (circle markers) in Singapore (A, B). Scale bars are approximate. Images in panels A and B are from Landsat Copernicus courtesy of Google Earth Pro 7.3.4.864.

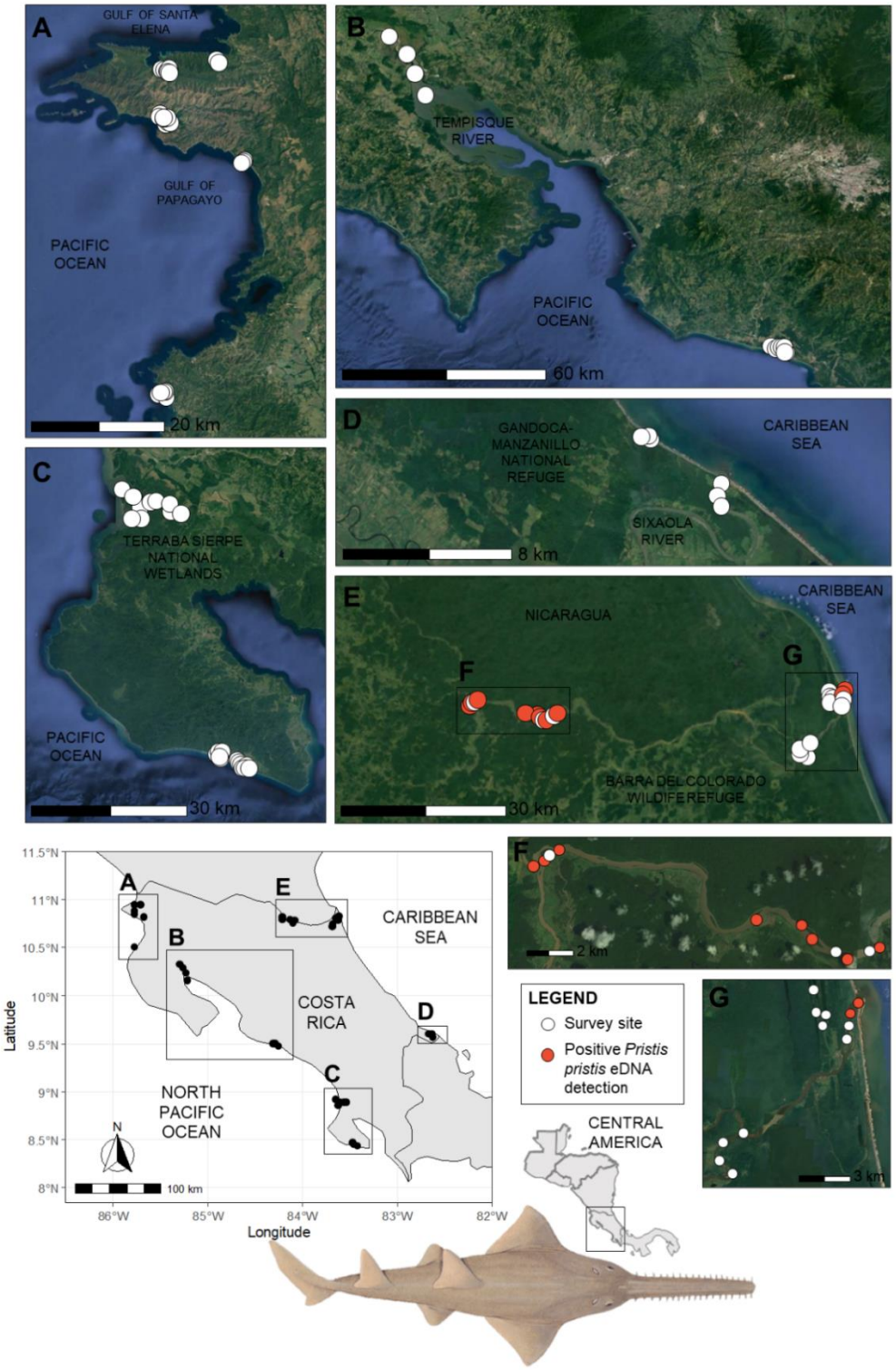

Figure S6.8. Map of environmental DNA (eDNA) survey sites (circle markers) in Costa Rica, including A) Northern Pacific region, B) Tempisque River and Central Pacific region, C) Terraba Sierpe National Wetlands and Southern Pacific region, D) Gandoca-Manzanillo National Refuge and Sixaola River region, and E) Colorado River region. Red circle markers indicate sites that tested positive for largetooth sawfish *Pristis pristis* eDNA. Scale bars are approximate. Largetooth sawfish drawing is from Last et al., (2016) by Lindsay Gutteridge. Images in panels A, B, C, D, and E are from Landsat Copernicus courtesy of Google Earth Pro 7.3.4.8642.

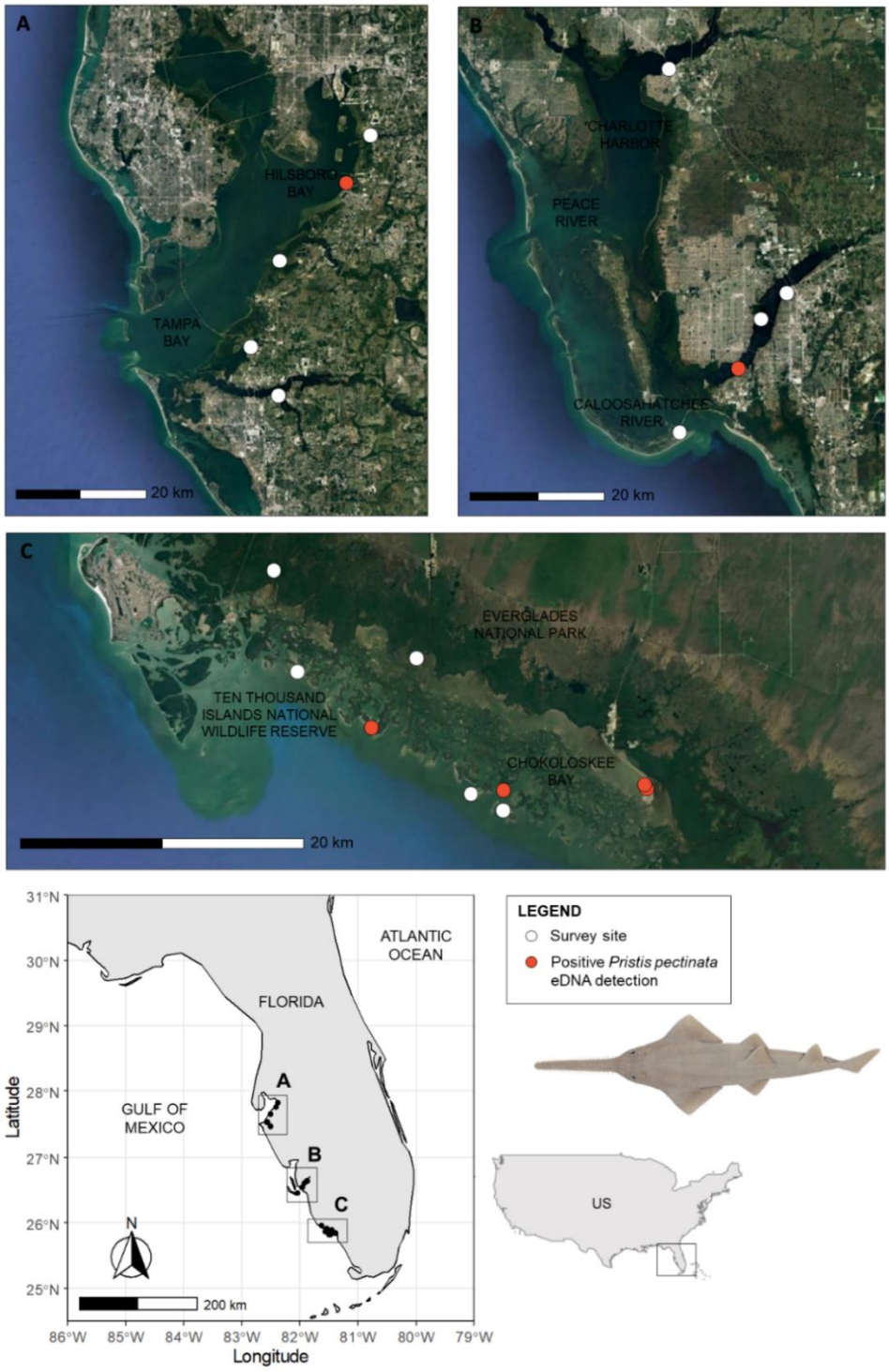

Figure S6.9. Map of environmental DNA (eDNA) survey sites (circle markers) in Florida, US, including A) Tampa Bay region, B) Charlotte Harbor-Caloosahatchee River region, and C) Everglades National Park region. Red circle markers indicate sites that tested positive for smalltooth sawfish *Pristis pectinata* eDNA. Scale bars are approximate. Smalltooth sawfish drawing is from Last et al., (2016) by Lindsay Gutteridge. Images in panels A, B, and C are from Landsat Copernicus courtesy of Google Earth Pro 7.3.4.8642.

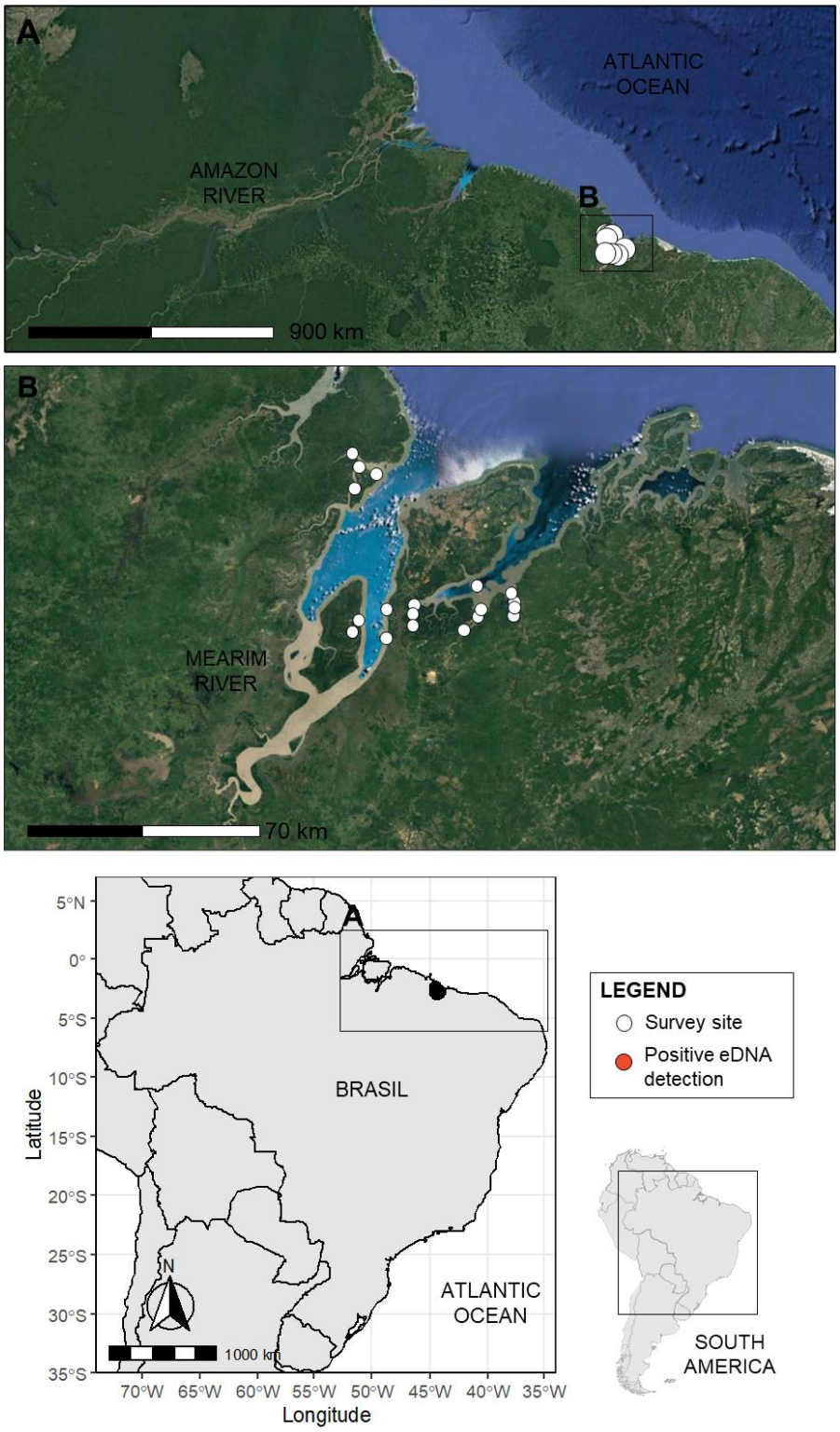

Figure S6.10. Map of environmental DNA (eDNA) survey sites (circle markers) in Brazil, Mearim River region (B). Scale bars are approximate. Images in panels A and B are from Landsat Copernicus courtesy of Google Earth Pro 7.3.4.8642

Data S6.5.3. Primer Dimer Analysis

Primers and probes were checked for the likelihood of dimerisation in multiplex conditions, as per the methods described in 3.7. The Primer Dimer check was completed using the freely available online software PrimerDimer (http://www.primer-dimer.com/) (Johnston et al., 2019; Lu et al., 2017) following the software instructions for multiplex analysis. Specifically, primer and probe sequences that were coupled together for the planned multiplex experiment were copied into the text in FASTA format using the guided software interface. Multiplex dimer analysis was selected and a downloadable dimer structure generated. The three lowest scores were estimated for dimer pairs 7, 8, and 11 (-13.58, -8.0, and -9.05, respectively) (Table S6.1).

Table S6.1. Multiplex primer dimer analysis, including score (kcal/mol) and visualisation of the 3' extension dimer formation, for each assay primer and probe pair. Analysis was completed using the freely available online software PrimerDimer (http://www.primer-dimer.com/) (Johnston et al., 2019; Lu et al., 2017). Pairs that were not complementary and therefore no likelihood of dimerisation and not shown here. Bold values indicate the three lowest scores and therefore the highest potential for dimerisation.

	Oligonucleotide #1	Oligonucleotide #2	Score (kcal/mol)	Dimer formation
1	*Anoxypristis cuspidata* Reverse	*Pristis zijsron* Forward	-4.45	5'> CCTGACGTGTTGGAGGTTAATC >3' 3'< AGATCCACCTAGATTCCGTGG <5'
2	*Anoxypristis cuspidata* Reverse	*Pristis zijsron* Reverse	-5.78	5'> CGACCTGACGTATTGAAGATAGAT >3' 3'< CTAATTGGAGGTTGTGCAGTCC <5'
3	*Pristis pristis* Forward	*Pristis clavata* Reverse	-3.64	5'> CTGACGTATTGAAGGTGGGTTCT >3' 3'< AGATCCACCCAGACTCCGTG <5'
4	*Pristis pristis* Reverse	*Pristis clavata* Forward	-0.95	5'> CATCATACTGTTCGTTTTTTCTTAGGAG >3' 3'< GAGATCCACCTAGATTCCGTGG <5'
5	*Pristis pristis* Reverse	*Pristis clavata* Reverse	-3.08	5'> CATCATACTGTTCGTTTTTTCTTAGGAG >3' 3'< TCTTGGGTGGAAGTTATGCAGTC <5'
6	*Pristis pristis* Reverse	*Pristis clavata* Probe	0.0	5'> CATCATACTGTTCGTTTTTTCTTAGGAG >3' 3'< CCAACTATCGTTCTTTAC <5'
7	*Pristis pristis* Probe	*Pristis clavata* Reverse	**-13.58**	5'> CTGACGTATTGAAGGTGGGTTCT >3' 3'< GCATAACTTCCAATCAAGTAAA <5'
8	*Pristis pristis* Forward	*Pristis pectinata* Forward	**-8.0**	5'> GGCGGTACCTTAGATCTCTCTAG >3' 3'< AGATCCACCCAGACTCCGTG <5'
9	*Pristis pristis* Reverse	*Pristis pectinata* Reverse	0.0	5'> CATCATACTGTTCGTTTTTTCTTAGGAG >3' 3'< GTAAGAGGTTATGCAGTCCAGC <5'

10	*Pristis pristis* Reverse	*Pristis pectinata* Probe	-1.2	5'> CATCATACTGTTCGTTTTTTCTTAGGAG >3' || | 3'< CCGCCAATTATCGTTCC <5'
11	*Pristis pristis* Probe	*Pristis pectinata* Reverse	**-9.05**	5'> CGACCTGACGTATTGGAGAATG >3' ||| ||| | || | 3'< GCATAACTTCCAATCAAGTAAA <5'

Table S6.2. Summary of data generated during experiments to validate multiplex qPCR reactions. Mean Cycle threshold (Ct) at each dilution point is provided, as well as the difference in Ct between singleplex and multiplex reactions.

***A. cuspidata* (with *P. zijsron*)**

Dilution point	Quantity (gene copies)	Singleplex (Ct mean)	Multiplex (Ct mean)	ΔCt
1	100,000,000	13.317	13.438	-0.121
2	10,000,000	16.068	17.181	-1.113
3	1,000,000	19.254	20.230	-0.976
4	100,000	24.360	25.071	-0.712
5	10,000	27.982	28.511	-0.529
6	1,000	31.384	32.532	-1.148
7	100	31.953	32.947	-0.994
			ΔCt Mean	-0.799

***P. zijsron* (with *A. cuspidata*)**

Dilution point	Quantity (gene copies)	Singleplex (Ct mean)	Multiplex (Ct mean)	ΔCt
1	100,000,000	15.181	15.083	0.099
2	10,000,000	16.848	17.374	-0.525
3	1,000,000	20.301	20.318	-0.017
4	100,000	23.300	23.981	-0.681
5	10,000	26.421	27.025	-0.604
6	1,000	29.938	30.296	-0.359
7	100	33.868	33.543	0.324
			ΔCt Mean	-0.252

***P. pristis* (with *P. clavata*)**

Dilution point	Quantity (gene copies)	Singleplex (Ct mean)	Multiplex (Ct mean)	ΔCt
1	100,000,000	13.063	22.479	-9.415
2	10,000,000	16.713	30.662	-13.949
3	1,000,000	20.493	34.020	-13.527
4	100,000	23.923	37.009	-13.086
5	10,000	26.268	43.836	-17.568
6	1,000	27.082	45.015	-17.934
			ΔCt Mean	-14.246

***P. clavata* (with *P. pristis*)**

Dilution point	Quantity (gene copies)	Singleplex (Ct mean)	Multiplex (Ct mean)	ΔCt
1	100,000,000	10.812	9.360	1.453
2	10,000,000	13.844	14.869	-1.026
3	1,000,000	16.969	17.321	-0.353
4	100,000	19.684	21.664	-1.980
5	10,000	23.129	24.856	-1.727
6	1,000	25.724	27.646	-1.923
7	100	27.657	29.225	-1.568
			ΔCt Mean	-1.018

P. pristis (with P. pectinata)

Dilution point	Quantity (gene copies)	Singleplex (Ct mean)	Multiplex (Ct mean)	ΔCt
1	100,000,000	15.928	15.331	0.597
2	10,000,000	18.933	19.001	-0.069
3	1,000,000	23.507	23.636	-0.129
4	100,000	27.927	28.177	-0.249
5	10,000	31.789	31.194	0.595
6	1,000	29.785	30.312	-0.528

			ΔCt Mean	0.036

P. pectinata (with P. pristis)

Dilution point	Quantity (gene copies)	Singleplex (Ct mean)	Multiplex (Ct mean)	ΔCt
1	100,000,000	11.993	12.078	-0.085
2	10,000,000	14.769	15.031	-0.262
3	1,000,000	19.352	20.024	-0.672
4	100,000	22.176	23.517	-1.340
5	10,000	25.476	26.802	-1.326
6	1,000	26.498	28.167	-1.669
			ΔCt Mean	-0.892

Figure S6.11. Largetooth sawfish (*Pristis pristis*) in Bangladesh markets in 2020 (A & B) and August 2018 (C). Photos were reposted on Sawfish Conservation Society Facebook group by Matthew McDavitt.

Figure S6.12. Largetooth sawfish (*Pristis pristis*) caught by local fishermen in Maranhão, Brazil, in November 2019. Image supplied by Leonardo Feitosa.

6.6. References

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Chapter 7

General Discussion

7.1. Summary of findings

Significant declines in the occurrence and distribution of sawfishes globally have driven the necessity for novel approaches such as eDNA to investigate and validate where sawfish population hotspots remain. Conservation practitioners and researchers working at the pointed edge of conservation for the species require a detection method that is efficient, economical, and accurate to enable the rapid scale-up of research and monitoring efforts. This thesis research investigated the utility of eDNA as a tool for the detection of sawfishes throughout river deltas and adjacent coastlines in key nations of the global tropics and subtropics that were considered as priority for species' survival, leveraging the willingness of in-country specialists to participate in the research. The outputs of this research offer insight into the applicability of eDNA as a tool for sawfish detection and monitoring and provide an overlapping perspective with other survey data on the persistence and rarity of sawfishes in their historic range. The sampling campaign also provided an opportunity build capacity of participants on eDNA sampling and the findings have been used to establish baselines for future sawfish monitoring. For example, eDNA survey efforts are ongoing with collaborators in Costa Rica. The assays developed are presently used in commercial, routine sawfish monitoring conducted by TropWATER, JCU, Townsville (Villacorta-Rath et al., 2020a, 2020b). Lastly, data were shared with the IUCN Shark Specialist Group to aid the re-assessment of sawfish occurrence and distribution for the IUCN Red List of Threatened Species.

This final discussion chapter draws together the main concepts and findings from each of the chapters, outlines the significance, and addresses the limitations of this research for applied eDNA studies. Future research directions related to the direct outputs of this thesis as well as the general field of eDNA research are summarised. Future directions related to sawfish monitoring and conservation outcomes are also summarised. In addition to the findings related to sawfishes, it is anticipated that the outputs here provide greater insight into the applicability of eDNA in general as an aquatic monitoring tool for rare and threatened species.

Several main findings from this thesis are summarised below:

1. Thorough and robust primer and probe design followed by *in silico* and *in vitro* testing is imperative for sensitive detection of rare and threatened species. Insufficient specificity and sensitivity of primers and probes can result in poor, erroneous performance of the assays and may yield false negative or positive results. The five qPCR TaqMan assays, which are forward and reverse primers that flank an internal probe, developed here as a central deliverable of the research are highly sensitive, capable of detection as few as 1.25–5 copies of the target *12S* barcode fragment (Chapter 3). The key finding was that assay specificity to the target sawfish species (i.e., competency to amplify and therefore detect only the target sequence) was not solely determined by the presence of base pair mismatches in the primer or probe binding region to non-target sequence, but that the position of the base pair mismatch was also influential (Kutyavin et al., 2000; Whiley & Sloots, 2005; Wilcox et al., 2013). Specifically, four or more base pair mismatches in primer binding sites resulted in ultimate species specificity when paired with a species-specific probe. The importance of this finding is related to the rarity of sawfish, wherein the assumption is that sawfish eDNA is very low in concentration in the water column and therefore in a sample, and co-captured eDNA from sympatric and closely related species may be present and more abundant, which solicits the requirement for sensitive and specific assays. Following stringent optimisation, the five assays

provided true positive detections of all five sawfish species down to single copies of eDNA, with no evidence of non-target amplification, in various regions throughout their range (for example, largetooth sawfish in northern Costa Rica, smalltooth sawfish in southeast US, dwarf sawfish in northern Australia, narrow sawfish in Papua New Guinea, and green sawfish in western Australia; Chapter 3, 5, and 6) showing the validity of the barcode region across different sub-populations. The finding has relevance beyond this research. Specifically, the recommendations regarding assay design are applicable across the discipline of eDNA with particular importance for applications to rare and threatened species detection and monitoring. This is pertinent in light of the rapid uptake in the use of eDNA for this application and the subsequent demand for standardisation and minimum criteria of eDNA assays before their routine use (De Brauwer et al., 2022b, 2022a; Thalinger et al., 2021).

2. Experimental optimisation and validation of laboratory workflow is also imperative for sensitive detection of rare and threatened species. In addition to highly sensitive qPCR assays, a key finding was that choice of laboratory workflow can have a profound impact on detection sensitivity. A direct comparison between a commonly used preservation and extraction method combination (i.e., preservation of filters membranes in 95% ethanol and extraction of DNA using Qiagen DNeasy Blood & Tissue Kit; Kumar et al., 2020; Lear et al., 2018) and an in-house validated workflow using standard, cost-effective molecular procedures for bulk DNA extraction from complex sample types (i.e., preservation Longmire's buffer and extraction using a glycogen-aided precipitation method; Edmunds & Burrows, 2020) on eDNA samples collected in a global sawfish hotspot revealed dwarf sawfish detections using the latter method only. This method also resulted in a 2x increase in total eDNA yield, suggesting that the improved detection sensitivity of this method may be attributed to the fact that more DNA is retained in this method (Natarajan et al., 2016; Renshaw et al., 2014). Minimising the probability of false negative detection is a central challenge to

eDNA workflows. It was revealed here that optimisation for the study objectives, i.e., maximising total eDNA yield, was an important step in ensuring accuracy of the method. This finding strengthens the current thinking in the eDNA discipline that there is no one-size-fits-all preservation and extraction method and that, provided the correct quality assurances and controls are included in the analyses, the workflow should be validated for the specific application (Klymus et al., 2020; Kumar et al., 2020).

3. eDNA in the environment exists in both large (≈20 µm) and small (≈1.2 µm) size fractions and best captured by filtration of large water volumes. This finding supports the general understanding that eDNA capture by filtration is predicated on the isolation of particles comprising intra- and extra-cellular DNA of various sizes on a filter net or membrane. At the time of this study, the state and size of eDNA particles in the environment represented a remarkably shallow aspect of our understanding. Few studies had indicated the size range of eDNA derived from teleost fishes (Jo et al., 2019; Sassoubre et al., 2016; Turner et al., 2014; Wilcox et al., 2015). The research in Chapter 4 reported for the first time for any elasmobranch species, the particle size distribution of largetooth sawfish eDNA and compared filtration and whole water precipitation methods on the efficacy to capture these particle sizes. It is suggested that mitochondrial DNA that comprises aqueous eDNA from sawfish is arranged in large aggregates of biological material such as waste products, sloughed epidermal tissues and secretions, and on the occasion of birth or death, reproductive material or post-mortem debris, which probably comprises the eDNA size portion that is greater than 20 µm. Upon release, the materials begin to rapidly degrade and disintegrate, breaking down into portions that are much smaller in size and only obtained by capture on a 1.2 µm or less filter membrane. The filtrate volumes in this study were controlled at a single litre, but there is opportunity to increase filtrate volume in field applications, which makes filtration a more desirable option than the whole water precipitation method that is limited to few hundred millilitres. Though, depending on

logistical and environmental constraints, precipitation may be the more user-friendly option, as this method indiscriminately captures all eDNA size classes. Overall, the application of eDNA to detection of aquatic species is strengthened by understanding physical attributes of eDNA and how best to capture it, which is an aspect that requires significant more attention.

4. eDNA-based monitoring and detection is complementary, not mutually exclusive, to conventional methods. It is important to understand how eDNA methods compare to conventional methods for aquatic species monitoring and detection. As this implies, the point of comparison is not to indicate that one is better than the one but rather to validate and ground-truth the newer method with the conventional. In the case of sawfishes, gillnet sampling is typically used by researchers during targeted research to study movement ecology (i.e., capture of individuals for implantation of acoustic or satellite tracking devices; Graham et al., 2021; Hays et al., 2019), collect valuable blood and tissue samples for genetics (Chen et al., 2016; Feutry et al., 2021), as well as morphological, meristic, health, and other population data). In this study, I only considered the sensitivity and efficiency of the method in terms of detection per deployment and per hour of effort, respectively. The detection of sawfish using eDNA was more sensitive and time-efficient (including subsequent time spent in the laboratory) than gillnetting. However, when sawfish were solitary and in low abundance, both methods had reduced reliability, which is not unexpected given the occurrence of false negative detection rates in both methods. Given the poor time-efficiency, potential to cause environmental and organismal harm, greater sampling effort required for gillnetting, eDNA may prove to be a more efficient choice of detection method when sawfish are anticipated to be rare and low in abundance in the environment to be surveyed. Overall, the utility of eDNA-based detection methods is a promising advancement for monitoring rare species as evidenced in other comparative studies (Deiner, Bik, et al., 2017; Dougherty et al., 2016; Jerde et al., 2011; Smart et al., 2015; Thomsen & Willerslev, 2015; Valentini et al., 2016). The growing

use of social media as a record of fisher activity as well as structured fisher interviews have proven valuable methods for filling in gaps of spatio-temporal records of occurrence where records are sparse. It is suggested that eDNA is not a replacement of any other method and instead it provides practitioners with an additional tool in the toolbox to assist in the strategic deployment of complementary research methods.

5. eDNA is a useful non-invasive tool to determine the contemporary distribution of threatened sawfishes where sightings and records are sparse. There is considerable doubt as to the contemporary distribution of sawfishes through their historical range as a result of decades of fisheries overexploitation (Dulvy et al., 2016). By leveraging a motivated global network of marine scientists, and specifically experts working toward the conservation of sawfishes in their home states, an eDNA survey of sawfishes throughout key nations in the global tropics and subtropics was possible. This was underpinned by the ability to send a complete sampling kit, comprising just two boxes of equipment and consumables, alongside a training video, which is a far simpler and scalable approach to complete this significant undertaking than the alternative approaches of fishing that require bulky gear, ethical considerations, and technical knowledge and experience. The results substantiated evidence for the persistence of largetooth sawfish *Pristis pristis* in a previously identified riverine hotspot in Costa Rica (Valerio-Vargas & Espinoza, 2019) and the persistence of *P. pristis* and *Anoxypristis cuspidata* in Papua New Guinea (Grant et al., 2021; Leeney et al., 2018). These data support suggestions that these regions are significant refuge for sawfishes, which reinforces efforts to increase education and awareness for local communities, including encouragement to live release sawfish that are captured, and strengthens the push for conservation and protection efforts on the ground and at the policy and management level. The results also confirm the presence of all four Indo-West Pacific species (largetooth sawfish *P.* pristis, dwarf sawfish *P. clavata*, green sawfish *P. zijsron* and narrow sawfish *A. cuspidata*) in

northern Australia and smalltooth sawfish *P. pectinata* in Florida, U.S., which are considered lifeboat populations for sawfishes (Dulvy et al., 2016).

7.2. Limitations

This thesis demonstrates the potential for simple, non-invasive eDNA surveys to improve our capability and efficiency to collect data on the occurrence and distribution of a family of highly threatened aquatic species. The method is also highly complementary to conventional survey approaches enabling us to supplement or confirm eDNA detection data with capture or sighting data. Yet, not unlike other detection methods, several limitations to the eDNA detection method remain. The limitations of eDNA in general are discussed, as well as the specific limitations of the research in this thesis.

First and foremost, the exclusive use of the single-species qPCR method was a key objective of this research but can also be viewed as a limitation because of the host of additional genomic information for other species that was disregarded. The nature of eDNA sampling means the eDNA of all species that are present at the time of sampling is indiscriminately captured in the same sample. Though not given a considerable amount of attention in the introduction to eDNA in Chapter 2 of this thesis, because it was out of the scope of this research, metabarcoding represents the current best-practice method for identifying all species present in an eDNA sample. Metabarcoding has revolutionised our ability to measure biodiversity (Lawson Handley, 2015; Yao et al., 2022). The inability to have completed metabarcoding on any or all of the eDNA samples collected during this research was a missed opportunity to produce a tremendous amount of biodiversity records, especially given the tropical biodiversity data gap (Collen et al., 2008; Culumber et al., 2019).

A key limitation during the design of the qPCR assays was the incompleteness of reference sequence databases and lack in geographic diversity of available tissue samples. Minimum criteria guidelines for the design of species-specific eDNA assays recommend rigorous, iterative testing of new primers and probes in the *in silico* and *in vitro* phase to identify and remove the risk, including re-design if necessary, of non-target amplification of sympatric or closely related species (Klymus et al., 2020). Failing to adequately complete this step, the assay is unlikely to have sufficient sensitivity to the target species and therefore fail to be broadly applicable. The research described in Chapter 3 of this thesis, which included *in silico* and *in vitro* testing of two separate assay design strategies, described how the incorporation of at least 4 base pair mismatches in both primer and probe binding regions can eliminate non-specific amplification among closely related and sympatric species. However, the *in silico* and *in vitro* tests conducted here were not exhaustive. Firstly, mitochondrial reference sequences for the target and non-target species identified in this thesis research were missing or incomplete (in sequence coverage and geographic representativeness). Sequence data published online in open-source repositories, such as NCBI GenBank, are generally not extensive or curated and relies on researchers to upload sequence data and update metadata generated from their studies, which is not always guaranteed. Furthermore, sequence repositories do not cover the entire tree of life, wherein lesser-studied organisms are lacking sequence data, and for most species only few sequences from few individuals are available, meaning that entire sub-populations are not represented. For the design of sawfish qPCR assays, few whole mitochondrial sequences of all five species were available on NCBI (Chapter 3). To supplement this, tissue samples from all five sawfish species were obtained from collaborators within the network of sawfish researchers to enable the generation of *12S* reference sequences and *in vitro* testing of gDNA with the newly developed assays. However, due to the rarity of the species, only a small number of genetic samples available for use and

so the tissue collection was not globally representative. It is therefore possible that the assays developed are not widely applicable to all sub-populations, though it is evident that the largetooth sawfish assay successfully amplified *12S* mitochondrial DNA from the western Atlantic sub-population in Costa Rica without prior validation that this would work. The highly conserved nature of the *12S* mitochondrial gene may be in favour of the assays, wherein the short fragment used here may be conserved across sub-populations of sawfish. Further testing using genomic DNA from archival specimens (e.g., Fearing et al., 2018) would strengthen the validity of the assays.

False positive or negative results have the potential to profoundly influence how eDNA data are interpreted (Ficetola et al., 2015; Guillera-Arroita et al., 2017; Piggott, 2016; Takahara et al., 2015). A potential and significant source of false negative detections is driven by the ecology of the target species, which was identified as a limitation of this research (Chapter 3 & 6). Specifically, seasonal, ontogenetic, and diurnal variation in habitat use of the target species has been identified as important information for monitoring programs and sampling schedules (Buxton et al., 2018; Takahara et al., 2019; Takeuchi et al., 2019; Uchii et al., 2017). The arrival of gravid females at pupping grounds in shallow riverine or estuarine pupping grounds was a seasonality component identified in Chapter 1, as were distinct preferences in habitat use, which informed the sampling strategy used here to sample in specific habitat types and at shallow depths where possible. Chapter 5 of this thesis demonstrated that neonate sawfish are readily detectable in their shallow, coastal pupping grounds. A 100% detection was observed across all samples collected in the Ten Thousand Islands National Wildlife Reserve, Florida, where the presence of dozens of smalltooth sawfish neonates within metres of sample collection was visually confirmed. A suggested outcome here is that eDNA surveys for sawfish will be strengthened when sampling campaigns are designed to target habitats during the species' pupping season. Outside of southeast U.S. and northern Australia, pupping grounds

for sawfish are not well-documented, making this approach challenging. A strategy for detection of adult sawfish is also not so clear. While Chapter 1 reviewed the literature that described some ontogenetic behaviours of smalltooth sawfish and generic tendencies of *Pristis* spp. to occupy mangrove-lined creeks, rivers, and estuaries, seagrass beds, and mudflat ecosystems (Carlson et al., 2014; Morgan et al., 2015, 2021; Papastamatiou et al., 2015; Simpfendorfer et al., 2011), a significant limitation is that the movement ecology of adult sawfishes remains largely unknown meaning that sampling for this age class was not well informed. Adult sawfishes are likely solitary and low in abundance and subsequently the probability of detection decreases significantly. For example, the detection rate of dwarf sawfish in Northern Territory, Australia, was low during the survey period, which was outside of pupping season for this species as well as largetooth sawfish that are known to use the habitat but were not detected. Additionally, narrow sawfish are almost exclusively marine-dwelling in adulthood, commonly found in offshore waters at depths up to 40 m (Peverell, 2005), meaning that this group were unlikely to be captured in surveys conducted for this research given the shallow, coastal and riverine focus. Alternatively, the incorporation of multiple/repeat sampling events, greater sampling intensity, and sample replication (increased technical replication shown to increase probability of detection; Chapter 5) are suitable strategies where information is limited. This of course is reliant on time, budget, and resources to increase sampling intensity and replication. In the laboratory false negative detection is simpler to constrain and control through sufficient optimisation of procedures to address inherent biases and errors at any step of the workflow (Chapter 3 & 5) and adequate removal or detection of inhibitory compounds (Chapter 5). A significant source of false positive detections arise from sample cross-contamination. The proper use of negative controls to detect contamination is a small yet critical detail (Sepulveda, Hutchins, et al., 2020). Sample cross-contamination should be simply identified through thorough use of best practice control measures, including blanks,

negative controls, and the strategic use of a synthetic DNA fragment as a positive control instead of genomic DNA of the target species (Furlan & Gleeson, 2017) (Chapters 3 & 5).

A limitation identified in the sample collection conducted for this thesis was the inability to achieve consistent filtrate volume due to the difficulties presented by the turbid environments that were sampled. Highly turbid coastal, estuarine, and riverine waters frequently impacted the ability to filter the desired 5 L due to rapid filter clogging. In some cases, the pressure differential between the filter paper and the peristaltic pump caused a fracture of the filter housing mesh (data not shown). At other times, the level of turbidity was almost entirely prohibitive to filtering even one litre of water (Chapter 5). Water turbidity is no doubt a serious drawback for the eDNA filtration method (Robson et al., 2016; Sanches & Schreier, 2020; Schwentner et al., 2021; Wittwer et al., 2017). Alternatives that could have been considered include Waterra filter capsules (Peixoto et al., 2020) or dead-end ultrafilters (Wittwer et al., 2017), which have an increased surface area that would permit a greater volume of water to be filtered before filter clogging, but these capture methods were not well-known and had not been thoroughly tested at the outset of this research. The collection of whole water for precipitation was considered but evidence suggests that filtration increases the chance of detection of rare eDNA (Kumar et al., 2020) and, additionally, the importation of whole water samples would be logistically and financially prohibitive considering more than 1000 individual samples were collected. More recently, passive filters have demonstrated efficiency in the capture of eDNA in water (Bessey et al., 2021; Kirtane et al., 2020), but their utility for rare species is yet to be demonstrated and many questions remain regarding the level of replication, best material type, and material-specific carrying capacity (i.e., how soon after deployment is the material saturated with DNA and no longer able to capture and retain DNA molecules). This finding is a signal to future research to address the significant limitations

associated with standardised sampling in turbid environments if we are to attempt to survey biodiversity in all the world's oceans, rivers, and lakes using eDNA.

Overall, a significant limitation for eDNA in the current state is the imprecise estimation of biomass, population size, and population structure, and inability to quantify sex, health status, or age class, which are principal reasons for the combined use of complementary research methods. Indeed, investigation of these questions were out of the scope of this research, where the intention was to generate presence-only data on sawfish distribution using an approach that was efficient and scalable. Yet, advancements in these research areas present some of the most promising opportunities for aquatic eDNA research, which are discussed in the following sections.

7.3. Future directions

Environmental DNA has the potential to revolutionise the efficiency and effectiveness of biodiversity management and conservation research, especially in the aquatic environment and for rare and threatened species such as sawfishes. Yet, the field is still in its infancy with many potential approaches and analyses that are yet to be systematically explored. Over the duration of this PhD research, innovation in field sampling methods including consideration of the environmental variables that influence eDNA collection proficiency have been central to improvements in quantity and quality of the isolated eDNA. Future research that harnesses these developments are likely to produce significant outcomes for the usability and scalability of eDNA research. Likewise, emerging genomics approaches, sequencing technologies, and computational proficiencies are continually modifying and improving the way that eDNA data can be analysed. Here I propose how the exploration of these novel approaches will progress the availability of critical data for sawfishes and rare and threatened species more generally.

7.3.1. Scaled sampling

Sparse records of some species of sawfish in parts of their historic range, for example 1–2 landings per month of largetooth sawfish in Bangladesh where fishing effort is high (Haque et al., 2020), indicates that the likelihood of their detection in an eDNA sample collected at a single time-point is very low. The non-detection of sawfish in samples collected during a single survey event is thus not conclusive (Chapter 6) and warrants further investigation, specifically by revisiting these regions and completing more systematic and intense sampling, acknowledging the availability of time, resources, and budget.

There is great appeal in 'set-and-forget' autonomous eDNA sampling methodologies that circumvent the requirement for hands-on filtration, which was a limiting component to the usability of eDNA in turbid coastal and riverine environments (Chapter 3, 4 & 6). Most notably, sophisticated autonomous sampling devices equipped into autonomous underwater vehicles (AUVs) and fixed on moorings have been successfully deployed to collect eDNA (e.g., Environmental Sample Processor; ESP) (Hansen et al., 2020; Sepulveda, Birch, et al., 2020; Yamahara et al., 2019). At present, the ESP can perform filtration, storage, and *in situ* genetic identification remotely and autonomously, termed 'ecogenomic sensing', and when integrated into a glider or long-range AUV can take dozens of samples while moving across programmed transects for several days (Eriksen et al., 2001; Scholin, 2009). Fleets of AUVs equipped with ESPs could drastically improve spatial and temporal coverage at which samples are collected, enabling the generation of baseline and time-series data at a grand scale. Other less-sophisticated commercially available autosamplers (e.g., Smith-Root; https://www.smith-root.com/, Ocean Diagnostics; https://www.oceandiagnostics.com/) are also more appealing than hands-on collection and filtration and may be more economically viable for non-profit conservation organisations to scale up sampling for sawfish detection. More broadly,

automation is positioned to be the key to unlock the next revolution of the eDNA discipline if the engineering feats and the expense outlay are minimised.

At the opposite end of the spectrum, cheaper and simpler alternatives called passive eDNA samplers (PEDS; Bessey et al., 2021; Kirtane et al., 2020) show promise for the future of low-cost, low-effort eDNA sampling. PEDS consisting of hydrophilic, negatively charged filter membranes or adsorbent-filled sachets containing activated carbon have been recently shown as promising alternatives to active filtration (Bessey et al., 2021; Kirtane et al., 2020). Natural samplers such as sponges (Phylum Porifera) have also been explored as alternative strategies to passively sample for eDNA (Mariani et al., 2019). These passive approaches are touted as affordable and universal alternatives to the current approach and are especially promising in the context of large-scale sampling leveraging citizen scientists or non-eDNA experts (Biggs et al., 2015). Challenges surrounding the standardisation of these approaches are apparent and the utility for rare species eDNA is yet to be demonstrated.

Finally, tremendous promise exists in the use of lateral flow technology that has been adapted from point-of-care medical diagnostics, which circumvents the requirement for sample collection, extraction, amplification, and sequencing altogether, and can therefore be rapidly applied in the field for immediate detection (Doyle & Uthicke, 2021). Adaptation of the sawfish assays developed in Chapter 3 to suit lateral flow technology is an especially promising future research direction.

7.3.2. Estimating population size and structure

One of the most asked questions of the eDNA discipline is – "can you tell me how many individuals there are?". This question is also true of discussions regarding conservation and management of sawfishes or any rare species of conservation concern, where obtaining

estimates of abundance and monitoring trends in population size are a prerequisite. The ability to use eDNA concentrations, either gene copy number or sequence reads, to quantify species abundance as a proxy for biomass or abundance would greatly aid sawfish conservation efforts. Currently, fisheries-dependent and -independent data and local ecological knowledge surveys are used to infer the trends in sawfish population numbers (Grant et al., 2021b; Leeney, 2016; Leeney & Downing, 2016), but presently we have no accurate idea of population sizes for any sawfish species. Numerous studies have begun exploring the utility of eDNA quantification, using qPCR, digital-droplet PCR, or metabarcoding, as a means of estimating relative abundance or biomass (Andres et al., 2021; Baldigo et al., 2017; Deutschmann et al., 2019; Levi et al., 2019; Pochardt et al., 2020; Spear et al., 2021; Thomsen et al., 2016; Weltz et al., 2017). A recent review by Rourke et al., (2021) indicated that there was consistent support for the utility of the approach with 92% of the reviewed studies reporting positive correlations between eDNA concentrations or read counts and abundance and/or biomass. However, accuracy with regard to absolute abundance has been difficult to establish and it is apparent that correlative relationships are species-specific (Barnes et al., 2020; Rourke et al., 2021) and impacted by the combined effects of biotic and abiotic influences (Hansen et al., 2018; Klymus et al., 2015; Rourke et al., 2021), warranting further investigation in controlled and *in situ* experiments. Developments elsewhere in the discipline have promise for supporting with accurate quantitative estimation, for example hierarchical sampling design (Hänfling et al., 2016) and negative binomial regression modelling (Chambert et al., 2018), which also require further optimisation and validation.

An effective population size estimate derived from eDNA haplotype data could be more reliable than quantitative estimates based on eDNA concentrations or read counts (Baker et al., 2018; Deiner et al., 2021; Dugal et al., 2021; Sigsgaard et al., 2017, 2020; Stat et al., 2017; Yoshitake et al., 2021). For example, the recently described HaDeC-Seq analytical method

utilised mitochondrial *D-loop* sequences to characterise population haplotypes and determine population size of Pacific bluefin tuna *Thunnus orientalis* (Yoshitake et al., 2019, 2021). Sigsgaard et al., (2017) also used the *D-loop* marker to infer haplotype diversity, population structure, and effective female population size of whale sharks *Rhincodon typus* and validated this with results from conventional tissue-based analyses. In the context of sawfishes, an eDNA-based haplotype study that offers insights into population size, structure and therefore management units throughout their range would greatly enable management decisions (Feutry et al., 2015; Feutry et al., 2021; Phillips et al., 2017). This is especially promising given the paucity of available tissue samples for all species throughout their range and the challenges of capturing individuals for tank-based eDNA abundance validation studies or tissue sampling given their rarity and the risk of fishing mortality.

Further to this, more sophisticated population-level information might be obtained from eDNA-based research in the future by harnessing loci within nuclear DNA (Andres et al., 2021; Sigsgaard et al., 2020) and utilising single-molecular real-time sequencing technology (e.g., PacBio and Oxford Nanopore long-read sequencing; Doorenspleet et al., 2021; Garlapati et al., 2019) which sequences longer eDNA fragments. The possibility of retrieving longer fragments from eDNA, i.e., a whole mitochondrial genome, has been demonstrated (Deiner, Renshaw, et al., 2017; Doorenspleet et al., 2021), qualifying the prospect of mitogenome-wide population genetics assessments. Further to this, target capture hybridisation approaches that offer more sequence coverage than qPCR and are more specific than metabarcoding have been developed to capture whole mitochondrial genomes and thousands of informative fragments of the mitochondrial or nuclear genome (Jensen et al., 2021; Sigsgaard et al., 2020), including ultraconserved elements (UCEs) for phylogenetic analyses (Allio et al., 2020). Commercially available kit-based target capture protocols can be made-to-order for your target species, but a significant amount of optimisation and validation with eDNA samples is warranted given that

these protocols are presently only used with genomic DNA from tissue sampling. For sawfishes, significant effort would be required to generate sufficient high-quality genomic resources that are required as the template for the design of target capture probe sets. With rigourous trial and validation these approaches paired with other methodological and analytical developments (e.g., automated pipelines; Allio et al., 2020, and machine learning; Cordier et al., 2017; Frühe et al., 2021), obtaining information regarding but not limited to demographic history, selection, phylogenetic relationships, and sex-ratios from eDNA may become a reality.

7.3.3. Functional genomics

Environmental DNA research is poised to attain its full potential by leveraging the much higher-resolution and functional information contained within environmental RNA (eRNA; Sigsgaard et al., 2020; Tsuri et al., 2021; Yates et al., 2021; Veilleux et al., 2021). Biomonitoring using eRNA has one major advantage over eDNA, that is the ability to provide functional information about a community or population from translational signatures. The eRNA that an organism produces could be targeted using specific assays to detect different conspecific forms that regulate or relate to life-history stages, sexes, phenotypes, or physiological status (Yates et al., 2021). This field is remarkably underexplored probably due to the extra layers of technical challenges related to its collection and preservation, the lack of transcriptomic references libraries for most species, and the substantially higher infrastructure demands (Yates et al., 2021). However, for species that are rare or of conservation concern, the promise of eRNA is high. One such example for the applications of eRNA to sawfishes is to detect natal habitat either through the detection of reproductive translational signatures or neonatal age classes. Given the demonstration of natal philopatry sawfishes and the preferential use of shallow coastal habitats by young (Norton et al., 2012), eRNA detection data could assist

in identifying critical habitat for protection. The United States is presently the only nation with designated critical habitat for any of the sawfish species.

7.3.4. Fundamentals of eDNA ecology

Finally, before the full potential of eDNA can be realised, there remains many unanswered questions about the fundamental ecology of eDNA that need to be systematically explored with several potential validation and optimisation experiments. By nature of being a non-invasive detection technique, wherein the target species' does not need to be captured or sighted to report presence, it is impossible to verify contemporaneity or exact locality of the target taxa using eDNA. Depending on the various biotic and abiotic conditions of the environment (Andruszkiewicz et al., 2017; Barnes & Turner, 2016; Collins et al., 2018; Deiner & Altermatt, 2014; Deutschmann et al., 2019; Harrison et al., 2019; Salter, 2018), the temporal and spatial dynamics of eDNA can confound inference of recency or locality, respectively. Until such time that our knowledge of eDNA shedding and degradation rates in different water body types is well-informed and we can incorporate this with modelling that deals with errors and high stochasticity, it remains challenging to build fine-scale maps of species distribution based solely on eDNA. Evidence-based prediction of species distribution is a key component to conservation decision-making (Guisan et al., 2013). It is currently only reasonable that eDNA is used in applications to determine presence at a large spatial scale, but refinements to reliability and accuracy of eDNA methods and data processing tools (i.e., occupancy or process-based models; Burian et al., 2021) are anticipated to powerfully overcome challenges with evaluating the distribution of rare and threatened species that have limited occurrence data (McColl-Gausden et al., 2021; Neto et al., 2020; Strickland & Roberts, 2019). In this thesis, the detection of sawfish eDNA was used as an indicator of species occurrence, which is tremendously valuable in the context of the dire need for occurrence and distribution

information that has been difficult to attain by traditional means. However, in its current form eDNA-based detection may not be the principal tool but rather a complementary tool to guide fine-scale monitoring and management programs. There remains considerable scope to determine the physicochemical dynamics of eDNA in different ecosystems and for at least a representative species of a family or genera, which is important to decrease variability and error and improve the inferential scale (Hansen et al., 2018; Stewart, 2019).

7.4. Optimism and concern for the future of sawfishes

Globally, we are facing a growing list of threatened or extinct species, degradation of the environment, and uncertainty about the likelihood of success of conservation efforts with limited budgets, resources, and information. But without conservation action, it is certain that species and habitats will disappear. Localised extinctions of sawfishes have been documented and predicted (Dulvy et al., 2016; Everett et al., 2015; Yan et al., 2021) and their key habitats including mangrove forests and seagrass beds are under significant threat (Bunting et al., 2022; Short et al., 2011).

A few species come to mind that have received a disproportionate amount of conservation attention given their imperiled status. One example is the giant panda (*Ailuropoda melanoleuca*), which has suffered demographically at the hands of anthropogenic pressures predisposed by its habitat specialisation and reproductive constraints, was argued to be at an evolutionary "dead-end" with climate change and human activities driving it faster towards its inevitable extinction (Swaisgood et al., 2010; Zhang et al., 2007). The relative absence of genetic variation in the populations was also attributed to population decline and poor evolutionary potential (Zhang et al., 2007); however, extraordinary protection and habitat restoration measures have all but saved the species (Swaisgood et al., 2010, 2018). Compassion

and enthusiasm toward their conservation from the general public also indicates that there are potential wins to be had in saving a species through effective science communication. The genetic diversity of sawfishes is not generally consistent with the hypothesis of an evolutionary dead-end despite significant population declines of up to 90% (Chapman et al., 2011; Feutry et al., 2015; Green et al., 2018; Phillips et al., 2017); however, there are specific characteristics, namely habitat specificity, natal philopatry, long generation interval, and high susceptibility to lethal capture in fishing nets, that are contributing factors towards their extreme rarity and localised extinction throughout their range (Dulvy et al., 2016; Yan et al., 2021). The success of the smalltooth sawfish population rebound in Florida, U.S., is cause for optimism, owing to ESA (Endangered Species Act 2003; NMFS, 2009) mandated protection and recovery efforts, scientific advances in biological and ecological knowledge, and public education initiatives (Wiley & Brame, 2018). Elsewhere, particularly in data-poor nations such as those nations in the global tropics surveyed in this thesis, interventions that protect the remaining populations of sawfish must be fast-tracked. Here, there is an opportunity cost of earlier implementation of conservation and management actions that likely outweighs the further allocation of time and resources spent to amass knowledge (Bottrill et al., 2008), notwithstanding nuanced socio-ecological factors that are barriers to species and habitat protection that must be considered.

Conservation action is in the realm of decision makers and stakeholders but must be based on the best scientific evidence available at the time. In the past decade, an increased focus of scientific fieldwork on sawfishes (including but not limited to, Bonfil et al., 2018; Carlson & Simpfendorfer, 2015; Downing & Leeney, 2019; Fernandez-Carvalho et al., 2014; Graham et al., 2021; Grant, et al., 2021a, 2021b; Haque et al., 2020; Haque & Das, 2019; Haque et al., 2022; Hossain et al., 2015; Jabado et al., 2017, 2018; Leeney, 2017; Leeney & Downing, 2016; Lehman et al., 2020, 2022; Feitosa et al., 2017; Papastamatiou et al., 2020; Phillips et al., 2017; Tanna et al., 2021; Valerio-Vargas & Espinoza, 2019) has enabled the reconstruction

of long-term population trends and spatio-temporal occurrence. Collectively, this body of literature has assisted the evaluation of sawfish global status (Dulvy et al., 2016; Yan et al., 2021). Additionally, the rise of engagement of fishers and sawfish enthusiasts with social media platforms has offered tremendous value (Kroetz et al., 2021). The eDNA survey that was conducted across a vast geographic range in this PhD research produced preliminary presence/absence data where species presence records were sparse and the mobilisation of resources to deploy alternative detection methods was challenging. These data provide overlapping perspective on the contemporary spatio-temporal occurrence of sawfishes, but large gaps still exist that warrant subsequent detection and monitoring efforts.

There is considerable scope for eDNA-based research capacity building and long-term self-sufficiency for scientists in developing nations, minimising the dependence on external reseachers. To this end, the co-design and co-management of research and conservation projects as a lever to support stakeholder empowerment and address the gaps in knowledge cannot be overstated if we are to adequately face the global biodiversity crisis (Barber et al., 2014; Cazé et al., 2022; Stefanoudis et al., 2021) and specifically the risk of extinction to sawfishes in the wild.

7.5. References

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APPENDIX A: SUPPORTING INFORMATION FOR CHAPTER 6

Table A1. Environmental DNA sample metadata. Data are sorted by date. Dash indicates missing data.

Country	Region/Province	Site Name	Field replicate no.	Pore size (µm)	Filtrate vol. (L)	Date	Latitude	Longitude	Tide state	Depth (m)	Surface Temp (ºC)	Salinity (ppt)	Turbidity (m)	Collected by	Field Comment
Australia	Exmouth Gulf, Western Australia	Tent Island	TI1	10	2	10/05/2017	-22.01161	114.5395	Rising	1.2	24.1	-	1.0	Madalyn Cooper	
Australia	Exmouth Gulf, Western Australia	Tent Island	TI2	10	2	10/05/2017	-22.01161	114.5395	Rising	1.2	24.1	-	1.0	Madalyn Cooper	
Australia	Exmouth Gulf, Western Australia	Tent Island	TI3	10	2	10/05/2017	-22.01161	114.5395	Rising	1.2	24.1	-	1.0	Madalyn Cooper	
Australia	Exmouth Gulf, Western Australia	Tent Island	TI4	10	2	10/05/2017	-22.01161	114.5395	Rising	1.2	24.1	-	1.0	Madalyn Cooper	
Australia	Exmouth Gulf, Western Australia	Tent Island	TI5	10	2	10/05/2017	-22.01161	114.5395	Rising	1.2	24.1	-	1.0	Madalyn Cooper	
Australia	Exmouth Gulf, Western Australia	Tent Island	TI6	10	2	10/05/2017	-22.01161	114.5395	Rising	-	24.1	-	1.0	Madalyn Cooper	
Australia	Exmouth Gulf, Western Australia	Tent Island	TI7	10	2	10/05/2017	-22.01161	114.5395	Rising	-	24.1	-	1.0	Madalyn Cooper	
Australia	Exmouth Gulf, Western Australia	Tent Island	TI8	10	2	10/05/2017	-22.01161	114.5395	Rising	-	24.1	-	1.0	Madalyn Cooper	
Australia	Exmouth Gulf, Western Australia	Tent Island	TI9	10	2	10/05/2017	-22.01161	114.5395	Rising	-	24.1	-	1.0	Madalyn Cooper	
Australia	Exmouth Gulf, Western Australia	Tent Island	TI10	10	2	10/05/2017	-22.01161	114.5395	Rising high	-	24.1	-	1.0	Madalyn Cooper	
Australia	Exmouth Gulf, Western Australia	Tent Island	TI11	20	2	10/05/2017	-22.01161	114.5395	Rising high	-	24.1	-	1.0	Madalyn Cooper	
Australia	Exmouth Gulf, Western Australia	Tent Island	TI12	20	2	10/05/2017	-22.01161	114.5395	Rising high	-	24.1	-	1.0	Madalyn Cooper	
Australia	Exmouth Gulf, Western Australia	Tent Island	TI13	20	2	10/05/2017	-22.01161	114.5395	Rising high	-	24.1	-	1.0	Madalyn Cooper	
Australia	Exmouth Gulf, Western Australia	Tent Island	TI14	20	2	10/05/2017	-22.01161	114.5395	Rising high	-	24.1	-	1.0	Madalyn Cooper	
Australia	Exmouth Gulf, Western Australia	Tent Island	TI15	20	2	10/05/2017	-22.01161	114.5395	Rising high	-	24.1	-	1.0	Madalyn Cooper	
Australia	Exmouth Gulf, Western Australia	Tent Island	TI16	3	2	10/05/2017	-22.01161	114.5395	Rising high	-	24.1	-	1.0	Madalyn Cooper	
Australia	Exmouth Gulf, Western Australia	Tent Island	TI17	3	2	10/05/2017	-22.01161	114.5395	High	-	24.1	-	1.0	Madalyn Cooper	
Australia	Exmouth Gulf, Western Australia	Tent Island	TI18	3	2	10/05/2017	-22.01161	114.5395	High	-	24.1	-	1.0	Madalyn Cooper	
Australia	Exmouth Gulf, Western Australia	Tent Island	TI19	3	2	10/05/2017	-22.01161	114.5395	High	-	24.1	-	1.0	Madalyn Cooper	
Australia	Exmouth Gulf, Western Australia	Tent Island	TI20	3	2	10/05/2017	-22.01161	114.5395	High	-	24.1	-	1.0	Madalyn Cooper	
Australia	Exmouth Gulf, Western Australia	Sth. Of Turtle Creek	TC1	10	2	11/05/2017	-22.41021	114.1257	Rising	-	-	-	-	Madalyn Cooper	
Australia	Exmouth Gulf, Western Australia	Sth. Of Turtle Creek	TC2	10	2	11/05/2017	-22.41021	114.1257	Rising	-	-	-	-	Madalyn Cooper	
Australia	Exmouth Gulf, Western Australia	Sth. Of Turtle Creek	TC3	10	2	11/05/2017	-22.41021	114.1257	Rising	-	-	-	-	Madalyn Cooper	
Australia	Exmouth Gulf, Western Australia	Sth. Of Turtle Creek	TC4	10	2	11/05/2017	-22.41021	114.1257	Rising	-	-	-	-	Madalyn Cooper	
Australia	Exmouth Gulf, Western Australia	Sth. Of Turtle Creek	TC5	10	2	11/05/2017	-22.41021	114.1257	Rising	-	-	-	-	Madalyn Cooper	
Australia	Exmouth Gulf, Western Australia	Sth. Of Turtle Creek	TC6	10	2	11/05/2017	-22.41021	114.1257	Rising	-	-	-	-	Madalyn Cooper	
Australia	Exmouth Gulf, Western Australia	Sth. Of Turtle Creek	TC7	10	2	11/05/2017	-22.41021	114.1257	Rising	-	-	-	-	Madalyn Cooper	
Australia	Exmouth Gulf, Western Australia	Sth. Of Turtle Creek	TC8	10	2	11/05/2017	-22.41021	114.1257	Rising	-	-	-	-	Madalyn Cooper	

Australia	Exmouth Gulf, Western Australia	Sth. Of Turtle Creek	TC9	10	2	11/05/2017	-22.41021	114.1257	Rising	-	-	-	-	Madalyn Cooper	
Australia	Exmouth Gulf, Western Australia	Sth. Of Turtle Creek	TC10	10	2	11/05/2017	-22.41021	114.1257	Rising	1.1	19.5	-	1.1	Madalyn Cooper	
Australia	Exmouth Gulf, Western Australia	Sandalwood Landing Creek	SLC1	10	2	11/05/2017	-22.46796	114.2248	Falling	-	24.0	-	-	Madalyn Cooper	
Australia	Exmouth Gulf, Western Australia	Sandalwood Landing Creek	SLC2	10	2	11/05/2017	-22.46796	114.2248	Falling	-	24.0	-	-	Madalyn Cooper	
Australia	Exmouth Gulf, Western Australia	Sandalwood Landing Creek	SLC3	10	2	11/05/2017	-22.46796	114.2248	Falling	-	24.0	-	-	Madalyn Cooper	
Australia	Exmouth Gulf, Western Australia	Sandalwood Landing Creek	SLC4	10	2	11/05/2017	-22.46796	114.2248	Falling	-	24.0	-	-	Madalyn Cooper	
Australia	Exmouth Gulf, Western Australia	Sandalwood Landing Creek	SLC5	10	2	11/05/2017	-22.46796	114.2248	Falling	-	24.0	-	-	Madalyn Cooper	
Australia	Exmouth Gulf, Western Australia	Sandalwood Landing Creek	SLC6	10	2	11/05/2017	-22.46796	114.2248	Falling	-	24.0	-	-	Madalyn Cooper	
Australia	Exmouth Gulf, Western Australia	Sandalwood Landing Creek	SLC7	10	2	11/05/2017	-22.46796	114.2248	Falling	-	24.0	-	-	Madalyn Cooper	
Australia	Exmouth Gulf, Western Australia	Sandalwood Landing Creek	SLC8	10	2	11/05/2017	-22.46796	114.2248	Falling	-	24.0	-	-	Madalyn Cooper	
Australia	Exmouth Gulf, Western Australia	Sandalwood Landing Creek	SLC9	10	2	11/05/2017	-22.46796	114.2248	Falling	-	24.0	-	-	Madalyn Cooper	
Australia	Exmouth Gulf, Western Australia	Sandalwood Landing Creek	SLC10	10	2	11/05/2017	-22.46796	114.2248	Falling	-	24.0	-	-	Madalyn Cooper	
Australia	Exmouth Gulf, Western Australia	Turtle Creek	TC11	10	2	12/05/2017	-22.39911	114.1277	High	-	23.0	-	-	Madalyn Cooper	
Australia	Exmouth Gulf, Western Australia	Turtle Creek	TC12	10	2	12/05/2017	-22.39911	114.1277	High	-	23.0	-	-	Madalyn Cooper	
Australia	Exmouth Gulf, Western Australia	Turtle Creek	TC13	10	2	12/05/2017	-22.39911	114.1277	High	-	23.0	-	-	Madalyn Cooper	
Australia	Exmouth Gulf, Western Australia	Turtle Creek	TC14	10	2	12/05/2017	-22.39911	114.1277	High	-	23.0	-	-	Madalyn Cooper	
Australia	Exmouth Gulf, Western Australia	Turtle Creek	TC15	10	2	12/05/2017	-22.39911	114.1277	High	-	23.0	-	-	Madalyn Cooper	
Australia	Exmouth Gulf, Western Australia	Turtle Creek	TC16	10	2	12/05/2017	-22.39911	114.1277	High	-	23.0	-	-	Madalyn Cooper	
Australia	Exmouth Gulf, Western Australia	Turtle Creek	TC17	10	2	12/05/2017	-22.39911	114.1277	High	-	23.0	-	-	Madalyn Cooper	
Australia	Exmouth Gulf, Western Australia	Turtle Creek	TC18	10	2	12/05/2017	-22.39911	114.1277	High	-	23.0	-	-	Madalyn Cooper	
Australia	Exmouth Gulf, Western Australia	Turtle Creek	TC19	10	2	12/05/2017	-22.39911	114.1277	High	-	23.0	-	-	Madalyn Cooper	
Australia	Exmouth Gulf, Western Australia	Turtle Creek	TC20	10	2	12/05/2017	-22.39911	114.1277	High	-	23.0	-	-	Madalyn Cooper	
Australia	Exmouth Gulf, Western Australia	Turtle Creek Mouth	TC21	10	2	12/05/2017	-22.39966	114.1416	Falling	-	23.0	-	-	Madalyn Cooper	
Australia	Exmouth Gulf, Western Australia	Turtle Creek Mouth	TC22	10	2	12/05/2017	-22.39966	114.1416	Falling	-	23.0	-	-	Madalyn Cooper	
Australia	Exmouth Gulf, Western Australia	Turtle Creek Mouth	TC23	10	2	12/05/2017	-22.39966	114.1416	Falling	-	23.0	-	-	Madalyn Cooper	
Australia	Exmouth Gulf, Western Australia	Turtle Creek Mouth	TC24	10	2	12/05/2017	-22.39966	114.1416	Falling	-	23.0	-	-	Madalyn Cooper	
Australia	Exmouth Gulf, Western Australia	Turtle Creek Mouth	TC25	10	2	12/05/2017	-22.39966	114.1416	Falling	-	23.0	-	-	Madalyn Cooper	
Australia	Exmouth Gulf, Western Australia	Turtle Creek Mouth	TC26	10	2	12/05/2017	-22.39966	114.1416	Falling Low	-	23.0	-	-	Madalyn Cooper	
Australia	Exmouth Gulf, Western Australia	Turtle Creek Mouth	TC27	10	2	12/05/2017	-22.39966	114.1416	Falling Low	-	23.0	-	-	Madalyn Cooper	
Australia	Exmouth Gulf, Western Australia	Turtle Creek Mouth	TC28	10	2	12/05/2017	-22.39966	114.1416	Falling Low	-	23.0	-	-	Madalyn Cooper	
Australia	Exmouth Gulf, Western Australia	Turtle Creek Mouth	TC29	10	2	12/05/2017	-22.39966	114.1416	Falling Low	-	23.0	-	-	Madalyn Cooper	

Australia	Exmouth Gulf, Western Australia	Turtle Creek Mouth	TC30	10	2	12/05/2017	-22.39966	114.1416	Falling Low	-	23.0	-	-	Madalyn Cooper	
Australia	Exmouth Gulf, Western Australia	Exmouth Gulf East	EXE1	10	2	14/05/2017	-22.33922	114.3820	Rising Low	-	19.9	-	-	Madalyn Cooper	
Australia	Exmouth Gulf, Western Australia	Exmouth Gulf East	EXE2	10	2	14/05/2017	-22.33922	114.3820	Rising Low	-	19.9	-	-	Madalyn Cooper	
Australia	Exmouth Gulf, Western Australia	Exmouth Gulf East	EXE3	10	2	14/05/2017	-22.33922	114.3820	Rising Low	-	19.9	-	-	Madalyn Cooper	
Australia	Exmouth Gulf, Western Australia	Exmouth Gulf East	EXE4	10	2	14/05/2017	-22.33922	114.3820	Rising Low	-	19.9	-	-	Madalyn Cooper	
Australia	Exmouth Gulf, Western Australia	Exmouth Gulf East	EXE5	10	2	14/05/2017	-22.33922	114.3820	Rising Low	-	19.9	-	-	Madalyn Cooper	
Australia	Exmouth Gulf, Western Australia	Exmouth Gulf East	EXE6	10	2	14/05/2017	-22.33922	114.3820	Rising Low	-	19.9	-	-	Madalyn Cooper	
Australia	Exmouth Gulf, Western Australia	Exmouth Gulf East	EXE7	10	2	14/05/2017	-22.33922	114.3820	Rising Low	-	19.9	-	-	Madalyn Cooper	
Australia	Exmouth Gulf, Western Australia	Exmouth Gulf East	EXE8	10	2	14/05/2017	-22.33922	114.3820	Rising Low	1.2	19.9	-	1.2	Madalyn Cooper	
Australia	Exmouth Gulf, Western Australia	Exmouth Gulf East	EXE9	10	2	14/05/2017	-22.33922	114.3820	Rising Low	-	19.9	-	-	Madalyn Cooper	
Australia	Exmouth Gulf, Western Australia	Exmouth Gulf East	EXE10	10	2	14/05/2017	-22.33922	114.3820	Rising Low	-	19.9	-	-	Madalyn Cooper	
Australia	Exmouth Gulf, Western Australia	South Murion Island	MI1	10	2	15/05/2017	-21.67568	114.3372	Rising Low	-	23.7	-	-	Madalyn Cooper	
Australia	Exmouth Gulf, Western Australia	South Murion Island	MI2	10	2	15/05/2017	-21.67568	114.3372	Rising Low	-	23.7	-	-	Madalyn Cooper	
Australia	Exmouth Gulf, Western Australia	South Murion Island	MI3	10	2	15/05/2017	-21.67568	114.3372	Rising Low	-	23.7	-	-	Madalyn Cooper	
Australia	Exmouth Gulf, Western Australia	South Murion Island	MI4	10	2	15/05/2017	-21.67568	114.3372	Rising Low	-	23.7	-	-	Madalyn Cooper	
Australia	Exmouth Gulf, Western Australia	South Murion Island	MI5	10	2	15/05/2017	-21.67568	114.3372	Rising Low	-	23.7	-	-	Madalyn Cooper	
Australia	Exmouth Gulf, Western Australia	South Murion Island	MI6	10	2	15/05/2017	-21.67568	114.3372	Rising Low	-	23.7	-	-	Madalyn Cooper	
Australia	Exmouth Gulf, Western Australia	South Murion Island	MI7	10	2	15/05/2017	-21.67568	114.3372	Rising Low	-	23.7	-	-	Madalyn Cooper	
Australia	Exmouth Gulf, Western Australia	South Murion Island	MI8	10	2	15/05/2017	-21.67568	114.3372	Rising Low	-	23.7	-	-	Madalyn Cooper	
Australia	Exmouth Gulf, Western Australia	South Murion Island	MI9	10	2	15/05/2017	-21.67568	114.3372	Rising Low	1.5	23.7	-	1.5	Madalyn Cooper	
Australia	Exmouth Gulf, Western Australia	South Murion Island	MI10	10	2.5	15/05/2017	-21.67568	114.3372	Rising Low	-	23.7	-		Madalyn Cooper	
Papua New Guinea	East Sepik Province, Momase	Ramu River Mouth	R1	10	-	16/09/2017	-4.01859	144.6669	-	-	30.7	2.5	0.3	Michael Grant	
Papua New Guinea	East Sepik Province, Momase	Ramu River Mouth	R2	10	-	16/09/2017	-4.01892	144.6521	-	-	30.7	-	0.3	Michael Grant	
Papua New Guinea	East Sepik Province, Momase	Ramu River Mouth	R3	10	-	16/09/2017	-4.01892	144.6521	-	-	30.7	-	0.3	Michael Grant	
Papua New Guinea	East Sepik Province, Momase	Ramu River Mouth	R4	10	-	16/09/2017	-4.01892	144.6521	-	-	30.7	-	0.3	Michael Grant	
Papua New Guinea	East Sepik Province, Momase	Ramu River Mouth	R5	10	-	16/09/2017	-4.01892	144.6521	-	-	30.7	-	0.3	Michael Grant	
Papua New Guinea	East Sepik Province, Momase	Murik Lakes	M1	10	-	17/09/2017	-3.78389	144.2681	-	-	29.0	-	0.1	Michael Grant	
Papua New Guinea	East Sepik Province, Momase	Murik Lakes	M2	10	-	17/09/2017	-3.78411	144.2673	-	-	29.0	-	0.1	Michael Grant	
Papua New Guinea	East Sepik Province, Momase	Murik Lakes	M3	10	-	17/09/2017	-3.78424	144.2682	-	-	29.0	-	0.1	Michael Grant	
Papua New Guinea	East Sepik Province, Momase	Murik Lakes	M4	10	-	17/09/2017	-3.78450	144.2685	-	-	29.0	-	0.1	Michael Grant	
Papua New Guinea	East Sepik Province, Momase	Mindimbid Village	M1	10	-	20/09/2017	-4.26822	143.4107	-	-	-	-	-	Michael Grant	

Papua New Guinea	East Sepik Province, Momase	Mindimbid Village	M2	10	-	20/09/2017	-4.26822	143.4107	-	-	-	-	-	Michael Grant	
Papua New Guinea	East Sepik Province, Momase	Mindimbid Village	M3	10	-	20/09/2017	-4.26822	143.4107	-	-	-	-	-	Michael Grant	
Papua New Guinea	East Sepik Province, Momase	Mindimbid Village	M4	10	-	20/09/2017	-4.26822	143.4107	-	-	-	-	-	Michael Grant	
Papua New Guinea	East Sepik Province, Momase	Keram River Mouth	KM1	10	-	21/09/2017	-4.08700	144.0678		-	29.3	-	0.2	Michael Grant	
Papua New Guinea	East Sepik Province, Momase	Keram River Mouth	KM2	10	-	21/09/2017	-4.08700	144.0678		-	29.3	-	0.2	Michael Grant	
Papua New Guinea	East Sepik Province, Momase	Keram River Mouth	KM3	10	-	21/09/2017	-4.08700	144.0678		-	29.3	-	0.2	Michael Grant	
Papua New Guinea	East Sepik Province, Momase	Keram River Mouth	KM4	10	-	21/09/2017	-4.08700	144.0678		-	29.3	-	0.2	Michael Grant	
Papua New Guinea	East Sepik Province, Momase	Keram River Mouth	KM5	10	-	21/09/2017	-4.08700	144.0678		-	29.3	-	0.2	Michael Grant	
Papua New Guinea	East Sepik Province, Momase	Tawai Village	T1	10	-	21/09/2017	-4.03092	144.2472	-	-	-	-	-	Michael Grant	
Papua New Guinea	East Sepik Province, Momase	Tawai Village	T2	10	-	21/09/2017	-4.03092	144.2472	-	-	-	-	-	Michael Grant	
Papua New Guinea	East Sepik Province, Momase	Tawai Village	T3	10	-	21/09/2017	-4.03092	144.2472	-	-	-	-	-	Michael Grant	
Papua New Guinea	East Sepik Province, Momase	Tawai Village	T4	10	-	21/09/2017	-4.03092	144.2472	-	-	-	-	-	Michael Grant	
Papua New Guinea	East Sepik Province, Momase	Tawai Village	T5	10	-	21/09/2017	-4.03092	144.2472	-	-	-	-	-	Michael Grant	
Papua New Guinea	East Sepik Province, Momase	Chambri Lake	C1	10	-	24/09/2017	-4.27620	143.1473	-	-	-	-	-	Michael Grant	
Papua New Guinea	East Sepik Province, Momase	Chambri Lake	C2	10	-	24/09/2017	-4.27620	143.1473	-	-	-	-	-	Michael Grant	
Papua New Guinea	East Sepik Province, Momase	Chambri Lake	C3	10	-	24/09/2017	-4.27620	143.1473	-	-	-	-	-	Michael Grant	
Papua New Guinea	East Sepik Province, Momase	Chambri Lake	C4	10	-	24/09/2017	-4.27620	143.1473	-	-	-	-	-	Michael Grant	
Papua New Guinea	East Sepik Province, Momase	Chambri Lake	C5	10	-	24/09/2017	-4.27620	143.1473	-	-	-	-	-	Michael Grant	
Papua New Guinea	East Sepik Province, Momase	Chambri Canal (East)	CC1	10	-	24/09/2017	-4.46444	143.2923	-	-	-	-	-	Michael Grant	
Papua New Guinea	East Sepik Province, Momase	Chambri Canal (East)	CC2	10	-	24/09/2017	-4.46444	143.2923	-	-	-	-	-	Michael Grant	
Papua New Guinea	East Sepik Province, Momase	Chambri Canal (East)	CC3	10	-	24/09/2017	-4.46444	143.2923	-	-	-	-	-	Michael Grant	
Papua New Guinea	East Sepik Province, Momase	Chambri Canal (East)	CC4	10	-	24/09/2017	-4.46444	143.2923	-	-	-	-	-	Michael Grant	
Papua New Guinea	East Sepik Province, Momase	Chambri Canal (East)	CC5	10	-	24/09/2017	-4.46444	143.2923	-	-	-	-	-	Michael Grant	
Papua New Guinea	East Sepik Province, Momase	Chambri Canal (opening to Sepik mainstem)	CM1	5	-	24/09/2017	-4.41444	143.8440	-	-	-	-	-	Michael Grant	
Papua New Guinea	Western Province, South Western	Sibidiri	S1	10	-	26/11/2017	-8.95231	142.2353	-	-	-	-	-	Michael Grant	
Papua New Guinea	Western Province, South Western	Sibidiri	S2	10	-	26/11/2017	-8.95231	142.2353	-	-	-	-	-	Michael Grant	
Papua New Guinea	Western Province, South Western	Sibidiri	S3	10	-	26/11/2017	-8.95231	142.2353	-	-	-	-	-	Michael Grant	
Papua New Guinea	Western Province, South Western	Sibidiri	S4	10	-	26/11/2017	-8.95231	142.2353	-	-	-	-	-	Michael Grant	
Papua New Guinea	Western Province, South Western	Sibidiri	S5	10	-	26/11/2017	-8.95231	142.2353	-	-	-	-	-	Michael Grant	
Papua New Guinea	Western Province, South Western	Oriomo River (middle)	OM1	10	-	26/11/2017	-8.91942	143.2190	-	-	-	-	-	Michael Grant	

Papua New Guinea	Western Province, South Western	Oriomo River (middle)	OM2	10	-	26/11/2017	-8.91942	143.2190	-	-	-	-	-	Michael Grant	
Papua New Guinea	Western Province, South Western	Oriomo River (middle)	OM3	10	-	26/11/2017	-8.91942	143.2190	-	-	-	-	-	Michael Grant	
Papua New Guinea	Western Province, South Western	Oriomo River (middle)	OM4	10	-	26/11/2017	-8.91942	143.2190	-	-	-	-	-	Michael Grant	
Papua New Guinea	Western Province, South Western	Oriomo River	OR1	10	-	26/11/2017	-8.98601	143.2352	-	-	-	-	-	Michael Grant	
Papua New Guinea	Western Province, South Western	Oriomo River	OR2	10	-	26/11/2017	-8.98601	143.2352	-	-	-	-	-	Michael Grant	
Papua New Guinea	Western Province, South Western	Oriomo River	OR3	10	-	26/11/2017	-8.98601	143.2352	-	-	-	-	-	Michael Grant	
Papua New Guinea	Western Province, South Western	Oriomo River	OR4	10	-	26/11/2017	-8.98601	143.2352	-	-	-	-	-	Michael Grant	
Papua New Guinea	Western Province, South Western	Oriomo River	OR5	10	-	26/11/2017	-8.98601	143.2352	-	-	-	-	-	Michael Grant	
Papua New Guinea	Western Province, South Western	Mia Kussa Mouth	MM1	10	-	30/11/2017	-9.13600	142.2024	-	-	-	-	-	Michael Grant	
Papua New Guinea	Western Province, South Western	Geawi River	G1	10	-	5/12/2017	-8.93875	143.4014	-	-	-	-	-	Michael Grant	
Papua New Guinea	Western Province, South Western	Geawi River	G2	10	-	5/12/2017	-8.93875	143.4014	-	-	-	-	-	Michael Grant	
Papua New Guinea	Western Province, South Western	Geawi River	G3	10	-	5/12/2017	-8.93875	143.4014	-	-	-	-	-	Michael Grant	
Papua New Guinea	Western Province, South Western	Geawi River	G4	10	-	5/12/2017	-8.93875	143.4014	-	-	-	-	-	Michael Grant	
Papua New Guinea	Western Province, South Western	Geawi River	G5	10	-	5/12/2017	-8.93875	143.4014	-	-	-	-	-	Michael Grant	
Papua New Guinea	Western Province, South Western	Oriomo River (fresh)	OF1	10	-	7/12/2017	-8.80216	143.1337	-	-	-	-	-	Michael Grant	
Papua New Guinea	Western Province, South Western	Oriomo River (fresh)	OF2	10	-	7/12/2017	-8.80216	143.1337	-	-	-	-	-	Michael Grant	
Papua New Guinea	Western Province, South Western	Oriomo River (fresh)	OF3	10	-	7/12/2017	-8.80216	143.1337	-	-	-	-	-	Michael Grant	
Papua New Guinea	Western Province, South Western	Oriomo River (fresh)	OF4	10	-	7/12/2017	-8.80216	143.1337	-	-	-	-	-	Michael Grant	
Papua New Guinea	Western Province, South Western	Oriomo River (fresh)	OF5	10	-	7/12/2017	-8.80216	143.1337	-	-	-	-	-	Michael Grant	
Australia	Kakadu, Northern Territory	Adelaide River (marine)	1.1	20	4	11/12/2017	-12.26593	131.2833	Incoming	3.4	-	-	High	Madalyn Cooper	
Australia	Kakadu, Northern Territory	Adelaide River (marine)	1.2	20	5	11/12/2017	-12.26250	131.2825	Incoming	1.2	-	-	High	Madalyn Cooper	
Australia	Kakadu, Northern Territory	Adelaide River (marine)	1.3	20	3	11/12/2017	-12.26476	131.2764	Incoming	2.2	-	-	High	Madalyn Cooper	
Australia	Kakadu, Northern Territory	Adelaide River (receiver site)	2.1	20	1	11/12/2017	-12.42678	131.3015	-	1.5	-	-	High	Madalyn Cooper	
Australia	Kakadu, Northern Territory	Adelaide River (receiver site)	2.2	20	1	11/12/2017	-12.42651	131.3054	-	2.0	-	-	High	Madalyn Cooper	
Australia	Kakadu, Northern Territory	Adelaide River (receiver site)	2.3	20	1	11/12/2017	-12.42444	131.3070	-	1.4	-	-	High	Madalyn Cooper	
Australia	Darwin, Northern Territory	East Arm Darwin Harbour - Middle Island	3.1	20	8	12/12/2017	-12.61757	130.9469	-	1.1	-	-	High	Madalyn Cooper	
Australia	Darwin, Northern Territory	East Arm Darwin Harbour - Middle Island	3.2	20	4	12/12/2017	-12.61884	130.9941	-	1.2	-	-	High	Madalyn Cooper	
Australia	Darwin, Northern Territory	East Arm Darwin Harbour - Middle Island.	3.3	20	4	12/12/2017	-12.61415	130.9427	-	0.7	-	-	High	Madalyn Cooper	
Australia	Darwin, Northern Territory	East Arm Darwin Harbour - Main Channel, Sand Island	4.1	20	1	12/12/2017	-12.59342	130.8680	-	0.4	-	-	High	Madalyn Cooper	

Australia	Darwin, Northern Territory	East Arm Darwin Harbour - Main Channel, Sand Island	4.2	20	1	12/12/2017	-12.59068	130.8696	-	0.5	-	-	High	Madalyn Cooper	
Australia	Darwin, Northern Territory	East Arm Darwin Harbour - Main Channel, Sand Island	4.3	20	1	12/12/2017	-12.58363	130.8653	-	1.3	-	-	High	Madalyn Cooper	
Australia	Darwin, Northern Territory	Mickets Creek - Shoal Bay	5.1	5	1	12/12/2017	-12.33960	130.9449	-	0.5	-	-	High	Madalyn Cooper	
Australia	Darwin, Northern Territory	Mickets Creek - Shoal Bay	5.2	5	1	12/12/2017	-12.33789	130.9473	-	0.6	-	-	High	Madalyn Cooper	
Australia	Darwin, Northern Territory	Mickets Creek - Shoal Bay	5.3	5	1	12/12/2017	-12.34050	130.9497	-	0.9	-	-	High	Madalyn Cooper	
Australia	Darwin, Northern Territory	Casuarina Beach	6.1	5	0.5	12/12/2017	-12.33902	130.8833	-	0.4	-	-	High	Madalyn Cooper	
Australia	Darwin, Northern Territory	Casuarina Beach	6.2	5	0.5	12/12/2017	-12.35625	130.8666	-	0.5	-	-	High	Madalyn Cooper	
Australia	Darwin, Northern Territory	Casuarina Beach	6.3	5	0.5	12/12/2017	-12.36885	130.8586	-	0.5	-	-	High	Madalyn Cooper	
Australia	Kakadu, Northern Territory	Adelaide River (freshwater)	7.1	20	4	13/12/2017	-12.68184	131.3346	-	1.0	-	-	High	Madalyn Cooper	
Australia	Kakadu, Northern Territory	Adelaide River (freshwater)	7.2	20	4	13/12/2017	-12.68260	131.3340	-	0.5	-	-	High	Madalyn Cooper	
Australia	Kakadu, Northern Territory	Adelaide River (freshwater)	7.3	20	4	13/12/2017	-12.68318	131.3331	-	0.5	-	-	High	Madalyn Cooper	
Australia	Kakadu, Northern Territory	South Alligator River - 'Round the World'	8.1	20	0.6	14/12/2017	-12.61425	132.4502	-	1.3	-	10.0	High	Madalyn Cooper	
Australia	Kakadu, Northern Territory	South Alligator River - 'Round the World'	8.2	20	1	14/12/2017	-12.61544	132.4463	-	1.0	-	10.0	High	Madalyn Cooper	
Australia	Kakadu, Northern Territory	South Alligator River - 'Round the World'	8.3	20	0.6	14/12/2017	-12.60762	132.4419	-	0.5	-	10.0	High	Madalyn Cooper	
Australia	Kakadu, Northern Territory	South Alligator River - 'Clavata Point'	9.1	20	2	14/12/2017	-12.52319	132.4122	-	1.0	-	-	High	Madalyn Cooper	
Australia	Kakadu, Northern Territory	South Alligator River - 'Clavata Point'	9.2	20	2	14/12/2017	-12.52221	132.4112	-	0.4	-	-	High	Madalyn Cooper	
Australia	Kakadu, Northern Territory	South Alligator River - 'Clavata Point'	9.3	20	2	14/12/2017	-12.51998	132.4136	-	0.5	-	-	High	Madalyn Cooper	
Australia	Kakadu, Northern Territory	South Alligator River (equidistant from 2 previous)	10.1	20	0.6	14/12/2017	-12.45810	132.4208	-	0.5	-	-	High	Madalyn Cooper	
Australia	Kakadu, Northern Territory	South Alligator River (equidistant from 2 previous)	10.2	20	1.5	14/12/2017	-12.46031	132.4217	-	0.4	-	-	High	Madalyn Cooper	
Australia	Kakadu, Northern Territory	South Alligator River (equidistant from 2 previous)	10.3	20	0.5	14/12/2017	-12.46192	132.4329	-	0.4	-	-	High	Madalyn Cooper	
Australia	Kakadu, Northern Territory	South Alligator River - mainstem	11.1	20	1	14/12/2017	-12.38384	132.3715	-	0.5	-	-	High	Madalyn Cooper	
Australia	Kakadu, Northern Territory	South Alligator River - mainstem	11.2	20	0.5	14/12/2017	-12.37737	132.3706	-	0.7	-	-	High	Madalyn Cooper	

Australia	Kakadu, Northern Territory	South Alligator River - mainstem	11.3	20	0.5	14/12/2017	-12.37343	132.3174	-	0.4	-	-	High	Madalyn Cooper	
Australia	Kakadu, Northern Territory	Mud Island	12.1	20	5	14/12/2017	-12.22595	132.3777	-	0.7	-	-	High	Madalyn Cooper	
Australia	Kakadu, Northern Territory	Mud Island	12.2	20	6	14/12/2017	-12.22753	132.3826	-	0.4	-	-	High	Madalyn Cooper	
Australia	Kakadu, Northern Territory	Mud Island	12.3	20	4	14/12/2017	-12.21922	132.3706	-	0.6	-	-	High	Madalyn Cooper	
Australia	Kakadu, Northern Territory	Brooke's Creek	13.1	20	1.5	14/12/2017	-12.20428	132.4150	-	0.5	-	-	High	Madalyn Cooper	
Australia	Kakadu, Northern Territory	Brooke's Creek	13.2	20	5	14/12/2017	-12.21007	132.4156	-	0.8	-	-	High	Madalyn Cooper	
Australia	Kakadu, Northern Territory	Brooke's Creek	13.3	20	0.3	14/12/2017	-12.20158	132.4204	-	0.6	-	-	High	Madalyn Cooper	
Australia	Kakadu, Northern Territory	Midnight Point	14.1	10	1	14/12/2017	-12.15751	132.4356	-	0.5	-	-	High	Madalyn Cooper	
Australia	Kakadu, Northern Territory	Midnight Point	14.2	10	1	14/12/2017	-12.15267	132.4363	-	0.8	-	-	High	Madalyn Cooper	
Australia	Kakadu, Northern Territory	Midnight Point	14.3	10	0.1	14/12/2017	-12.14911	132.4379	-	0.8	-	-	High	Madalyn Cooper	
Australia	Kakadu, Northern Territory	Field Island	15.1	10	2	14/12/2017	-12.13409	132.3762	-	0.5	-	-	High	Madalyn Cooper	
Australia	Kakadu, Northern Territory	Field Island	15.2	10	1	14/12/2017	-12.13452	132.3789	-	0.4	-	-	High	Madalyn Cooper	
Australia	Kakadu, Northern Territory	Field Island	15.3	10	2	14/12/2017	-12.13856	132.3840	-	0.4	-	-	High	Madalyn Cooper	
Papua New Guinea	Gulf Province, South Western	Vailala River	V1	10	-	15/12/2017	-7.94303	145.4129	-	-	-	-	-	Michael Grant	
Papua New Guinea	Gulf Province, South Western	Vailala River	V2	10	-	15/12/2017	-7.94303	145.4129	-	-	-	-	-	Michael Grant	
Papua New Guinea	Gulf Province, South Western	Vailala River	V3	10	-	15/12/2017	-7.94303	145.4129	-	-	-	-	-	Michael Grant	
Papua New Guinea	Gulf Province, South Western	Vailala River	V4	10	-	15/12/2017	-7.94303	145.4129	-	-	-	-	-	Michael Grant	
Papua New Guinea	Gulf Province, South Western	Vailala River	V4	10	-	15/12/2017	-7.94303	145.4129	-	-	-	-	-	Michael Grant	
Australia	Kakadu, Northern Territory	West Alligator River (East mud banks)	16.1	20	2	15/12/2017	-12.21320	132.3114	-	0.8	-	-	High	Madalyn Cooper	
Australia	Kakadu, Northern Territory	West Alligator River (East mud banks)	16.2	20	0.2	15/12/2017	-12.21209	132.3153	-	0.5	-	-	High	Madalyn Cooper	
Australia	Kakadu, Northern Territory	West Alligator River (East mud banks)	16.3	20	2	15/12/2017	-12.21388	132.3067	-	0.5	-	-	High	Madalyn Cooper	
Australia	Kakadu, Northern Territory	West Alligator River Head	17.1	20	2	15/12/2017	-12.18979	132.2659	-	0.4	-	-	High	Madalyn Cooper	
Australia	Kakadu, Northern Territory	West Alligator River Head	17.2	20	7	15/12/2017	-12.18990	132.2630	-	0.4	-	-	High	Madalyn Cooper	
Australia	Kakadu, Northern Territory	West Alligator River Head	17.3	20	6	15/12/2017	-12.18594	132.2646	-	0.5	-	-	High	Madalyn Cooper	
Australia	Kakadu, Northern Territory	West Alligator River Mouth	18.1	20	0.3	15/12/2017	-12.23241	132.2851	-	0.4	-	-	High	Madalyn Cooper	
Australia	Kakadu, Northern Territory	West Alligator River Mouth	18.2	20	1.5	15/12/2017	-12.23405	132.2786	-	0.4	-	-	High	Madalyn Cooper	
Australia	Kakadu, Northern Territory	West Alligator River Mouth	18.3	20	1.5	15/12/2017	-12.23843	132.2773	-	0.7	-	-	High	Madalyn Cooper	
Australia	Kakadu, Northern Territory	West Alligator River (6 km upstream)	19.1	20	2	15/12/2017	-12.28952	132.2591	-	0.5	-	-	High	Madalyn Cooper	

Australia	Kakadu, Northern Territory	West Alligator River (6 km upstream)	19.2	20	0.5	15/12/2017	-12.28755	132.2568	-	0.9	-	-	High	Madalyn Cooper	
Australia	Kakadu, Northern Territory	West Alligator River (6 km upstream)	19.3	20	1.5	15/12/2017	-12.29107	132.2558	-	0.9	-	-	High	Madalyn Cooper	
Australia	Kakadu, Northern Territory	West Alligator River (12 km upstream)	20.1	20	1	15/12/2017	-12.33067	132.2325	-	0.7	-	-	High	Madalyn Cooper	
Australia	Kakadu, Northern Territory	West Alligator River (12 km upstream)	20.2	20	2	15/12/2017	-12.33370	132.2262	-	0.4	-	-	High	Madalyn Cooper	
Australia	Kakadu, Northern Territory	West Alligator River (12 km upstream)	20.3	20	4	15/12/2017	-12.33406	132.2275	-	0.7	-	-	High	Madalyn Cooper	
Papua New Guinea	Gulf Province, South Western	Lelefiru River	L1	10	-	16/12/2017	-8.09231	146.0656	-	-	-	-	-	Michael Grant	
Papua New Guinea	Gulf Province, South Western	Lelefiru River	L2	10	-	16/12/2017	-8.09231	146.0656	-	-	-	-	-	Michael Grant	
Papua New Guinea	Gulf Province, South Western	Lelefiru River	L3	10	-	16/12/2017	-8.09231	146.0656	-	-	-	-	-	Michael Grant	
Papua New Guinea	Gulf Province, South Western	Lelefiru River	L4	10	-	16/12/2017	-8.09231	146.0656	-	-	-	-	-	Michael Grant	
Papua New Guinea	Gulf Province, South Western	Lelefiru River	L5	10	-	16/12/2017	-8.09231	146.0656	-	-	-	-	-	Michael Grant	
Australia	Townsville, Queensland	Ross River Flats 1	1.1	5	4.5	15/03/2018	-19.26510	146.8400	-	-	-	-	-	Madalyn Cooper	
Australia	Townsville, Queensland	Ross River Flats 1	1.2	5	4.9	15/03/2018	-19.26491	146.8466	-	-	-	-	-	Madalyn Cooper	
Australia	Townsville, Queensland	Ross River Flats 1	1.3	5	5	15/03/2018	-19.26507	146.8469	-	-	-	-	-	Madalyn Cooper	
Australia	Townsville, Queensland	Ross River Flats 1	1.4	5	5	15/03/2018	-19.26485	146.8466	-	-	-	-	-	Madalyn Cooper	
Australia	Townsville, Queensland	Ross River Flats 1	1.5	5	5	15/03/2018	-19.26476	146.8462	-	-	-	-	-	Madalyn Cooper	
Australia	Townsville, Queensland	Ross River Flats 2	2.1	5	3	15/03/2018	-19.26871	146.8576	-	-	-	-	-	Madalyn Cooper	
Australia	Townsville, Queensland	Ross River Flats 2	2.1	5	2.8	15/03/2018	-19.26824	146.8578	-	-	-	-	-	Madalyn Cooper	
Australia	Townsville, Queensland	Ross River Flats 2	2.1	5	3	15/03/2018	-19.26810	146.8578	-	-	-	-	-	Madalyn Cooper	
Australia	Townsville, Queensland	Ross River Flats 2	2.1	5	6.5	15/03/2018	-19.27680	146.8578	-	-	-	-	-	Madalyn Cooper	
Australia	Townsville, Queensland	Ross River Flats 2	2.1	5	6	15/03/2018	-19.26725	146.8578	-	-	-	-	-	Madalyn Cooper	
Australia	Townsville, Queensland	Ross River Flats 3	3.1	5	3	15/03/2018	-19.27527	146.8691	-	-	-	-	-	Madalyn Cooper	
Australia	Townsville, Queensland	Ross River Flats 3	3.2	5	3	15/03/2018	-19.27488	146.8691	-	-	-	-	-	Madalyn Cooper	
Australia	Townsville, Queensland	Ross River Flats 3	3.3	5	3	15/03/2018	-19.27479	146.8591	-	-	-	-	-	Madalyn Cooper	
Australia	Townsville, Queensland	Ross River Flats 3	3.4	5	2.8	15/03/2018	-19.27461	146.8691	-	-	-	-	-	Madalyn Cooper	
Australia	Townsville, Queensland	Ross River Flats 3	3.5	5	3	15/03/2018	-19.27461	146.8691	-	-	-	-	-	Madalyn Cooper	
Australia	Townsville, Queensland	Ross River Flats 4	4.1	5	3	15/03/2018	-19.28584	146.8794	-	-	-	-	-	Madalyn Cooper	
Australia	Townsville, Queensland	Ross River Flats 4	4.1	5	3	15/03/2018	-19.28584	146.8794	-	-	-	-	-	Madalyn Cooper	
Australia	Townsville, Queensland	Ross River Flats 4	4.1	5	4	15/03/2018	-19.28584	146.8794	-	-	-	-	-	Madalyn Cooper	
Australia	Townsville, Queensland	Ross River Flats 4	4.1	5	4	15/03/2018	-19.28584	146.8793	-	-	-	-	-	Madalyn Cooper	

Australia	Townsville, Queensland	Ross River Flats 4	4.1	5	4	15/03/2018	-19.28582	146.8792	-	-	-	-	-	Madalyn Cooper	
United States	Tampa Bay, Florida	Alafia River	1.1	5	2	27/03/2018	27.86025	-82.38554	-	-	-	-	-	Madalyn Cooper	
United States	Tampa Bay, Florida	Alafia River	1.2	10	2	27/03/2018	27.86025	-82.38554	-	-	-	-	-	Madalyn Cooper	
United States	Tampa Bay, Florida	Alafia River	1.3	10	1	27/03/2018	27.86025	-82.38554	-	-	-	-	-	Madalyn Cooper	
United States	Tampa Bay, Florida	Alafia River	1.4	10	1	27/03/2018	27.86025	-82.38554	-	-	-	-	-	Madalyn Cooper	
United States	Tampa Bay, Florida	Alafia River	1.5	10	1	27/03/2018	27.86025	-82.38554	-	-	-	-	-	Madalyn Cooper	
United States	Tampa Bay, Florida	Apollo Beach Nature Park	2.1	5	2	27/03/2018	27.79249	-82.41884	-	-	-	-	-	Madalyn Cooper	
United States	Tampa Bay, Florida	Apollo Beach Nature Park	2.2	5	2	27/03/2018	27.79249	-82.41884	-	-	-	-	-	Madalyn Cooper	
United States	Tampa Bay, Florida	Apollo Beach Nature Park	2.3	5	2	27/03/2018	27.79249	-82.41884	-	-	-	-	-	Madalyn Cooper	
United States	Tampa Bay, Florida	Apollo Beach Nature Park	2.4	5	2	27/03/2018	27.79249	-82.41884	-	-	-	-	-	Madalyn Cooper	
United States	Tampa Bay, Florida	Apollo Beach Nature Park	2.5	5	2	27/03/2018	27.79249	-82.41884	-	-	-	-	-	Madalyn Cooper	
United States	Tampa Bay, Florida	Cockroach Bay	3.1	5	2	27/03/2018	27.68689	-82.52037	-	-	-	-	-	Madalyn Cooper	
United States	Tampa Bay, Florida	Cockroach Bay	3.2	5	1.5	27/03/2018	27.68689	-82.52037	-	-	-	-	-	Madalyn Cooper	
United States	Tampa Bay, Florida	Cockroach Bay	3.3	5	2	27/03/2018	27.68689	-82.52037	-	-	-	-	-	Madalyn Cooper	
United States	Tampa Bay, Florida	Cockroach Bay	3.4	5	2	27/03/2018	27.68689	-82.52037	-	-	-	-	-	Madalyn Cooper	
United States	Tampa Bay, Florida	Cockroach Bay	3.5	5	2	27/03/2018	27.68689	-82.52037	-	-	-	-	-	Madalyn Cooper	
United States	Tampa Bay, Florida	State Road 64 Boat Ramp	4.1	5	1	27/03/2018	27.49615	-82.52552	-	-	-	-	-	Madalyn Cooper	
United States	Tampa Bay, Florida	State Road 64 Boat Ramp	4.2	5	2	27/03/2018	27.49615	-82.52552	-	-	-	-	-	Madalyn Cooper	
United States	Tampa Bay, Florida	State Road 64 Boat Ramp	4.3	5	2	27/03/2018	27.49615	-82.52552	-	-	-	-	-	Madalyn Cooper	
United States	Tampa Bay, Florida	State Road 64 Boat Ramp	4.4	5	2	27/03/2018	27.49615	-82.52552	-	-	-	-	-	Madalyn Cooper	
United States	Tampa Bay, Florida	State Road 64 Boat Ramp	4.5	5	2	27/03/2018	27.49615	-82.52552	-	-	-	-	-	Madalyn Cooper	
United States	Tampa Bay, Florida	5120 Beacon Road (Tonya's House)	5.1	5	2	27/03/2018	27.56100	-82.57137	-	-	-	-	-	Madalyn Cooper	
United States	Tampa Bay, Florida	5120 Beacon Road (Tonya's House)	5.2	5	1	27/03/2018	27.56100	-82.57137	-	-	-	-	-	Madalyn Cooper	
United States	Tampa Bay, Florida	5120 Beacon Road (Tonya's House)	5.3	5	1.5	27/03/2018	27.56100	-82.57137	-	-	-	-	-	Madalyn Cooper	
United States	Tampa Bay, Florida	5120 Beacon Road (Tonya's House)	5.4	5	1	27/03/2018	27.56100	-82.57137	-	-	-	-	-	Madalyn Cooper	
United States	Tampa Bay, Florida	5120 Beacon Road (Tonya's House)	5.5	5	1.25	27/03/2018	27.56100	-82.57137	-	-	-	-	-	Madalyn Cooper	
United States	Caloosahatchee River, Florida	Sanibel Causeway boat ramp, San Carlos Bay	6.1	5	2	28/03/2018	26.45365	-82.03566	-	-	-	-	-	Madalyn Cooper	
United States	Caloosahatchee River, Florida	Sanibel Causeway boat ramp, San Carlos Bay	6.2	5	1.5	28/03/2018	26.45365	-82.03566	-	-	-	-	-	Madalyn Cooper	
United States	Caloosahatchee River, Florida	Sanibel Causeway boat ramp, San Carlos Bay	6.3	5	2	28/03/2018	26.45365	-82.03566	-	-	-	-	-	Madalyn Cooper	

United States	Caloosahatchee River, Florida	Sanibel Causeway boat ramp, San Carlos Bay	6.4	5	2	28/03/2018	26.45365	-82.03566	-	-	-	-	-	Madalyn Cooper	
United States	Caloosahatchee River, Florida	Sanibel Causeway boat ramp, San Carlos Bay	6.5	5	2	28/03/2018	26.45365	-82.03566	-	-	-	-	-	Madalyn Cooper	
United States	Caloosahatchee River, Florida	Cape Coral Yacht Club boat ramp, Caloosahatchee River	7.1	5	2	28/03/2018	26.54255	-81.95244	-	-	-	-	-	Madalyn Cooper	
United States	Caloosahatchee River, Florida	Cape Coral Yacht Club boat ramp, Caloosahatchee River	7.2	5	2	28/03/2018	26.54255	-81.95244	-	-	-	-	-	Madalyn Cooper	
United States	Caloosahatchee River, Florida	Cape Coral Yacht Club boat ramp, Caloosahatchee River	7.3	5	1.75	28/03/2018	26.54255	-81.95244	-	-	-	-	-	Madalyn Cooper	
United States	Caloosahatchee River, Florida	Cape Coral Yacht Club boat ramp, Caloosahatchee River	7.4	5	2	28/03/2018	26.54255	-81.95244	-	-	-	-	-	Madalyn Cooper	
United States	Caloosahatchee River, Florida	Cape Coral Yacht Club boat ramp, Caloosahatchee River	7.5	5	2	28/03/2018	26.54255	-81.95244	-	-	-	-	-	Madalyn Cooper	
United States	Caloosahatchee River, Florida	Horton Park boat ramp, Cape Coral, Caloosahatchee River	8.1	5	1.75	28/03/2018	26.60756	-81.91343	-	-	-	-	-	Madalyn Cooper	
United States	Caloosahatchee River, Florida	Horton Park boat ramp, Cape Coral, Caloosahatchee River	8.2	5	1.75	28/03/2018	26.60756	-81.91343	-	-	-	-	-	Madalyn Cooper	
United States	Caloosahatchee River, Florida	Horton Park boat ramp, Cape Coral, Caloosahatchee River	8.3	5	2	28/03/2018	26.60756	-81.91343	-	-	-	-	-	Madalyn Cooper	
United States	Caloosahatchee River, Florida	Horton Park boat ramp, Cape Coral, Caloosahatchee River	8.4	5	2	28/03/2018	26.60756	-81.91343	-	-	-	-	-	Madalyn Cooper	
United States	Caloosahatchee River, Florida	Horton Park boat ramp, Cape Coral, Caloosahatchee River	8.5	5	1.75	28/03/2018	26.60756	-81.91343	-	-	-	-	-	Madalyn Cooper	
United States	Caloosahatchee River, Florida	Centennial Park boat ramp, Fort Myers, Caloosahatchee River	9.1	5	2	28/03/2018	26.64635	-81.87257	-	-	-	-	-	Madalyn Cooper	

United States	Caloosahatchee River, Florida	Centennial Park boat ramp, Fort Myers, Caloosahatchee River	9.2	5	2	28/03/2018	26.64635	-81.87257	-	-	-	-	-	Madalyn Cooper	
United States	Caloosahatchee River, Florida	Centennial Park boat ramp, Fort Myers, Caloosahatchee River	9.3	5	1.5	28/03/2018	26.64635	-81.87257	-	-	-	-	-	Madalyn Cooper	
United States	Caloosahatchee River, Florida	Centennial Park boat ramp, Fort Myers, Caloosahatchee River	9.4	5	2	28/03/2018	26.64635	-81.87257	-	-	-	-	-	Madalyn Cooper	
United States	Caloosahatchee River, Florida	Centennial Park boat ramp, Fort Myers, Caloosahatchee River	9.5	5	2	28/03/2018	26.64635	-81.87257	-	-	-	-	-	Madalyn Cooper	
United States	Charlotte Harbour, Florida	Laishley Park, Peace River	10.1	5	1.25	28/03/2018	26.64632	-81.87260	-	-	-	-	-	Madalyn Cooper	
United States	Charlotte Harbour, Florida	Laishley Park, Peace River	10.2	5	0.5	28/03/2018	26.64632	-81.87260	-	-	-	-	-	Madalyn Cooper	
United States	Charlotte Harbour, Florida	Laishley Park, Peace River	10.3	5	0.5	28/03/2018	26.64632	-81.87260	-	-	-	-	-	Madalyn Cooper	
United States	Charlotte Harbour, Florida	Laishley Park, Peace River	10.4	10	1.2	28/03/2018	26.64632	-81.87260	-	-	-	-	-	Madalyn Cooper	
United States	Charlotte Harbour, Florida	Laishley Park, Peace River	10.5	10	1	28/03/2018	26.64632	-81.87260	-	-	-	-	-	Madalyn Cooper	
United States	Ten Thousand Islands National Wildlife Reserve, Florida	Goodland Outer Island	11.1	20	5	31/03/2018	25.88822	-81.43087		-	24.6	36.3	0.6	Madalyn Cooper	
United States	Ten Thousand Islands National Wildlife Reserve, Florida	Goodland Outer Island	11.2	20	5	31/03/2018	25.88822	-81.43087	-	-	-	-	-	Madalyn Cooper	
United States	Ten Thousand Islands National Wildlife Reserve, Florida	Goodland Outer Island	11.3	20	5	31/03/2018	25.88822	-81.43087	-	-	-	-	-	Madalyn Cooper	
United States	Ten Thousand Islands National Wildlife Reserve, Florida	Goodland Outer Island	11.4	20	5	31/03/2018	25.88822	-81.43087	-	-	-	-	-	Madalyn Cooper	
United States	Ten Thousand Islands National Wildlife Reserve, Florida	Goodland Outer Island	11.5	20	5	31/03/2018	25.88822	-81.43087	-	-	-	-	-	Madalyn Cooper	
United States	Ten Thousand Islands National Wildlife Reserve, Florida	Stop Keys	12.1	10	5	31/03/2018	25.81307	-81.47900		-	24.7	35.5	1.0	Madalyn Cooper	
United States	Ten Thousand Islands National Wildlife Reserve, Florida	Stop Keys	12.2	10	5	31/03/2018	25.81307	-81.47900	-	-	-	-	-	Madalyn Cooper	
United States	Ten Thousand Islands National Wildlife Reserve, Florida	Stop Keys	12.3	10	5	31/03/2018	25.81307	-81.47900	-	-	-	-	-	Madalyn Cooper	
United States	Ten Thousand Islands National Wildlife Reserve, Florida	Stop Keys	12.4	10	5	31/03/2018	25.81307	-81.47900	-	-	-	-	-	Madalyn Cooper	
United States	Ten Thousand Islands National Wildlife Reserve, Florida	Stop Keys	12.5	10	5	31/03/2018	25.81307	-81.47900	-	-	-	-	-	Madalyn Cooper	
United States	Ten Thousand Islands National Wildlife Reserve, Florida	Grocery Creek	13.1	20	5	31/03/2018	25.95108	-81.61483		-	25.8	37.1	0.4	Madalyn Cooper	

United States	Ten Thousand Islands National Wildlife Reserve, Florida	Grocery Creek	13.2	20	5	31/03/2018	25.95108	-81.61483	-	-	-	-	-	Madalyn Cooper	
United States	Ten Thousand Islands National Wildlife Reserve, Florida	Grocery Creek	13.3	20	5	31/03/2018	25.95108	-81.61483	-	-	-	-	-	Madalyn Cooper	
United States	Ten Thousand Islands National Wildlife Reserve, Florida	Grocery Creek	13.4	20	5	31/03/2018	25.95108	-81.61483	-	-	-	-	-	Madalyn Cooper	
United States	Ten Thousand Islands National Wildlife Reserve, Florida	Grocery Creek	13.5	20	5	31/03/2018	25.95108	-81.61483	-	-	-	-	-	Madalyn Cooper	
United States	Ten Thousand Islands National Wildlife Reserve, Florida	Faka Union Bay	14.1	10	4	31/03/2018	25.89863	-81.51713		-	24.9	35.1	0.8	Madalyn Cooper	
United States	Ten Thousand Islands National Wildlife Reserve, Florida	Faka Union Bay	14.2	1.2	1	31/03/2018	25.89863	-81.51713	-	-	-	-	-	Madalyn Cooper	
United States	Ten Thousand Islands National Wildlife Reserve, Florida	Faka Union Bay	14.3	10	4	31/03/2018	25.89863	-81.51713	-	-	-	-	-	Madalyn Cooper	
United States	Ten Thousand Islands National Wildlife Reserve, Florida	Faka Union Bay	14.4	10	4	31/03/2018	25.89863	-81.51713	-	-	-	-	-	Madalyn Cooper	
United States	Ten Thousand Islands National Wildlife Reserve, Florida	Faka Union Bay	14.5	10	4	31/03/2018	25.89863	-81.51713	-	-	-	-	-	Madalyn Cooper	
United States	Ten Thousand Islands National Wildlife Reserve, Florida	Faka Union Bay	14.6	10	4	31/03/2018	25.89863	-81.51713	-	-	-	-	-	Madalyn Cooper	
United States	Ten Thousand Islands National Wildlife Reserve, Florida	Kingston Key	15.1	5	3	1/04/2018	25.80322	-81.45647		0.7	28.7	35.9		Madalyn Cooper	
United States	Ten Thousand Islands National Wildlife Reserve, Florida	Kingston Key	15.2	10	5	1/04/2018	25.80322	-81.45647	-	-	-	-	-	Madalyn Cooper	
United States	Ten Thousand Islands National Wildlife Reserve, Florida	Kingston Key	15.3	10	5	1/04/2018	25.80322	-81.45647	-	-	-	-	-	Madalyn Cooper	
United States	Ten Thousand Islands National Wildlife Reserve, Florida	Kingston Key	15.4	1.2	1	1/04/2018	25.80322	-81.45647	-	-	-	-	-	Madalyn Cooper	
United States	Ten Thousand Islands National Wildlife Reserve, Florida	Kingston Key	15.5	10	5	1/04/2018	25.80322	-81.45647	-	-	-	-	-	Madalyn Cooper	
United States	Ten Thousand Islands National Wildlife Reserve, Florida	Kingston Key	15.6	10	5	1/04/2018	25.80322	-81.45647	-	-	-	-	-	Madalyn Cooper	
United States	Ten Thousand Islands National Wildlife Reserve, Florida	Kingston Key	15.7	10	5	1/04/2018	25.80322	-81.45647	-	-	-	-	-	Madalyn Cooper	
United States	Ten Thousand Islands National Wildlife Reserve, Florida	Stop Key	16.1	10	5	1/04/2018	25.81497	-81.45647		1.1	26.6	36.1		Madalyn Cooper	
United States	Ten Thousand Islands National Wildlife Reserve, Florida	Stop Key	16.2	10	5	1/04/2018	25.81497	-81.45647	-	-	-	-	-	Madalyn Cooper	
United States	Ten Thousand Islands National Wildlife Reserve, Florida	Stop Key	16.3	10	5	1/04/2018	25.81497	-81.45647	-	-	-	-	-	Madalyn Cooper	
United States	Ten Thousand Islands National Wildlife Reserve, Florida	Stop Key	16.4	1.2	0.5	1/04/2018	25.81497	-81.45647	-	-	-	-	-	Madalyn Cooper	

United States	Ten Thousand Islands National Wildlife Reserve, Florida	Stop Key	16.5	10	5	1/04/2018	25.81497	-81.45647	-	-	-	-	-	Madalyn Cooper	
United States	Ten Thousand Islands National Wildlife Reserve, Florida	Stop Key	16.6	10	5	1/04/2018	25.81497	-81.45647	-	-	-	-	-	Madalyn Cooper	
United States	Ten Thousand Islands National Wildlife Reserve, Florida	Panther Key	17.1	10	5	1/04/2018	25.85417	-81.54740		-	26.6	36.2		Madalyn Cooper	
United States	Ten Thousand Islands National Wildlife Reserve, Florida	Panther Key	17.2	10	5	1/04/2018	25.85417	-81.54740	-	-	-	-	-	Madalyn Cooper	
United States	Ten Thousand Islands National Wildlife Reserve, Florida	Panther Key	17.3	1.2	1	1/04/2018	25.85417	-81.54740	-	-	-	-	-	Madalyn Cooper	
United States	Ten Thousand Islands National Wildlife Reserve, Florida	Panther Key	17.4	10	5	1/04/2018	25.85417	-81.54740	-	-	-	-	-	Madalyn Cooper	
United States	Ten Thousand Islands National Wildlife Reserve, Florida	Panther Key	17.5	10	5	1/04/2018	25.85417	-81.54740	-	-	-	-	-	Madalyn Cooper	
United States	Ten Thousand Islands National Wildlife Reserve, Florida	Panther Key	17.6	10	5	1/04/2018	25.85417	-81.54740	-	-	-	-	-	Madalyn Cooper	
United States	Chokoloskee Bay, Florida	Causeway, Chokoloskee Island	18.1	5	0.5	1/04/2018	25.82748	-81.36375	-	0.2	-	-	-	Madalyn Cooper	20+ juvenile smalltooth sawfish within sight of sampling location
United States	Chokoloskee Bay, Florida	Causeway, Chokoloskee Island	18.2	10	2	1/04/2018	25.82748	-81.36375	-	-	-	-	-	Madalyn Cooper	20+ juvenile smalltooth sawfish within sight of sampling location
United States	Chokoloskee Bay, Florida	Causeway, Chokoloskee Island	18.3	10	2	1/04/2018	25.82748	-81.36375	-	-	-	-	-	Madalyn Cooper	20+ juvenile smalltooth sawfish within sight of sampling location
United States	Chokoloskee Bay, Florida	Causeway, Chokoloskee Island	18.4	10	2	1/04/2018	25.82748	-81.36375	-	-	-	-	-	Madalyn Cooper	20+ juvenile smalltooth sawfish within sight of sampling location
United States	Chokoloskee Bay, Florida	Causeway, Chokoloskee Island	18.5	1.2	1	1/04/2018	25.82748	-81.36375	-	-	-	-	-	Madalyn Cooper	20+ juvenile smalltooth sawfish within sight of sampling location
United States	Chokoloskee Bay, Florida	Causeway, Chokoloskee Island	18.6	10	1.5	1/04/2018	25.82748	-81.36375	-	-	-	-	-	Madalyn Cooper	20+ juvenile smalltooth sawfish within sight of sampling location
United States	Chokoloskee Bay, Florida	Causeway, Chokoloskee Island	18.7	10	1.5	1/04/2018	25.82748	-81.36375	-	-	-	-	-	Madalyn Cooper	20+ juvenile smalltooth sawfish within sight of sampling location
United States	Chokoloskee Bay, Florida	Causeway, Chokoloskee Island	18.8	20	2	1/04/2018	25.82748	-81.36375	-	-	-	-	-	Madalyn Cooper	20+ juvenile smalltooth sawfish within sight of sampling location
United States	Chokoloskee Bay, Florida	Causeway, Chokoloskee Island	19.1	10	1.5	1/04/2018	25.82748	-81.36375		0.2	25.9	35.6		Madalyn Cooper	20+ juvenile smalltooth sawfish within sight of sampling location
United States	Chokoloskee Bay, Florida	Causeway, Chokoloskee Island	19.2	10	3	1/04/2018	25.82748	-81.36375	-	-	-	-	-	Madalyn Cooper	20+ juvenile smalltooth sawfish within sight of sampling location
United States	Chokoloskee Bay, Florida	Causeway, Chokoloskee Island	19.3	10	3	1/04/2018	25.82748	-81.36375	-	-	-	-	-	Madalyn Cooper	20+ juvenile smalltooth sawfish within sight of sampling location
United States	Chokoloskee Bay, Florida	Causeway, Chokoloskee Island	19.4	10	3	1/04/2018	25.82748	-81.36375	-	-	-	-	-	Madalyn Cooper	20+ juvenile smalltooth sawfish within sight of sampling location
United States	Chokoloskee Bay, Florida	Causeway, Chokoloskee Island	19.6	10	4	1/04/2018	25.82748	-81.36375	-	-	-	-	-	Madalyn Cooper	20+ juvenile smalltooth sawfish within sight of sampling location

United States	Chokoloskee Bay, Florida	Causeway, Chokoloskee Island	19.5	1.2	1	2/04/2018	25.82748	-81.36375	-	-	-	-	-	Madalyn Cooper	20+ juvenile smalltooth sawfish within sight of sampling location
Australia	Townsville, Queensland	Toomulla Beach	1.6	5	1	8/11/2018	-19.07530	146.4683	Rising	0.5	-	-	-	Madalyn Cooper, Colin Simpfendorfer, Melissa Joyce	
Australia	Townsville, Queensland	Toomulla Beach	1.1	10	1	8/11/2018	-19.07530	146.4683	Rising	0.5	-	-	-	Madalyn Cooper, Colin Simpfendorfer, Melissa Joyce	
Australia	Townsville, Queensland	Toomulla Beach	1.2	10	1	8/11/2018	-19.07530	146.4683	Rising	0.5	-	-	-	Madalyn Cooper, Colin Simpfendorfer, Melissa Joyce	
Australia	Townsville, Queensland	Toomulla Beach	1.3	10	1	8/11/2018	-19.07530	146.4683	Rising	0.5	-	-	-	Madalyn Cooper, Colin Simpfendorfer, Melissa Joyce	
Australia	Townsville, Queensland	Toomulla Beach	1.4	10	1	8/11/2018	-19.07530	146.4683	Rising	0.5	-	-	-	Madalyn Cooper, Colin Simpfendorfer, Melissa Joyce	
Australia	Townsville, Queensland	Toomulla Beach	1.5	10	1	8/11/2018	-19.07530	146.4683	Rising	0.5	-	-	-	Madalyn Cooper, Colin Simpfendorfer, Melissa Joyce	
Australia	Townsville, Queensland	Toomulla Beach boat ramp	2.6	20	1	8/11/2018	-19.08364	146.4767	Rising	0.5	-	-	-	Madalyn Cooper, Colin Simpfendorfer, Melissa Joyce	
Australia	Townsville, Queensland	Toomulla Beach boat ramp	2.1	10	1	8/11/2018	-19.08364	146.4767	Rising	0.5	-	-	-	Madalyn Cooper, Colin Simpfendorfer, Melissa Joyce	
Australia	Townsville, Queensland	Toomulla Beach boat ramp	2.2	10	1	8/11/2018	-19.08364	146.4767	Rising	0.5	-	-	-	Madalyn Cooper, Colin Simpfendorfer, Melissa Joyce	
Australia	Townsville, Queensland	Toomulla Beach boat ramp	2.3	10	1	8/11/2018	-19.08364	146.4767	Rising	0.5	-	-	-	Madalyn Cooper, Colin Simpfendorfer, Melissa Joyce	
Australia	Townsville, Queensland	Toomulla Beach boat ramp	2.4	10	1	8/11/2018	-19.08364	146.4767	Rising	0.5	-	-	-	Madalyn Cooper, Colin Simpfendorfer, Melissa Joyce	
Australia	Townsville, Queensland	Toomulla Beach boat ramp	2.5	10	1	8/11/2018	-19.08364	146.4767	Rising	0.5	-	-	-	Madalyn Cooper, Colin Simpfendorfer, Melissa Joyce	
Australia	Townsville, Queensland	Toolakea Beach	3.1	10	1	8/11/2018	-19.14569	146.5753	Rising	0.5	-	-	-	Madalyn Cooper, Colin Simpfendorfer, Melissa Joyce	
Australia	Townsville, Queensland	Toolakea Beach	3.2	10	1	8/11/2018	-19.14569	146.5753	Rising	0.5	-	-	-	Madalyn Cooper, Colin Simpfendorfer, Melissa Joyce	
Australia	Townsville, Queensland	Toolakea Beach	3.3	10	1	8/11/2018	-19.14569	146.5753	Rising	0.5	-	-	-	Madalyn Cooper, Colin Simpfendorfer, Melissa Joyce	
Australia	Townsville, Queensland	Toolakea Beach	3.4	10	1	8/11/2018	-19.14569	146.5753	Rising	0.5	-	-	-	Madalyn Cooper, Colin Simpfendorfer, Melissa Joyce	
Australia	Townsville, Queensland	Toolakea Beach	3.5	10	1	8/11/2018	-19.14569	146.5753	Rising	0.5	-	-	-	Madalyn Cooper, Colin Simpfendorfer, Melissa Joyce	
Australia	Townsville, Queensland	Saunders Beach	4.1	10	1	8/11/2018	-19.15339	146.6044	High	0.8	-	-	-	Madalyn Cooper, Colin Simpfendorfer, Melissa Joyce	
Australia	Townsville, Queensland	Saunders Beach	4.2	10	1	8/11/2018	-19.15339	146.6044	High	0.8	-	-	-	Madalyn Cooper, Colin Simpfendorfer, Melissa Joyce	
Australia	Townsville, Queensland	Saunders Beach	4.3	10	1	8/11/2018	-19.15339	146.6044	High	0.8	-	-	-	Madalyn Cooper, Colin Simpfendorfer, Melissa Joyce	

Australia	Townsville, Queensland	Saunders Beach	4.4	10	1	8/11/2018	-19.15339	146.6044	High	0.8	-	-	-	Madalyn Cooper, Colin Simpfendorfer, Melissa Joyce	
Australia	Townsville, Queensland	Saunders Beach	4.5	10	1	8/11/2018	-19.15339	146.6044	High	0.8	-	-	-	Madalyn Cooper, Colin Simpfendorfer, Melissa Joyce	
Australia	Townsville, Queensland	Saunders Beach	4.6	5	1	8/11/2018	-19.15339	146.6044	High	0.8	-	-	-	Madalyn Cooper, Colin Simpfendorfer, Melissa Joyce	
Australia	Townsville, Queensland	Althaus River Mouth	5.1	5	1	8/11/2018	-19.15259	146.6030	High	0.7	-	-	-	Madalyn Cooper, Colin Simpfendorfer, Melissa Joyce	
Australia	Townsville, Queensland	Althaus River Mouth	5.2	5	1	8/11/2018	-19.15259	146.6030	High	0.7	-	-	-	Madalyn Cooper, Colin Simpfendorfer, Melissa Joyce	
Australia	Townsville, Queensland	Althaus River Mouth	5.3	5	1	8/11/2018	-19.15259	146.6030	High	0.7	-	-	-	Madalyn Cooper, Colin Simpfendorfer, Melissa Joyce	
Australia	Townsville, Queensland	Althaus River Mouth	5.4	5	1	8/11/2018	-19.15259	146.6030	High	0.7	-	-	-	Madalyn Cooper, Colin Simpfendorfer, Melissa Joyce	
Australia	Townsville, Queensland	Althaus River Mouth	5.5	5	1	8/11/2018	-19.15259	146.6030	High	0.7	-	-	-	Madalyn Cooper, Colin Simpfendorfer, Melissa Joyce	
Australia	Townsville, Queensland	Beach Holm	6.1	5	1	8/11/2018	-19.17985	146.6533	High	0.5	-	-	-	Madalyn Cooper, Colin Simpfendorfer, Melissa Joyce	
Australia	Townsville, Queensland	Beach Holm	6.2	5	1	8/11/2018	-19.17985	146.6533	High	0.5	-	-	-	Madalyn Cooper, Colin Simpfendorfer, Melissa Joyce	
Australia	Townsville, Queensland	Beach Holm	6.3	5	1	8/11/2018	-19.17985	146.6533	High	0.5	-	-	-	Madalyn Cooper, Colin Simpfendorfer, Melissa Joyce	
Australia	Townsville, Queensland	Beach Holm	6.4	5	1	8/11/2018	-19.17985	146.6533	High	0.5	-	-	-	Madalyn Cooper, Colin Simpfendorfer, Melissa Joyce	
Australia	Townsville, Queensland	Beach Holm	6.5	5	1	8/11/2018	-19.17985	146.6533	High	0.5	-	-	-	Madalyn Cooper, Colin Simpfendorfer, Melissa Joyce	
Australia	Queensland	Baffle group Islands, Norman River	1.1	20	-	21/11/2018	-17.62372	141.0040	Incoming	1	33.6	18.4	0.57	Barbara Wueringer	
Australia	Queensland	Baffle group Islands, Norman River	1.2	20	-	21/11/2018	-17.62372	141.0040	Incoming	1	33.6	18.4	0.57	Barbara Wueringer	
Australia	Queensland	Baffle group Islands, Norman River	1.3	20	-	21/11/2018	-17.62372	141.0040	Incoming	1	33.6	18.4	0.57	Barbara Wueringer	
Australia	Queensland	Baffle group Islands, Norman River	1.4	20	-	21/11/2018	-17.62372	141.0040	Incoming	1	33.6	18.4	0.57	Barbara Wueringer	
Australia	Queensland	Baffle group Islands, Norman River	1.5	20	-	21/11/2018	-17.62372	141.0040	Incoming	1	33.6	18.4	0.57	Barbara Wueringer	
Australia	Queensland	Charlie's land, Norman River	2.1	20	-	21/11/2018	-17.65807	141.0546	Incoming	0.8	33.2	16.5	0.57	Barbara Wueringer	
Australia	Queensland	Charlie's land, Norman River	2.2	20	-	21/11/2018	-17.65807	141.0546	Incoming	0.8	33.2	16.5	0.57	Barbara Wueringer	
Australia	Queensland	Charlie's land, Norman River	2.3	20	-	21/11/2018	-17.65807	141.0546	Incoming	0.8	33.2	16.5	0.57	Barbara Wueringer	
Australia	Queensland	Charlie's land, Norman River	2.4	20	-	21/11/2018	-17.65807	141.0546	Incoming	0.8	33.2	16.5	0.57	Barbara Wueringer	

Australia	Queensland	Charlie's land, Norman River	2.5	20	-	21/11/2018	-17.65807	141.0546	Incoming	0.8	33.2	16.5	0.57	Barbara Wueringer	
Australia	Queensland	Karumba beach, Norman river	3.1	10	-	21/11/2018	-17.47747	140.8398	Incoming	1.2	32.2	36	-	Barbara Wueringer	
Australia	Queensland	Karumba beach, Norman river	3.2	20	-	21/11/2018	-17.47747	140.8398	Incoming	1.2	32.2	36	-	Barbara Wueringer	
Australia	Queensland	Karumba beach, Norman river	3.3	20	-	21/11/2018	-17.47747	140.8398	Incoming	1.2	32.2	36	-	Barbara Wueringer	
Australia	Queensland	Karumba beach, Norman river	3.4	20	-	21/11/2018	-17.47747	140.8398	Incoming	1.2	32.2	36	-	Barbara Wueringer	
Australia	Queensland	Karumba beach, Norman river	3.5	20	-	21/11/2018	-17.47747	140.8398	Incoming	1.2	32.2	36	-	Barbara Wueringer	
Costa Rica	Southern Pacific	Terraba-Sierpe National Wetlands	1.1	10	6	28/01/2019	8.89769	-83.60652	high tide	6.7	29.7	NA	3	Jorge V, Mario, Marta, Mariel and Esteban	
Costa Rica	Southern Pacific	Terraba-Sierpe National Wetlands	1.2	10	5	28/01/2019	8.89769	-83.60652	high tide	6.7	29.7	NA	3	Jorge V, Mario, Marta, Mariel and Esteban	
Costa Rica	Southern Pacific	Terraba-Sierpe National Wetlands	1.3	10	5	28/01/2019	8.89769	-83.60652	high tide	6.7	29.7	NA	3	Jorge V, Mario, Marta, Mariel and Esteban	
Costa Rica	Southern Pacific	Terraba-Sierpe National Wetlands	1.4	10	5	28/01/2019	8.89769	-83.60652	high tide	6.7	29.7	NA	3	Jorge V, Mario, Marta, Mariel and Esteban	
Costa Rica	Southern Pacific	Terraba-Sierpe National Wetlands	1.5	10	5	28/01/2019	8.89769	-83.60652	high tide	6.7	29.7	NA	3	Jorge V, Mario, Marta, Mariel and Esteban	
Costa Rica	Southern Pacific	Terraba-Sierpe National Wetlands	2.1	20	3.5	28/01/2019	8.88526	-83.58542	Falling	6.4	29.9	NA	1.5	Jorge V, Mario, Marta, Mariel and Esteban	
Costa Rica	Southern Pacific	Terraba-Sierpe National Wetlands	2.2	20	3.8	28/01/2019	8.88526	-83.58542	Falling	6.4	29.9	NA	1.5	Jorge V, Mario, Marta, Mariel and Esteban	
Costa Rica	Southern Pacific	Terraba-Sierpe National Wetlands	2.3	20	5	28/01/2019	8.88526	-83.58542	Falling	6.4	29.9	NA	1.5	Jorge V, Mario, Marta, Mariel and Esteban	
Costa Rica	Southern Pacific	Terraba-Sierpe National Wetlands	2.4	20	5.2	28/01/2019	8.88526	-83.58542	Falling	6.4	29.9	NA	1.5	Jorge V, Mario, Marta, Mariel and Esteban	
Costa Rica	Southern Pacific	Terraba-Sierpe National Wetlands	2.5	20	5	28/01/2019	8.88526	-83.58542	Falling	6.4	29.9	NA	1.5	Jorge V, Mario, Marta, Mariel and Esteban	
Costa Rica	Southern Pacific	Terraba-Sierpe National Wetlands	3.1	10	1.5	28/01/2019	8.87626	-83.55611	Falling	5.2	30.3	NA	1.25	Jorge V, Mario, Marta, Mariel and Esteban	
Costa Rica	Southern Pacific	Terraba-Sierpe National Wetlands	3.2	20	5	28/01/2019	8.87626	-83.55611	Falling	5.2	30.3	NA	1.25	Jorge V, Mario, Marta, Mariel and Esteban	
Costa Rica	Southern Pacific	Terraba-Sierpe National Wetlands	3.3	20	5	28/01/2019	8.87626	-83.55611	Falling	5.2	30.3	NA	1.25	Jorge V, Mario, Marta, Mariel and Esteban	
Costa Rica	Southern Pacific	Terraba-Sierpe National Wetlands	3.4	20	5	28/01/2019	8.87626	-83.55611	Falling	5.2	30.3	NA	1.25	Jorge V, Mario, Marta, Mariel and Esteban	
Costa Rica	Southern Pacific	Terraba-Sierpe National Wetlands	3.5	20	5	28/01/2019	8.87626	-83.55611	Falling	5.2	30.3	NA	1.25	Jorge V, Mario, Marta, Mariel and Esteban	

Costa Rica	Southern Pacific	Terraba-Sierpe National Wetlands	4.1	10	2	28/01/2019	8.87827	-83.54834	Falling	1.6	30.4	NA	0.5	Jorge V, Mario, Marta, Mariel and Esteban	
Costa Rica	Southern Pacific	Terraba-Sierpe National Wetlands	4.2	10	4	28/01/2019	8.87827	-83.54834	Falling	1.6	30.4	NA	0.5	Jorge V, Mario, Marta, Mariel and Esteban	
Costa Rica	Southern Pacific	Terraba-Sierpe National Wetlands	4.3	10	2	28/01/2019	8.87827	-83.54834	Falling	1.6	30.4	NA	0.5	Jorge V, Mario, Marta, Mariel and Esteban	
Costa Rica	Southern Pacific	Terraba-Sierpe National Wetlands	4.4	20	5	28/01/2019	8.87827	-83.54834	Falling	1.6	30.4	NA	0.5	Jorge V, Mario, Marta, Mariel and Esteban	
Costa Rica	Southern Pacific	Terraba-Sierpe National Wetlands	4.5	20	5.2	28/01/2019	8.87827	-83.54834	Falling	1.6	30.4	NA	0.5	Jorge V, Mario, Marta, Mariel and Esteban	
Costa Rica	Southern Pacific	Terraba-Sierpe National Wetlands	5.1	10	2.1	28/01/2019	8.87319	-83.52245	Rising	5	30.5	NA	1	Jorge V, Mario, Marta, Mariel and Esteban	
Costa Rica	Southern Pacific	Terraba-Sierpe National Wetlands	5.2	20	4.2	28/01/2019	8.87319	-83.52245	Rising	5	30.5	NA	1	Jorge V, Mario, Marta, Mariel and Esteban	
Costa Rica	Southern Pacific	Terraba-Sierpe National Wetlands	5.3	20	4.7	28/01/2019	8.87319	-83.52245	Rising	5	30.5	NA	1	Jorge V, Mario, Marta, Mariel and Esteban	
Costa Rica	Southern Pacific	Terraba-Sierpe National Wetlands	5.4	20	4.3	28/01/2019	8.87319	-83.52245	Rising	5	30.5	NA	1	Jorge V, Mario, Marta, Mariel and Esteban	
Costa Rica	Southern Pacific	Terraba-Sierpe National Wetlands	5.5	20	4.2	28/01/2019	8.87319	-83.52245	Rising	5	30.5	NA	1	Jorge V, Mario, Marta, Mariel and Esteban	
Costa Rica	Southern Pacific	Terraba-Sierpe National Wetlands	6.1	10	3,150	29/01/2019	8.84786	-83.58899	Rising	5.1	30.3	NA	2.15	Jorge V, Mario, Mariel and Esteban	
Costa Rica	Southern Pacific	Terraba-Sierpe National Wetlands	6.2	10	3,300	29/01/2019	8.84786	-83.58899	Rising	5.1	30.3	NA	2.15	Jorge V, Mario, Mariel and Esteban	
Costa Rica	Southern Pacific	Terraba-Sierpe National Wetlands	6.3	10	4,250	29/01/2019	8.84786	-83.58899	Rising	5.1	30.3	NA	2.15	Jorge V, Mario, Mariel and Esteban	
Costa Rica	Southern Pacific	Terraba-Sierpe National Wetlands	6.4	10	4,500	29/01/2019	8.84786	-83.58899	Rising	5.1	30.3	NA	2.15	Jorge V, Mario, Mariel and Esteban	
Costa Rica	Southern Pacific	Terraba-Sierpe National Wetlands	6.5	10	4,100	29/01/2019	8.84786	-83.58899	Rising	5.1	30.3	NA	2.15	Jorge V, Mario, Mariel and Esteban	
Costa Rica	Southern Pacific	Terraba-Sierpe National Wetlands	7.1	20	3	29/01/2019	8.84796	-83.5742	Rising	12.2	30.3	NA	2	Jorge V, Mario, Mariel and Esteban	
Costa Rica	Southern Pacific	Terraba-Sierpe National Wetlands	7.2	20	5.1	29/01/2019	8.84796	-83.5742	Rising	12.2	30.3	NA	2	Jorge V, Mario, Mariel and Esteban	
Costa Rica	Southern Pacific	Terraba-Sierpe National Wetlands	7.3	20	5.1	29/01/2019	8.84796	-83.5742	Rising	12.2	30.3	NA	2	Jorge V, Mario, Mariel and Esteban	
Costa Rica	Southern Pacific	Terraba-Sierpe National Wetlands	7.4	20	5.1	29/01/2019	8.84796	-83.5742	Rising	12.2	30.3	NA	2	Jorge V, Mario, Mariel and Esteban	
Costa Rica	Southern Pacific	Terraba-Sierpe National Wetlands	7.5	20	5	29/01/2019	8.84796	-83.5742	Rising	12.2	30.3	NA	2	Jorge V, Mario, Mariel and Esteban	
Costa Rica	Southern Pacific	Terraba-Sierpe National Wetlands	8.1	10	3.2	29/01/2019	8.86701	-83.57455	Falling	6.3	30.3	NA	2.3	Jorge V, Mario, Mariel and Esteban	

Costa Rica	Southern Pacific	Terraba-Sierpe National Wetlands	8.2	10	3.2	29/01/2019	8.86701	-83.57455	Falling	6.3	30.3	NA	2.3	Jorge V, Mario, Mariel and Esteban	
Costa Rica	Southern Pacific	Terraba-Sierpe National Wetlands	8.3	20	5.5	29/01/2019	8.86701	-83.57455	Falling	6.3	30.3	NA	2.3	Jorge V, Mario, Mariel and Esteban	
Costa Rica	Southern Pacific	Terraba-Sierpe National Wetlands	8.4	20	5	29/01/2019	8.86701	-83.57455	Falling	6.3	30.3	NA	2.3	Jorge V, Mario, Mariel and Esteban	
Costa Rica	Southern Pacific	Terraba-Sierpe National Wetlands	8.5	20	5	29/01/2019	8.86701	-83.57455	Falling	6.3	30.3	NA	2.3	Jorge V, Mario, Mariel and Esteban	
Costa Rica	Southern Pacific	Terraba-Sierpe National Wetlands	9.1	10	2	29/01/2019	8.86156	-83.51999	Falling	5.5	30.7	NA	1.15	Jorge V, Mario, Mariel and Esteban	
Costa Rica	Southern Pacific	Terraba-Sierpe National Wetlands	9.2	20	4	29/01/2019	8.86156	-83.51999	Falling	5.5	30.7	NA	1.15	Jorge V, Mario, Mariel and Esteban	
Costa Rica	Southern Pacific	Terraba-Sierpe National Wetlands	9.3	20	3	29/01/2019	8.86156	-83.51999	Falling	5.5	30.7	NA	1.15	Jorge V, Mario, Mariel and Esteban	
Costa Rica	Southern Pacific	Terraba-Sierpe National Wetlands	9.4	20	3.5	29/01/2019	8.86156	-83.51999	Falling	5.5	30.7	NA	1.15	Jorge V, Mario, Mariel and Esteban	
Costa Rica	Southern Pacific	Terraba-Sierpe National Wetlands	9.5	20	3.5	29/01/2019	8.86156	-83.51999	Falling	5.5	30.7	NA	1.15	Jorge V, Mario, Mariel and Esteban	
Costa Rica	Southern Pacific	Terraba-Sierpe National Wetlands	10.1	20	2.5	29/01/2019	8.85763	-83.50382	Falling	2.4	30.8	NA	0.85	Jorge V, Mario, Mariel and Esteban	
Costa Rica	Southern Pacific	Terraba-Sierpe National Wetlands	10.2	20	2.4	29/01/2019	8.85763	-83.50382	Falling	2.4	30.8	NA	0.85	Jorge V, Mario, Mariel and Esteban	
Costa Rica	Southern Pacific	Terraba-Sierpe National Wetlands	10.3	20	2.3	29/01/2019	8.85763	-83.50382	Falling	2.4	30.8	NA	0.85	Jorge V, Mario, Mariel and Esteban	
Costa Rica	Southern Pacific	Terraba-Sierpe National Wetlands	10.4	20	2.3	29/01/2019	8.85763	-83.50382	Falling	2.4	30.8	NA	0.85	Jorge V, Mario, Mariel and Esteban	
Costa Rica	Southern Pacific	Terraba-Sierpe National Wetlands	10.5	20	2.25	29/01/2019	8.85763	-83.50382	Falling	2.4	30.8	NA	0.85	Jorge V, Mario, Mariel and Esteban	
Costa Rica	Southern Pacific	Terraba-Sierpe National Wetlands	11.1	20	2.4	29/01/2019	8.86701	-83.57455	Falling	-	28	NA	0.5	Jorge V, Mario, Mariel and Esteban	
Costa Rica	Southern Pacific	Terraba-Sierpe National Wetlands	11.2	20	2.4	29/01/2019	8.86701	-83.57455	Falling	-	28	NA	0.5	Jorge V, Mario, Mariel and Esteban	
Costa Rica	Southern Pacific	Terraba-Sierpe National Wetlands	11.3	20	2.3	29/01/2019	8.86701	-83.57455	Falling	-	28	NA	0.5	Jorge V, Mario, Mariel and Esteban	
Costa Rica	Southern Pacific	Terraba-Sierpe National Wetlands	11.4	20	2.6	29/01/2019	8.86701	-83.57455	Falling	-	28	NA	0.5	Jorge V, Mario, Mariel and Esteban	
Costa Rica	Southern Pacific	Terraba-Sierpe National Wetlands	11.5	20	2.6	29/01/2019	8.86701	-83.57455	Falling	-	28	NA	0.5	Jorge V, Mario, Mariel and Esteban	
Pakistan	Balochistan Province	Door 1	1.1	10	5	7/02/2019	25.19967	62.4380	Normal	-	-	5	-	Meesum Kazmi, Jawad Khan, Areeba Moiz	
Pakistan	Balochistan Province	Door 1	1.2	10	5	7/02/2019	25.19967	62.4380	Normal	-	-	5	-	Meesum Kazmi, Jawad Khan, Areeba Moiz	

Pakistan	Balochistan Province	Door 1	1.3	10	5	7/02/2019	25.19967	62.4380	Normal	-	-	5	-	Meesum Kazmi, Jawad Khan, Areeba Moiz	
Pakistan	Balochistan Province	Door 1	1.4	10	5	7/02/2019	25.19967	62.4380	Normal	-	-	5	-	Meesum Kazmi, Jawad Khan, Areeba Moiz	
Pakistan	Balochistan Province	Door 1	1.5	10	5	7/02/2019	25.19967	62.4380	Normal	-	-	5	-	Meesum Kazmi, Jawad Khan, Areeba Moiz	
Pakistan	Balochistan Province	Door 2	2.1	10	5	7/02/2019	25.20557	62.4696	Normal	-	-	7	-	Meesum Kazmi, Jawad Khan, Areeba Moiz	
Pakistan	Balochistan Province	Door 2	2.2	10	5	7/02/2019	25.20557	62.4696	Normal	-	-	7	-	Meesum Kazmi, Jawad Khan, Areeba Moiz	
Pakistan	Balochistan Province	Door 2	2.3	10	5	7/02/2019	25.20557	62.4696	Normal	-	-	7	-	Meesum Kazmi, Jawad Khan, Areeba Moiz	
Pakistan	Balochistan Province	Door 2	2.4	10	5	7/02/2019	25.20557	62.4696	Normal	-	-	7	-	Meesum Kazmi, Jawad Khan, Areeba Moiz	
Pakistan	Balochistan Province	Door 2	2.5	20	5	7/02/2019	25.20557	62.4696	Normal	-	-	7	-	Meesum Kazmi, Jawad Khan, Areeba Moiz	
Pakistan	Balochistan Province	Sur Bandar	3.1	10	5	7/02/2019	25.22420	62.4871	Normal	-	-	10	-	Meesum Kazmi, Jawad Khan, Areeba Moiz	
Pakistan	Balochistan Province	Sur Bandar	3.2	10	5	7/02/2019	25.22420	62.4871	Normal	-	-	10	-	Meesum Kazmi, Jawad Khan, Areeba Moiz	
Pakistan	Balochistan Province	Sur Bandar	3.3	10	5	7/02/2019	25.22420	62.4871	Normal	-	-	10	-	Meesum Kazmi, Jawad Khan, Areeba Moiz	
Pakistan	Balochistan Province	Sur Bandar	3.4	10	5	7/02/2019	25.22420	62.4871	Normal	-	-	10	-	Meesum Kazmi, Jawad Khan, Areeba Moiz	
Pakistan	Balochistan Province	Sur Bandar	3.5	10	5	7/02/2019	25.22420	62.4871	Normal	-	-	10	-	Meesum Kazmi, Jawad Khan, Areeba Moiz	
Pakistan	Sindh Province	Chan 1	4.1	10	5	14/02/2019	24.24552	67.3171	Steady	14.5	18	1.4		Saeed Islam, Meesum Kazmi	
Pakistan	Sindh Province	Chan 1	4.2	10	5	14/02/2019	24.24552	67.3171	Steady	14.5	18	1.4		Saeed Islam, Meesum Kazmi	
Pakistan	Sindh Province	Chan 1	4.3	10	5	14/02/2019	24.24552	67.3171	Steady	14.5	18	1.4		Saeed Islam, Meesum Kazmi	
Pakistan	Sindh Province	Chan 1	4.4	10	5	14/02/2019	24.24552	67.3171	Steady	14.5	18	1.4		Saeed Islam, Meesum Kazmi	
Pakistan	Sindh Province	Chan 1	4.5	10	5	14/02/2019	24.24552	67.3171	Steady	14.5	18	1.4		Saeed Islam, Meesum Kazmi	
Pakistan	Sindh Province	Chan 2	5.1	10	5	14/02/2019	23.92239	67.3190	Stable	18	18	2	-	Saeed Islam, Meesum Kazmi	
Pakistan	Sindh Province	Chan 2	5.2	10	5	14/02/2019	23.92239	67.3190	Stable	18	18	2	-	Saeed Islam, Meesum Kazmi	
Pakistan	Sindh Province	Chan 2	5.3	10	5	14/02/2019	23.92239	67.3190	Stable	18	18	2	-	Saeed Islam, Meesum Kazmi	
Pakistan	Sindh Province	Chan 2	5.4	10	5	14/02/2019	23.92239	67.3190	Stable	18	18	2	-	Saeed Islam, Meesum Kazmi	
Pakistan	Sindh Province	Chan 2	5.5	10	5	14/02/2019	23.92239	67.3190	Stable	18	18	2	-	Saeed Islam, Meesum Kazmi	
Pakistan	Sindh Province	Wari 1	6.1	10	5	15/02/2019	23.90031	67.8406	Steady	8.4	18	0.9		Saeed Islam, Meesum Kazmi	
Pakistan	Sindh Province	Wari 1	6.2	10	5	15/02/2019	23.90031	67.8406	Steady	8.4	18	0.9		Saeed Islam, Meesum Kazmi	

Pakistan	Sindh Province	Wari 1	6.3	10	5	15/02/2019	23.90031	67.8406	Steady	8.4	18	0.9		Saeed Islam, Meesum Kazmi	
Pakistan	Sindh Province	Wari 1	6.4	10	5	15/02/2019	23.90031	67.8406	Steady	8.4	18	0.9		Saeed Islam, Meesum Kazmi	
Pakistan	Sindh Province	Wari 1	6.5	10	5	15/02/2019	23.90031	67.8406	Steady	8.4	18	0.9		Saeed Islam, Meesum Kazmi	
Pakistan	Sindh Province	Wari 2	7.1	10	5	15/02/2019	23.90078	67.8512	Steady	7.4	18	0.7	-	Saeed Islam, Meesum Kazmi	
Pakistan	Sindh Province	Wari 2	7.2	10	5	15/02/2019	23.90078	67.8512	Steady	7.4	18	0.7	-	Saeed Islam, Meesum Kazmi	
Pakistan	Sindh Province	Wari 2	7.3	10	5	15/02/2019	23.90078	67.8512	Steady	7.4	18	0.7	-	Saeed Islam, Meesum Kazmi	
Pakistan	Sindh Province	Wari 2	7.4	10	5	15/02/2019	23.90078	67.8512	Steady	7.4	18	0.7	-	Saeed Islam, Meesum Kazmi	
Pakistan	Sindh Province	Wari 2	7.5	10	5	15/02/2019	23.90078	67.8512	Steady	7.4	18	0.7	-	Saeed Islam, Meesum Kazmi	
Pakistan	Sindh Province	Khajar 1	8.1	10	5	15/02/2019	23.93353	68.0118	Steady	6.3	20	1.2	-	Saeed Islam, Meesum Kazmi	
Pakistan	Sindh Province	Khajar 1	8.2	10	5	15/02/2019	23.93353	68.0118	Steady	6.3	20	1.2	-	Saeed Islam, Meesum Kazmi	
Pakistan	Sindh Province	Khajar 1	8.3	10	5	15/02/2019	23.93353	68.0118	Steady	6.3	20	1.2	-	Saeed Islam, Meesum Kazmi	
Pakistan	Sindh Province	Khajar 1	8.4	10	5	15/02/2019	23.93353	68.0118	Steady	6.3	20	1.2	-	Saeed Islam, Meesum Kazmi	
Pakistan	Sindh Province	Khajar 1	8.5	10	5	15/02/2019	23.93353	68.0118	Steady	6.3	20	1.2	-	Saeed Islam, Meesum Kazmi	
Pakistan	Sindh Province	Khajar 2	9.1	10	5	15/02/2019	23.92603	68.0165	Steady	6.3	20	1.2	-	Saeed Islam, Meesum Kazmi	
Pakistan	Sindh Province	Khajar 2	9.2	10	5	15/02/2019	23.92603	68.0165	Steady	6.3	20	1.2	-	Saeed Islam, Meesum Kazmi	
Pakistan	Sindh Province	Khajar 2	9.3	10	5	15/02/2019	23.92603	68.0165	Steady	6.3	20	1.2	-	Saeed Islam, Meesum Kazmi	
Pakistan	Sindh Province	Khajar 2	9.4	10	5	15/02/2019	23.92603	68.0165	Steady	6.3	20	1.2	-	Saeed Islam, Meesum Kazmi	
Pakistan	Sindh Province	Khajar 2	9.5	10	5	15/02/2019	23.92603	68.0165	Steady	6.3	20	1.2	-	Saeed Islam, Meesum Kazmi	
Pakistan	Sindh Province	Khajar 3	10.1	10	5	15/02/2019	23.94869	68.0136	Steady	11.5	20	1.5	-	Saeed Islam, Meesum Kazmi	
Pakistan	Sindh Province	Khajar 3	10.2	10	5	15/02/2019	23.94869	68.0136	Steady	11.5	20	1.5	-	Saeed Islam, Meesum Kazmi	
Pakistan	Sindh Province	Khajar 3	10.3	10	5	15/02/2019	23.94869	68.0136	Steady	11.5	20	1.5	-	Saeed Islam, Meesum Kazmi	
Pakistan	Sindh Province	Khajar 3	10.4	10	5	15/02/2019	23.94869	68.0136	Steady	11.5	20	1.5	-	Saeed Islam, Meesum Kazmi	
Pakistan	Sindh Province	Khajar 3	10.5	10	5	15/02/2019	23.94869	68.0136	Steady	11.5	20	1.5	-	Saeed Islam, Meesum Kazmi	
Costa Rica	Central Pacific	Tempisque	12.1	20	1.05	19/02/2019	10.20852	-85.24013	Falling	4.5	-	NA	0.75	Jorge V, Daniela Solis, Gabino/Jacinto Perez.	
Costa Rica	Central Pacific	Tempisque	12.2	20	1	19/02/2019	10.20852	-85.24013	Falling	4.5	-	NA	0.75	Jorge V, Daniela Solis, Gabino/Jacinto Perez.	
Costa Rica	Central Pacific	Tempisque	12.3	20	2.2	19/02/2019	10.20852	-85.24013	Falling	4.5	-	NA	0.75	Jorge V, Daniela Solis, Gabino/Jacinto Perez.	
Costa Rica	Central Pacific	Tempisque	12.4	20	2.2	19/02/2019	10.20852	-85.24013	Falling	4.5	-	NA	0.75	Jorge V, Daniela Solis, Gabino/Jacinto Perez.	
Costa Rica	Central Pacific	Tempisque	12.5	20	3.9	19/02/2019	10.20852	-85.24013	Falling	4.5	-	NA	0.75	Jorge V, Daniela Solis, Gabino/Jacinto Perez.	

Costa Rica	Central Pacific	Tempisque	13.1	20	0.75	19/02/2019	10.2553	-85.25935	Rising	2.2	30.6	NA	0.5	Jorge V, Daniela Solis, Gabino/Jacinto Perez.	
Costa Rica	Central Pacific	Tempisque	13.2	20	4.5	19/02/2019	10.2553	-85.25935	Rising	2.2	30.6	NA	0.5	Jorge V, Daniela Solis, Gabino/Jacinto Perez.	
Costa Rica	Central Pacific	Tempisque	13.3	20	4.8	19/02/2019	10.2553	-85.25935	Rising	2.2	30.6	NA	0.5	Jorge V, Daniela Solis, Gabino/Jacinto Perez.	
Costa Rica	Central Pacific	Tempisque	13.4	20	4.8	19/02/2019	10.2553	-85.25935	Rising	2.2	30.6	NA	0.5	Jorge V, Daniela Solis, Gabino/Jacinto Perez.	
Costa Rica	Central Pacific	Tempisque	13.5	20	5	19/02/2019	10.2553	-85.25935	Rising	2.2	30.6	NA	0.5	Jorge V, Daniela Solis, Gabino/Jacinto Perez.	
Costa Rica	Central Pacific	Tempisque	14.1	20	4.8	19/02/2019	10.15025	-85.21119	Falling	4.8	29.7	NA	0.6	Jorge V, Daniela Solis, Jacinto Perez.	
Costa Rica	Central Pacific	Tempisque	14.2	20	4.8	19/02/2019	10.15025	-85.21119	Falling	4.8	29.7	NA	0.6	Jorge V, Daniela Solis, Jacinto Perez.	
Costa Rica	Central Pacific	Tempisque	14.3	20	3.8	19/02/2019	10.15025	-85.21119	Falling	4.8	29.7	NA	0.6	Jorge V, Daniela Solis, Jacinto Perez.	
Costa Rica	Central Pacific	Tempisque	14.4	20	5.1	19/02/2019	10.15025	-85.21119	Falling	4.8	29.7	NA	0.6	Jorge V, Daniela Solis, Jacinto Perez.	
Costa Rica	Central Pacific	Tempisque	14.5	20	5.2	19/02/2019	10.15025	-85.21119	Falling	4.8	29.7	NA	0.6	Jorge V, Daniela Solis, Jacinto Perez.	
Papua New Guinea	Western Province	Fly River, Gesoa Village tributary	1.1	20	0.5	22/02/2019	-8.39641	143.6876	Rising	0.6	29.1	22.1	0.03	Michael Grant, Adam Harman	
Papua New Guinea	Western Province	Fly River, Gesoa Village tributary	1.2	20	0.55	22/02/2019	-8.39641	143.6876	Rising	0.6	29.1	22.1	0.03	Michael Grant, Adam Harman	
Papua New Guinea	Western Province	Fly River, Gesoa Village tributary	1.3	20	0.65	22/02/2019	-8.39641	143.6876	Rising	0.6	29.1	22.1	0.03	Michael Grant, Adam Harman	
Papua New Guinea	Western Province	Fly River, Gesoa Village tributary	1.4	20	0.55	22/02/2019	-8.39641	143.6876	Rising	0.6	29.1	22.1	0.03	Michael Grant, Adam Harman	
Papua New Guinea	Western Province	Fly River, Gesoa Village channel	2.1	20	0.5	22/02/2019	-8.39401	143.5710	Rising	1.8	29.3	22	0.05	Michael Grant, Adam Harman	
Papua New Guinea	Western Province	Fly River, Gesoa Village channel	2.2	20	0.75	22/02/2019	-8.39401	143.5710	Rising	1.8	29.3	22	0.05	Michael Grant, Adam Harman	
Papua New Guinea	Western Province	Fly River, Gesoa Village channel	2.3	20	0.8	22/02/2019	-8.39401	143.5710	Rising	1.8	29.3	22	0.05	Michael Grant, Adam Harman	
Papua New Guinea	Western Province	Fly River, Gesoa Village channel	2.4	20	0.7	22/02/2019	-8.39401	143.5710	Rising	1.8	29.3	22	0.05	Michael Grant, Adam Harman	
Papua New Guinea	Western Province	Fly River, Gesoa Village channel	2.5	20	0.85	22/02/2019	-8.39401	143.5710	Rising	1.8	29.3	22	0.05	Michael Grant, Adam Harman	
Papua New Guinea	Western Province	Fly River, main estuary	3.1	20	0.55	22/02/2019	-8.39163	143.5715	Slack high	1.5	29.7	21.3	0.05	Michael Grant, Adam Harman	
Papua New Guinea	Western Province	Fly River, main estuary	3.2	20	0.5	22/02/2019	-8.39163	143.5715		-	-	-	-	Michael Grant, Adam Harman	
Papua New Guinea	Western Province	Fly River, main estuary	3.3	20	-	22/02/2019	-8.39163	143.5715	-	-	-	-	-	Michael Grant, Adam Harman	
Papua New Guinea	Western Province	Fly River, main estuary	3.4	20	1	22/02/2019	-8.39163	143.5715	-	-	-	-	-	Michael Grant, Adam Harman	
Papua New Guinea	Western Province	Fly River, main estuary	3.5	20	1	22/02/2019	-8.39163	143.5715	-	-	-	-	-	Michael Grant, Adam Harman	

Papua New Guinea	Western Province	Fly River, main estuary	4.1	20	2.5	22/02/2019	-8.38612	143.5724	Falling	2	29.2	21.9	0.05	Michael Grant, Adam Harman	
Papua New Guinea	Western Province	Fly River, main estuary	4.2	20	2	22/02/2019	-8.38612	143.5724	-	-	-	-	-	Michael Grant, Adam Harman	
Papua New Guinea	Western Province	Fly River, main estuary	4.3	20	2	22/02/2019	-8.38612	143.5724	-	-	-	-	-	Michael Grant, Adam Harman	
Papua New Guinea	Western Province	Fly River, main estuary	4.4	20	2	22/02/2019	-8.38612	143.5724	-	-	-	-	-	Michael Grant, Adam Harman	
Papua New Guinea	Western Province	Fly River, main estuary	4.5	20	1.9	22/02/2019	-8.38612	143.5724	-	-	-	-	-	Michael Grant, Adam Harman	
Papua New Guinea	Western Province	Fly River	5.1	20	1.5	22/02/2019	-8.38230	143.5730	Falling	0.6	29.7	22.4	0.05	Michael Grant, Adam Harman	
Papua New Guinea	Western Province	Fly River	5.2	20	1.3	22/02/2019	-8.38230	143.5730	-	-	-	-	-	Michael Grant, Adam Harman	
Papua New Guinea	Western Province	Fly River	5.3	20	-	22/02/2019	-8.38230	143.5730	-	-	-	-	-	Michael Grant, Adam Harman	
Papua New Guinea	Western Province	Fly River	5.4	20	1.5	22/02/2019	-8.38230	143.5730	-	-	-	-	-	Michael Grant, Adam Harman	
Papua New Guinea	Western Province	Fly River	5.5	20	1.2	22/02/2019	-8.38230	143.5730	-	-	-	-	-	Michael Grant, Adam Harman	
Papua New Guinea	Western Province	Oxbow 181, river mouth	6.1	20	0.75	25/02/2019	-7.79061	141.5580	-	2	27.9	0.1	0.07	Michael Grant, Adam Harman	
Papua New Guinea	Western Province	Oxbow 181, river mouth	6.2	20	0.55	25/02/2019	-7.79061	141.5580	-	2	27.9	0.1	0.07	Michael Grant, Adam Harman	
Papua New Guinea	Western Province	Oxbow 181, river mouth	6.3	20	0.65	25/02/2019	-7.79061	141.5580	-	2	27.9	0.1	0.07	Michael Grant, Adam Harman	
Papua New Guinea	Western Province	Oxbow 181, river mouth	6.4	20	0.62	25/02/2019	-7.79061	141.5580	-	2	27.9	0.1	0.07	Michael Grant, Adam Harman	
Papua New Guinea	Western Province	Oxbow 181, river mouth	6.5	20	0.9	25/02/2019	-7.79061	141.5580	-	2	27.9	0.1	0.07	Michael Grant, Adam Harman	
Papua New Guinea	Western Province	Oxbow 181, inside	7.1	20	5	25/02/2019	-7.81337	141.5746	-	2	31.2	0.1	0.05	Michael Grant, Adam Harman	
Papua New Guinea	Western Province	Oxbow 181, inside	7.2	20	5	25/02/2019	-7.81337	141.5746	-	2	31.2	0.1	0.05	Michael Grant, Adam Harman	
Papua New Guinea	Western Province	Oxbow 181, inside	7.3	20	5	25/02/2019	-7.81337	141.5746	-	2	31.2	0.1	0.05	Michael Grant, Adam Harman	
Papua New Guinea	Western Province	Oxbow 181, inside	7.4	20	5	25/02/2019	-7.81337	141.5746	-	2	31.2	0.1	0.05	Michael Grant, Adam Harman	
Papua New Guinea	Western Province	Oxbow 181, inside	7.5	20	5	25/02/2019	-7.81337	141.5746	-	2	31.2	0.1	0.05	Michael Grant, Adam Harman	
Papua New Guinea	Western Province	Oxbow 181, inside	7.6	1.2	0.5	25/02/2019	-7.81337	141.5746	-	2	31.2	0.1	0.05	Michael Grant, Adam Harman	
Papua New Guinea	Western Province	Oxbow 181, inside	8.1	20	5	25/02/2019	-7.81671	141.5771	-	1	29.6	0.1	0.4	Michael Grant, Adam Harman	
Papua New Guinea	Western Province	Oxbow 181, inside	8.2	20	5	25/02/2019	-7.81671	141.5771	-	1	29.6	0.1	0.4	Michael Grant, Adam Harman	
Papua New Guinea	Western Province	Oxbow 181, inside	8.3	20	5	25/02/2019	-7.81671	141.5771	-	1	29.6	0.1	0.4	Michael Grant, Adam Harman	
Papua New Guinea	Western Province	Oxbow 181, inside	8.4	20	5.5	25/02/2019	-7.81671	141.5771	-	1	29.6	0.1	0.4	Michael Grant, Adam Harman	
Papua New Guinea	Western Province	Oxbow 181, inside	8.5	20	5.5	25/02/2019	-7.81671	141.5771	-	1	29.6	0.1	0.4	Michael Grant, Adam Harman	
Papua New Guinea	Western Province	Oxbow 181	9.1	20	5	25/02/2019	-7.81170	141.5786	-	2	29.5	0.1	0.4	Michael Grant, Adam Harman	
Papua New Guinea	Western Province	Oxbow 181	9.2	20	5	25/02/2019	-7.81170	141.5786	-	2	29.5	0.1	0.4	Michael Grant, Adam Harman	
Papua New Guinea	Western Province	Oxbow 181	9.3	20	5	25/02/2019	-7.81170	141.5786	-	2	29.5	0.1	0.4	Michael Grant, Adam Harman	
Papua New Guinea	Western Province	Oxbow 181	9.4	20	5	25/02/2019	-7.81170	141.5786	-	2	29.5	0.1	0.4	Michael Grant, Adam Harman	
Papua New Guinea	Western Province	Oxbow 181	9.5	20	5	25/02/2019	-7.81170	141.5786	-	2	29.5	0.1	0.4	Michael Grant, Adam Harman	

Costa Rica	Northern Plains	Boca San Carlos	15.1	20	3.2	4/03/2019	10.78982	-84.19172	Non-tidal	2	28	NA	0.7	Jorge V, Huberth Gonzalez, Davis Morera, Jorge Chavarría	
Costa Rica	Northern Plains	Boca San Carlos	15.2	20	3.25	4/03/2019	10.78982	-84.19172	Non-tidal	2	28	NA	0.7	Jorge V, Huberth Gonzalez, Davis Morera, Jorge Chavarría	
Costa Rica	Northern Plains	Boca San Carlos	15.3	20	3.2	4/03/2019	10.78982	-84.19172	Non-tidal	2	28	NA	0.7	Jorge V, Huberth Gonzalez, Davis Morera, Jorge Chavarría	
Costa Rica	Northern Plains	Boca San Carlos	15.4	20	3	4/03/2019	10.78982	-84.19172	Non-tidal	2	28	NA	0.7	Jorge V, Huberth Gonzalez, Davis Morera, Jorge Chavarría	
Costa Rica	Northern Plains	Boca San Carlos	15.5	20	3.25	4/03/2019	10.78982	-84.19172	Non-tidal	2	28	NA	0.7	Jorge V, Huberth Gonzalez, Davis Morera, Jorge Chavarría	
Costa Rica	Northern Plains	Boca San Carlos	16.1	20	3.1	4/03/2019	10.78757	-84.19518	Non-tidal	0.75	28	NA	0.5	Jorge V, Huberth Gonzalez, Davis Morera, Jorge Chavarría	
Costa Rica	Northern Plains	Boca San Carlos	16.2	20	3.1	4/03/2019	10.78757	-84.19518	Non-tidal	0.75	28	NA	0.5	Jorge V, Huberth Gonzalez, Davis Morera, Jorge Chavarría	
Costa Rica	Northern Plains	Boca San Carlos	16.3	20	3	4/03/2019	10.78757	-84.19518	Non-tidal	0.75	28	NA	0.5	Jorge V, Huberth Gonzalez, Davis Morera, Jorge Chavarría	
Costa Rica	Northern Plains	Boca San Carlos	16.4	20	3	4/03/2019	10.78757	-84.19518	Non-tidal	0.75	28	NA	0.5	Jorge V, Huberth Gonzalez, Davis Morera, Jorge Chavarría	
Costa Rica	Northern Plains	Boca San Carlos	16.5	20	2.5	4/03/2019	10.78757	-84.19518	Non-tidal	0.75	28	NA	0.5	Jorge V, Huberth Gonzalez, Davis Morera, Jorge Chavarría	
Costa Rica	Northern Plains	Boca San Carlos	17.1	20	3	4/03/2019	10.78543	-84.19724	Non-tidal	0.3	27.6	NA	0.25	Jorge V, Huberth Gonzalez, Davis Morera, Jorge Chavarría	
Costa Rica	Northern Plains	Boca San Carlos	17.2	20	3	4/03/2019	10.78543	-84.19724	Non-tidal	0.3	27.6	NA	0.25	Jorge V, Huberth Gonzalez, Davis Morera, Jorge Chavarría	
Costa Rica	Northern Plains	Boca San Carlos	17.3	20	3	4/03/2019	10.78543	-84.19724	Non-tidal	0.3	27.6	NA	0.25	Jorge V, Huberth Gonzalez, Davis Morera, Jorge Chavarría	
Costa Rica	Northern Plains	Boca San Carlos	17.4	20	3.5	4/03/2019	10.78543	-84.19724	Non-tidal	0.3	27.6	NA	0.25	Jorge V, Huberth Gonzalez, Davis Morera, Jorge Chavarría	
Costa Rica	Northern Plains	Boca San Carlos	17.5	20	3.5	4/03/2019	10.78543	-84.19724	Non-tidal	0.3	27.6	NA	0.25	Jorge V, Huberth Gonzalez, Davis Morera, Jorge Chavarría	
Costa Rica	Northern Plains	Boca Cureñita	18.1	20	3.7	5/03/2019	10.76593	-84.06249	Non-tidal	9	28	NA	0.5	Jorge V, Huberth Gonzalez, Davis Morera, Jorge Chavarría	

Costa Rica	Northern Plains	Boca Cureñita	18.2	20	4	5/03/2019	10.76593	-84.06249	Non-tidal	9	28	NA	0.5	Jorge V, Huberth Gonzalez, Davis Morera, Jorge Chavarría	
Costa Rica	Northern Plains	Boca Cureñita	18.3	20	3.5	5/03/2019	10.76593	-84.06249	Non-tidal	9	28	NA	0.5	Jorge V, Huberth Gonzalez, Davis Morera, Jorge Chavarría	
Costa Rica	Northern Plains	Boca Cureñita	18.4	20	3.5	5/03/2019	10.76593	-84.06249	Non-tidal	9	28	NA	0.5	Jorge V, Huberth Gonzalez, Davis Morera, Jorge Chavarría	
Costa Rica	Northern Plains	Boca Cureñita	18.5	20	3.7	5/03/2019	10.76593	-84.06249	Non-tidal	9	28	NA	0.5	Jorge V, Huberth Gonzalez, Davis Morera, Jorge Chavarría	
Bangladesh	Barisal	Andharmanik Khal	1.10	10	1	15/03/2019	22.32350	89.7058	High	2.83	26.8	9.9	0.4	Md.Kutub Uddin	
Bangladesh	Barisal	Andharmanik Khal	1.20	20	2	15/03/2019	22.32350	89.7058	High	2.83	26.8	9.9	0.4	Md.Kutub Uddin	
Bangladesh	Barisal	Andharmanik Khal	1.30	20	2	15/03/2019	22.32350	89.7058	High	2.83	26.98	9.9	0.4	Md.Kutub Uddin	
Bangladesh	Barisal	Andharmanik Khal	1.1	10	1	15/03/2019	22.32350	89.7058	High	2.83	26.8	9.9	0.4	Md.Kutub Uddin	
Bangladesh	Barisal	Andharmanik Khal	1.2	20	2	15/03/2019	22.32350	89.7058	High	2.83	26.8	9.9	0.4	Md.Kutub Uddin	
Bangladesh	Barisal	Andharmanik Khal	1.3	20	2	15/03/2019	22.32350	89.7058	High	2.83	26.8	9.9	0.4	Md.Kutub Uddin	
Bangladesh	Barisal	Andharmanik Khal	1.4			15/03/2019	22.32350	89.7058	High	2.83	26.8	9.9	0.4	Md.Kutub Uddin	
Bangladesh	Barisal	Beri Khal	2.1	20	3.5	16/03/2019	22.21758	89.6856	Low	1.7	26.2	10.1	0.2	Md.Kutub Uddin	
Bangladesh	Barisal	Beri Khal	2.2	20	3.5	16/03/2019	22.21758	89.6856	Low	1.7	26.2	10.1	0.2	Md.Kutub Uddin	
Bangladesh	Barisal	Beri Khal	2.3	20	3.5	16/03/2019	22.21758	89.6856	Low	1.7	26.2	10.1	0.2	Md.Kutub Uddin	
Bangladesh	Barisal	Beri Khal	2.4	20	5	16/03/2019	22.21758	89.6856	Low	1.7	26.2	10.1	0.2	Md.Kutub Uddin	
Bangladesh	Barisal	Beri Khal	2.5	10	1	16/03/2019	22.21758	89.6856	Low	1.7	26.2	10.1	0.2	Md.Kutub Uddin	
Bangladesh	Barisal	Beri Khal	2.6	1.2	0.5	16/03/2019	22.21758	89.6856	Low	1.7	26.2	10.1	0.2	Md.Kutub Uddin	
Bangladesh	Barisal	Beri Khal	2.7	1.2	0.5	16/03/2019	22.21758	89.6856	Low	1.7	26.2	10.1	0.2	Md.Kutub Uddin	
Bangladesh	Barisal	Beri Khal	2.8	1.2	0.5	16/03/2019	22.21758	89.6856	Low	1.7	26.2	10.1	0.2	Md.Kutub Uddin	
Bangladesh	Barisal	Armal Khal	3.1	20	5	16/03/2019	22.08717	89.6659	Low	2.71	26.5	10.3	0.4	Md.Kutub Uddin	
Bangladesh	Barisal	Armal Khal	3.2	20	5	16/03/2019	22.08717	89.6659	Low	2.71	26.5	10.3	0.4	Md.Kutub Uddin	
Bangladesh	Barisal	Armal Khal	3.3	10	0.5	16/03/2019	22.08717	89.6659	Low	2.71	26.5	10.3	0.4	Md.Kutub Uddin	
Bangladesh	Barisal	Armal Khal	3.4	20	3	16/03/2019	22.08717	89.6659	Low	2.71	26.5	10.3	0.4	Md.Kutub Uddin	
Bangladesh	Barisal	Armal Khal	3.5	20	4.5	16/03/2019	22.08717	89.6659	Low	2.71	26.5	10.3	0.4	Md.Kutub Uddin	
Bangladesh	Barisal	Harintana Khal	4.1	20	5	16/03/2019	22.13280	89.7337	Low	5.86	27.6	6	0.4	Md.Kutub Uddin	
Bangladesh	Barisal	Harintana Khal	4.2	20	5	16/03/2019	22.13280	89.7337	Low	5.86	27.6	6	0.4	Md.Kutub Uddin	
Bangladesh	Barisal	Harintana Khal	4.3	20	5	16/03/2019	22.13280	89.7337	Low	5.86	27.6	6	0.4	Md.Kutub Uddin	
Bangladesh	Barisal	Harintana Khal	4.4	20	5	16/03/2019	22.13280	89.7337	Low	5.86	27.6	6	0.4	Md.Kutub Uddin	
Bangladesh	Barisal	Harintana Khal	4.5	10	1.5	16/03/2019	22.13280	89.7337	Low	5.86	27.6	6	0.4	Md.Kutub Uddin	
Bangladesh	Barisal	Harintana Khal	4.6	1.2	1	16/03/2019	22.13280	89.7337	Low	5.86	27.6	6	0.4	Md.Kutub Uddin	
Bangladesh	Barisal	Supati Khal	5.1	20	5	16/03/2019	22.05219	89.8197	Low	2.89	28	4	0.46	Md.Kutub Uddin	
Bangladesh	Barisal	Supati Khal	5.2	20	5	16/03/2019	22.05219	89.8197	Low	2.89	28	4	0.46	Md.Kutub Uddin	
Bangladesh	Barisal	Supati Khal	5.3	20	5	16/03/2019	22.05219	89.8197	Low	2.89	28	4	0.46	Md.Kutub Uddin	
Bangladesh	Barisal	Supati Khal	5.4	20	5	16/03/2019	22.05219	89.8197	Low	2.89	28	4	0.46	Md.Kutub Uddin	
Bangladesh	Barisal	Supati Khal	5.5	10	2	16/03/2019	22.05219	89.8197	Low	2.89	28	4	0.46	Md.Kutub Uddin	
Bangladesh	Barisal	Choto Katka Khal	6.1	20	5	17/03/2019	21.93913	89.7965	Low	7.2	27.1	13	0.33	Md.Kutub Uddin	

Bangladesh	Barisal	Siala Khal	7.1	20	5	17/03/2019	21.98226	89.6414	Low	3.46	27.9	19	0.4	Md.Kutub Uddin	
Bangladesh	Barisal	Siala Khal	7.2	20	5	17/03/2019	21.98226	89.6414	Low	3.46	27.9	19	0.4	Md.Kutub Uddin	
Bangladesh	Barisal	Siala Khal	7.3	20	5	17/03/2019	21.98226	89.6414	Low	3.46	27.9	19	0.4	Md.Kutub Uddin	
Bangladesh	Barisal	Khajurbari Khal	8.1	20	5	17/03/2019	21.89402	89.5853	High	2.94	28.1	20	0.73	Md.Kutub Uddin	
Bangladesh	Barisal	Khajurbari Khal	8.2	20	5	17/03/2019	21.89402	89.5853	High	2.94	28.1	20	0.73	Md.Kutub Uddin	
Bangladesh	Barisal	Khajurbari Khal	8.3	20	5	17/03/2019	21.89402	89.5853	High	2.94	28.1	20	0.73	Md.Kutub Uddin	
Bangladesh	Barisal	Khajurbari Khal	8.4	20	5	17/03/2019	21.89402	89.5853	High	2.94	28.1	20	0.73	Md.Kutub Uddin	
Bangladesh	Barisal	Khajurbari Khal	8.5	10	5	17/03/2019	21.89402	89.5853	High	2.94	28.1	20	0.73	Md.Kutub Uddin	
Bangladesh	Barisal	Khajurbari Khal	8.6	1.2	0.5	17/03/2019	21.89402	89.5853	High	2.94	28.1	20	0.73	Md.Kutub Uddin	
Bangladesh	Barisal	Kagadonia Khal	9.1	20	2.5	18/03/2019	21.97608	89.4011	High	4.53	27.1	26	0.39	Md.Kutub Uddin	
Bangladesh	Barisal	Kagadonia Khal	9.2	20	2.5	18/03/2019	21.97608	89.4011	High	4.53	27.1	26	0.39	Md.Kutub Uddin	
Bangladesh	Barisal	Kagadonia Khal	9.3	20	2.4	18/03/2019	21.97608	89.4011	High	4.53	27.1	26	0.39	Md.Kutub Uddin	
Bangladesh	Barisal	Kagadonia Khal	9.4	20	2.4	18/03/2019	21.97608	89.4011	High	4.53	27.1	26	0.39	Md.Kutub Uddin	
Bangladesh	Barisal	Kagadonia Khal	9.5	10	1.2	18/03/2019	21.97608	89.4011	High	4.53	27.1	26	0.39	Md.Kutub Uddin	
Bangladesh	Barisal	Khajuria Khal	10.1	20	5	18/03/2019	21.87776	89.3847	High	3.44	27.4	28	0.45	Md.Kutub Uddin	
Bangladesh	Barisal	Khajuria Khal	10.2	20	5	18/03/2019	21.87776	89.3847	High	3.44	27.4	28	0.45	Md.Kutub Uddin	
Bangladesh	Barisal	Taltola varani	11.1	20	5	18/03/2019	21.83312	89.2548	Low	3.69	27	27	6.1	Md.Kutub Uddin	
Bangladesh	Barisal	Taltola varani	11.2	20	5	18/03/2019	21.83312	89.2548	Low	3.69	27	27	6.1	Md.Kutub Uddin	
Bangladesh	Barisal	Taltola varani	11.3	20	5	18/03/2019	21.83312	89.2548	Low	3.69	27	27	6.1	Md.Kutub Uddin	
Bangladesh	Barisal	Taltola varani	11.4	20	5	18/03/2019	21.83312	89.2548	Low	3.69	27	27	6.1	Md.Kutub Uddin	
Bangladesh	Barisal	Taltola varani	11.5	10	4	18/03/2019	21.83312	89.2548	Low	3.69	27	27	6.1	Md.Kutub Uddin	
Bangladesh	Barisal	Taltola varani	11.6	1.2	1	18/03/2019	21.83312	89.2548	Low	3.69	27	27	6.1	Md.Kutub Uddin	
Bangladesh	Barisal	Angrakona Khal	12.1	20	5	19/03/2019	21.94642	89.2308	High	3.43	26.1	25.5	0.28	Md.Kutub Uddin	
Bangladesh	Barisal	Jamuna River	13.1	20	5	19/03/2019	22.05236	89.1511	High	1.68	26.5	22	0.42	Md.Kutub Uddin	
Bangladesh	Barisal	Jamuna River	13.2	20	5	19/03/2019	22.05236	89.1511	High	1.68	26.5	22	0.42	Md.Kutub Uddin	
Bangladesh	Barisal	Jamuna River	13.3	20	5	19/03/2019	22.05236	89.1511	High	1.68	26.5	22	0.42	Md.Kutub Uddin	
Bangladesh	Barisal	Jamuna River	13.4	20	5	19/03/2019	22.05236	89.1511	High	1.68	26.5	22	0.42	Md.Kutub Uddin	
Bangladesh	Barisal	Jamuna River	13.5	10	1.5	19/03/2019	22.05236	89.1511	High	1.68	26.5	22	0.42	Md.Kutub Uddin	
Bangladesh	Barisal	Jamuna River	13.6	1.2	0.7	19/03/2019	22.05236	89.1511	High	1.68	26.5	22	0.42	Md.Kutub Uddin	
Bangladesh	Barisal	Kali Khal	14.1	20	5	19/03/2019	22.17077	89.2783	Low	3.43	27.2	21	0.55	Md.Kutub Uddin	
Bangladesh	Barisal	Kali Khal	14.2	20	5	19/03/2019	22.17077	89.2783	Low	3.43	27.2	21	0.55	Md.Kutub Uddin	
Bangladesh	Barisal	Kali Khal	14.3	20	5	19/03/2019	22.17077	89.2783	Low	3.43	27.2	21	0.55	Md.Kutub Uddin	
Bangladesh	Barisal	Kali Khal	14.4	20	5	19/03/2019	22.17077	89.2783	Low	3.43	27.2	21	0.55	Md.Kutub Uddin	
Bangladesh	Barisal	Kali Khal	14.5	10	5	19/03/2019	22.17077	89.2783	Low	3.43	27.2	21	0.55	Md.Kutub Uddin	
Bangladesh	Barisal	Jhap Khal	15.1	20	5	20/03/2019	22.19710	89.3523	High	2.36	26.4	21	0.35	Md.Kutub Uddin	
Bangladesh	Barisal	Adachai	16.1	20	5	20/03/2019	22.26494	89.4915	High	2.55	26.9	20	0.4	Md.Kutub Uddin	
Pakistan	Balochistan Province	Miani 4	11.1	10	5	26/03/2019	25.49539	66.5453	Stable	16	24	1.4	-	Meesum Kazmi, Areeba Moiz	
Pakistan	Balochistan Province	Miani 4	11.2	10	5	26/03/2019	25.49539	66.5453	Stable	16	24	1.4	-	Meesum Kazmi, Areeba Moiz	
Pakistan	Balochistan Province	Miani 4	11.3	10	5	26/03/2019	25.49539	66.5453	Stable	16	24	1.4	-	Meesum Kazmi, Areeba Moiz	
Pakistan	Balochistan Province	Miani 4	11.4	10	5	26/03/2019	25.49539	66.5453	Stable	16	24	1.4	-	Meesum Kazmi, Areeba Moiz	
Pakistan	Balochistan Province	Miani 4	11.5	10	7	26/03/2019	25.49539	66.5453	Stable	16	24	1.4	-	Meesum Kazmi, Areeba Moiz	
Pakistan	Balochistan Province	Miani 3	12.1	10	5	26/03/2019	25.49978	66.4901	Steady	17	22	1.5		Meesum Kazmi, Areeba Moiz	
Pakistan	Balochistan Province	Miani 3	12.2	10	5	26/03/2019	25.49978	66.4901	Steady	17	22	1.5		Meesum Kazmi, Areeba Moiz	
Pakistan	Balochistan Province	Miani 3	12.3	20	5	26/03/2019	25.49978	66.4901	Steady	17	22	1.5		Meesum Kazmi, Areeba Moiz	

Pakistan	Balochistan Province	Miani 3	12.4	20	5	26/03/2019	25.49978	66.4901	Steady	17	22	1.5		Meesum Kazmi, Areeba Moiz	
Pakistan	Balochistan Province	Miani 3	12.5	10	5	26/03/2019	25.49978	66.4901	Steady	17	22	1.5		Meesum Kazmi, Areeba Moiz	
Pakistan	Balochistan Province	Miani 2	13.1	10	4	26/03/2019	25.4516	66.5455	Moderate	14	22	1.5		Meesum Kazmi, Areeba Moiz	
Pakistan	Balochistan Province	Miani 2	13.2	10	3	26/03/2019	25.4516	66.5455	Moderate	14	22	1.5		Meesum Kazmi, Areeba Moiz	
Australia	Queensland	No Name Creek, Ducie River	11.1	10	5	5/04/2019	-12.02248	142.1526	Rising	5.6	28.3	-	-	Barbara Wueringer	
Australia	Queensland	No Name Creek, Ducie River	11.2	10	5	5/04/2019	-12.02248	142.1526	Rising	5.6	28.3	-	-	Barbara Wueringer	
Australia	Queensland	No Name Creek, Ducie River	11.3	10	5	5/04/2019	-12.02248	142.1526	Rising	5.6	28.3	-	-	Barbara Wueringer	
Australia	Queensland	No Name Creek, Ducie River	11.4	10	5	5/04/2019	-12.02248	142.1526	Rising	5.6	28.3	-	-	Barbara Wueringer	
Australia	Queensland	No Name Creek, Ducie River	11.5	10	5	5/04/2019	-12.02248	142.1526	Rising	5.6	28.3	-	-	Barbara Wueringer	
Australia	Queensland	No Name Creek, Ducie River	11.6	1.2	1	5/04/2019	-12.02248	142.1526	Rising	5.6	28.3	-	-	Barbara Wueringer	
Australia	Queensland	No Name Creek, Ducie River	11.7	1.2	1	5/04/2019	-12.02248	142.1526	Rising	5.6	28.3	-	-	Barbara Wueringer	
Australia	Queensland	Mapoon/Trout Beach	12.1	10	5	11/04/2019	-11.64433	141.8914	Rising	1.5	27.3	7.6	1	Barbara Wueringer	
Australia	Queensland	Mapoon/Trout Beach	12.2	10	5	11/04/2019	-11.64433	141.8914	Rising	1.5	27.3	7.6	1	Barbara Wueringer	
Australia	Queensland	Mapoon/Trout Beach	12.3	10	5	11/04/2019	-11.64433	141.8914	Rising	1.5	27.3	7.6	1	Barbara Wueringer	
Australia	Queensland	Mapoon/Trout Beach	12.4	10	5	11/04/2019	-11.64433	141.8914	Rising	1.5	27.3	7.6	1	Barbara Wueringer	
Australia	Queensland	Mapoon/Trout Beach	12.5	10	5	11/04/2019	-11.64433	141.8914	Rising	1.5	27.3	7.6	1	Barbara Wueringer	
Australia	Queensland	Skardon Beach	13.1	10	5	12/04/2019	-	-	Falling	2.2	28	-	2	Barbara Wueringer	
Australia	Queensland	Skardon Beach	13.2	10	5	12/04/2019	-	-	Falling	2.2	28	-	2	Barbara Wueringer	
Australia	Queensland	Skardon Beach	13.3	10	5	12/04/2019	-	-	Falling	2.2	28	-	2	Barbara Wueringer	
Australia	Queensland	Skardon Beach	13.4	10	5	12/04/2019	-	-	Falling	2.2	28	-	2	Barbara Wueringer	
Australia	Queensland	Skardon Beach	13.5	10	5	12/04/2019	-	-	Falling	2.2	28	-	2	Barbara Wueringer	
Australia	Queensland	Darn Flats, Port Musgrave	14.1	20	5	14/04/2019	-	-	Falling	0.8	-	-	20	Barbara Wueringer	
Australia	Queensland	Darn Flats, Port Musgrave	14.2	20	5	14/04/2019	-	-	Falling	0.8	-	-	20	Barbara Wueringer	
Australia	Queensland	Darn Flats, Port Musgrave	14.3	20	5	14/04/2019	-	-	Falling	0.8	-	-	20	Barbara Wueringer	
Australia	Queensland	Darn Flats, Port Musgrave	14.4	20	5	14/04/2019	-	-	Falling	0.8	-	-	20	Barbara Wueringer	
Australia	Queensland	Darn Flats, Port Musgrave	14.5	20	5	14/04/2019	-	-	Falling	0.8	-	-	20	Barbara Wueringer	
Australia	Queensland	Darn Flats, Port Musgrave	14.6	1.2	1	14/04/2019	-	-	Falling	0.8	-	-	20	Barbara Wueringer	
Costa Rica	Central Pacific	Tempisque	20.1	20	0.3	15/04/2019	10.30581	-85.30579	Falling	4.7	30	NA	0.2	Jorge V, Natalia M, Jacinto Carrillo	
Costa Rica	Central Pacific	Tempisque	20.2	20	0.7	15/04/2019	10.30581	-85.30579	Falling	4.7	30	NA	0.2	Jorge V, Natalia M, Jacinto Carrillo	
Costa Rica	Central Pacific	Tempisque	20.3	20	0.9	15/04/2019	10.30581	-85.30579	Falling	4.7	30	NA	0.2	Jorge V, Natalia M, Jacinto Carrillo	

Costa Rica	Central Pacific	Tempisque	20.4	20	1	15/04/2019	10.30581	-85.30579	Falling	4.7	30	NA	0.2	Jorge V, Natalia M, Jacinto Carrillo	
Costa Rica	Central Pacific	Tempisque	20.5	20	0.9	15/04/2019	10.30581	-85.30579	Falling	4.7	30	NA	0.2	Jorge V, Natalia M, Jacinto Carrillo	
Costa Rica	Central Pacific	Palo Seco	21.1	10	7	16/04/2019	9.47074	-84.23907	Falling	2.6	30.2	NA	2.6	Jorge V, Natalia M, Carlos Viales	
Costa Rica	Central Pacific	Palo Seco	21.2	10	5	16/04/2019	9.47074	-84.23907	Falling	2.6	30.2	NA	2.6	Jorge V, Natalia M, Carlos Viales	
Costa Rica	Central Pacific	Palo Seco	21.3	10	5.4	16/04/2019	9.47074	-84.23907	Falling	2.6	30.2	NA	2.6	Jorge V, Natalia M, Carlos Viales	
Costa Rica	Central Pacific	Palo Seco	21.4	10	5.5	16/04/2019	9.47074	-84.23907	Falling	2.6	30.2	NA	2.6	Jorge V, Natalia M, Carlos Viales	
Costa Rica	Central Pacific	Palo Seco	21.5	10	5	16/04/2019	9.47074	-84.23907	Falling	2.6	30.2	NA	2.6	Jorge V, Natalia M, Carlos Viales	
Costa Rica	Central Pacific	Palo Seco	22.1	10	5	16/04/2019	9.47398	-84.24294	Rising	0.6	32	NA	0.6	Jorge V, Natalia M, Carlos Viales	
Costa Rica	Central Pacific	Palo Seco	22.2	10	5	16/04/2019	9.47398	-84.24294	Rising	0.6	32	NA	0.6	Jorge V, Natalia M, Carlos Viales	
Costa Rica	Central Pacific	Palo Seco	22.3	10	5	16/04/2019	9.47398	-84.24294	Rising	0.6	32	NA	0.6	Jorge V, Natalia M, Carlos Viales	
Costa Rica	Central Pacific	Palo Seco	22.4	10	5	16/04/2019	9.47398	-84.24294	Rising	0.6	32	NA	0.6	Jorge V, Natalia M, Carlos Viales	
Costa Rica	Central Pacific	Palo Seco	22.5	10	5	16/04/2019	9.47398	-84.24294	Rising	0.6	32	NA	0.6	Jorge V, Natalia M, Carlos Viales	
Costa Rica	Central Pacific	Palo Seco	23.1	10	5.5	16/04/2019	9.47836	-84.24993	Rising	0.8	32	NA	0.8	Jorge V, Natalia M, Carlos Viales	
Costa Rica	Central Pacific	Palo Seco	23.2	10	5	16/04/2019	9.47836	-84.24993	Rising	0.8	32	NA	0.8	Jorge V, Natalia M, Carlos Viales	
Costa Rica	Central Pacific	Palo Seco	23.3	10	5	16/04/2019	9.47836	-84.24993	Rising	0.8	32	NA	0.8	Jorge V, Natalia M, Carlos Viales	
Costa Rica	Central Pacific	Palo Seco	23.4	10	6	16/04/2019	9.47836	-84.24993	Rising	0.8	32	NA	0.8	Jorge V, Natalia M, Carlos Viales	
Costa Rica	Central Pacific	Palo Seco	23.5	10	5.5	16/04/2019	9.47836	-84.24993	Rising	0.8	32	NA	0.8	Jorge V, Natalia M, Carlos Viales	
Costa Rica	Central Pacific	Palo Seco	24.1	10	5	17/04/2019	9.4808	-84.2541	Rising	0.4	31.5	NA	0.4	Jorge V, Natalia M, Carlos Viales	
Costa Rica	Central Pacific	Palo Seco	24.2	10	5	17/04/2019	9.4808	-84.2541	Rising	0.4	31.5	NA	0.4	Jorge V, Natalia M, Carlos Viales	
Costa Rica	Central Pacific	Palo Seco	24.3	10	4	17/04/2019	9.4808	-84.2541	Rising	0.4	31.5	NA	0.4	Jorge V, Natalia M, Carlos Viales	
Costa Rica	Central Pacific	Palo Seco	24.4	10	4	17/04/2019	9.4808	-84.2541	Rising	0.4	31.5	NA	0.4	Jorge V, Natalia M, Carlos Viales	
Costa Rica	Central Pacific	Palo Seco	24.5	10	4	17/04/2019	9.4808	-84.2541	Rising	0.4	31.5	NA	0.4	Jorge V, Natalia M, Carlos Viales	
Costa Rica	Central Pacific	Palo Seco	25.1	10	4	17/04/2019	9.48221	-84.26725	Rising	0.8	33.4	NA	0.8	Jorge V, Natalia M, Carlos Viales	
Costa Rica	Central Pacific	Palo Seco	25.2	10	4	17/04/2019	9.48221	-84.26725	Rising	0.8	33.4	NA	0.8	Jorge V, Natalia M, Carlos Viales	
Costa Rica	Central Pacific	Palo Seco	25.3	10	4	17/04/2019	9.48221	-84.26725	Rising	0.8	33.4	NA	0.8	Jorge V, Natalia M, Carlos Viales	
Costa Rica	Central Pacific	Palo Seco	25.4	10	3.5	17/04/2019	9.48221	-84.26725	Rising	0.8	33.4	NA	0.8	Jorge V, Natalia M, Carlos Viales	
Costa Rica	Central Pacific	Palo Seco	25.5	10	3.5	17/04/2019	9.48221	-84.26725	Rising	0.8	33.4	NA	0.8	Jorge V, Natalia M, Carlos Viales	
Costa Rica	Central Pacific	Palo Seco	26.1	10	1.5	18/04/2019	9.48351	-84.27771	Falling	0.8	31.8	NA	0.5	Jorge V, Natalia M, Carlos Viales	
Costa Rica	Central Pacific	Palo Seco	26.2	20	3	18/04/2019	9.48351	-84.27771	Falling	0.8	31.8	NA	0.5	Jorge V, Natalia M, Carlos Viales	
Costa Rica	Central Pacific	Palo Seco	26.3	20	3	18/04/2019	9.48351	-84.27771	Falling	0.8	31.8	NA	0.5	Jorge V, Natalia M, Carlos Viales	
Costa Rica	Central Pacific	Palo Seco	26.4	20	3	18/04/2019	9.48351	-84.27771	Falling	0.8	31.8	NA	0.5	Jorge V, Natalia M, Carlos Viales	

Costa Rica	Central Pacific	Palo Seco	26.5	20	3	18/04/2019	9.48351	-84.27771	Falling	0.8	31.8	NA	0.5	Jorge V, Natalia M, Carlos Viales	
Costa Rica	Central Pacific	Palo Seco	27.1	20	5	18/04/2019	9.48539	-84.2914	Rising	1.6	31.9	NA	0.7	Jorge V, Natalia M, Carlos Viales	
Costa Rica	Central Pacific	Palo Seco	27.2	10	1.5	18/04/2019	9.48539	-84.2914	Rising	1.6	31.9	NA	0.7	Jorge V, Natalia M, Carlos Viales	
Costa Rica	Central Pacific	Palo Seco	27.3	20	2	18/04/2019	9.48539	-84.2914	Rising	1.6	31.9	NA	0.7	Jorge V, Natalia M, Carlos Viales	
Costa Rica	Central Pacific	Palo Seco	27.4	20	2	18/04/2019	9.48539	-84.2914	Rising	1.6	31.9	NA	0.7	Jorge V, Natalia M, Carlos Viales	
Costa Rica	Central Pacific	Palo Seco	27.5	20	2.5	18/04/2019	9.48539	-84.2914	Rising	1.6	31.9	NA	0.7	Jorge V, Natalia M, Carlos Viales	
Singapore	South West Distirct	Berlayer Creek	1.1	10	1.6	1/05/2019	1.26556	103.8069	Falling	0.2	-	-	1	Madalyn Cooper, Leah Carr	
Singapore	South West Distirct	Berlayer Creek	1.2	10	0.9	1/05/2019	1.26556	103.8069	Falling	0.2	-	-	1	Madalyn Cooper, Leah Carr	
Singapore	South West Distirct	Berlayer Creek	1.3	10	1.2	1/05/2019	1.26556	103.8069	Falling	0.2	-	-	1	Madalyn Cooper, Leah Carr	
Singapore	South West Distirct	Berlayer Creek	1.4	10	0.8	1/05/2019	1.26556	103.8069	Falling	0.2	-	-	1	Madalyn Cooper, Leah Carr	
Singapore	South West Distirct	Berlayer Creek	1.5	10	1	1/05/2019	1.26556	103.8069	Falling	0.2	-	-	1	Madalyn Cooper, Leah Carr	
Singapore	South West Distirct	Berlayer Creek	1.6	1.2	1	1/05/2019	1.26556	103.8069	Falling	0.2	-	-	1	Madalyn Cooper, Leah Carr	
Singapore	South West Distirct	Berlayer Creek	1.7	1.2	2	1/05/2019	1.26556	103.8069	Falling	0.2	-	-	1	Madalyn Cooper, Leah Carr	
Singapore	South West Distirct	Berlayer Creek	1.8	1.2	2	1/05/2019	1.26556	103.8069	Falling	0.2	-	-	1	Madalyn Cooper, Leah Carr	
Singapore	South East District	Sungei Jelutong, Palau Ubin	2.1	10	1.4	2/05/2019	1.40277	103.9574	High slack	3	-	-	<0.3	Madalyn Cooper, Leah Carr	
Singapore	South East District	Sungei Jelutong, Palau Ubin	2.2	10	1.65	2/05/2019	1.40277	103.9574	High slack	3	-	-	<0.3	Madalyn Cooper, Leah Carr	
Singapore	South East District	Sungei Jelutong, Palau Ubin	2.3	10	1.82	2/05/2019	1.40277	103.9574	High slack	3	-	-	<0.3	Madalyn Cooper, Leah Carr	
Singapore	South East District	Sungei Jelutong, Palau Ubin	2.4	10	2.11	2/05/2019	1.40277	103.9574	High slack	3	-	-	<0.3	Madalyn Cooper, Leah Carr	
Singapore	South East District	Sungei Jelutong, Palau Ubin	2.5	10	2	2/05/2019	1.40277	103.9574	High slack	3	-	-	<0.3	Madalyn Cooper, Leah Carr	
Singapore	South East District	Sungei Jelutong, Palau Ubin	2.6	1.2	0.5	2/05/2019	1.40277	103.9574	High slack	3	-	-	<0.3	Madalyn Cooper, Leah Carr	
Singapore	South East District	Sungei Jelutong, Palau Ubin	2.7	1.2	0.5	2/05/2019	1.40277	103.9574	High slack	3	-	-	<0.3	Madalyn Cooper, Leah Carr	
Singapore	South East District	Sungei Jelutong, Palau Ubin	2.8	1.2	0.5	2/05/2019	1.40277	103.9574	High slack	3	-	-	<0.3	Madalyn Cooper, Leah Carr	
Singapore	South East District	Sungei Puaka, Palau Ubin	3.1	10	5	2/05/2019	1.40795	103.9523	High falling	2	-	-	<0.3	Madalyn Cooper, Leah Carr	
Singapore	South East District	Sungei Puaka, Palau Ubin	3.2	10	4.25	2/05/2019	1.40795	103.9523	High falling	2	-	-	<0.3	Madalyn Cooper, Leah Carr	
Singapore	South East District	Sungei Puaka, Palau Ubin	3.3	10	3	2/05/2019	1.40795	103.9523	High falling	2	-	-	<0.3	Madalyn Cooper, Leah Carr	
Singapore	South East District	Sungei Puaka, Palau Ubin	3.4	10	3	2/05/2019	1.40795	103.9523	High falling	2	-	-	<0.3	Madalyn Cooper, Leah Carr	
Singapore	South East District	Sungei Puaka, Palau Ubin	3.5	10	3	2/05/2019	1.40795	103.9523	High falling	2	-	-	<0.3	Madalyn Cooper, Leah Carr	

Singapore	South East District	Sungei Puaka, Palau Ubin	3.6	1.2	0.75	2/05/2019	1.40795	103.9523	High falling	2	-	-	<0.3	Madalyn Cooper, Leah Carr	
Singapore	South East District	Sungei Puaka, Palau Ubin	3.7	1.2	1.25	2/05/2019	1.40795	103.9523	High falling	2	-	-	<0.3	Madalyn Cooper, Leah Carr	
Singapore	South East District	Sungei Puaka, Palau Ubin	3.8	1.2	0.76	2/05/2019	1.40795	103.9523	High falling	2	-	-	<0.3	Madalyn Cooper, Leah Carr	
Singapore	South East District	Sungei Perances, Palau Ubin	4.1	10	5	2/05/2019	1.40586	103.9739	Falling	2	-	-	<0.3	Madalyn Cooper, Leah Carr	
Singapore	South East District	Sungei Perances, Palau Ubin	4.2	10	1.75	2/05/2019	1.40586	103.9739	Falling	2	-	-	<0.3	Madalyn Cooper, Leah Carr	
Singapore	South East District	Sungei Perances, Palau Ubin	4.3	10	2	2/05/2019	1.40586	103.9739	Falling	2	-	-	<0.3	Madalyn Cooper, Leah Carr	
Singapore	South East District	Sungei Perances, Palau Ubin	4.4	10	3	2/05/2019	1.40586	103.9739	Falling	2	-	-	<0.3	Madalyn Cooper, Leah Carr	
Singapore	South East District	Sungei Perances, Palau Ubin	4.5	10	1.5	2/05/2019	1.40586	103.9739	Falling	2	-	-	<0.3	Madalyn Cooper, Leah Carr	
Singapore	South East District	Sungei Perances, Palau Ubin	4.6	1.2	3	2/05/2019	1.40586	103.9739	Falling	2	-	-	<0.3	Madalyn Cooper, Leah Carr	
Singapore	South East District	Sungei Perances, Palau Ubin	4.7	1.2	2.25	2/05/2019	1.40586	103.9739	Falling	2	-	-	<0.3	Madalyn Cooper, Leah Carr	
Singapore	South East District	Sungei Perances, Palau Ubin	4.8	1.2	0.9	2/05/2019	1.40586	103.9739	Falling	2	-	-	<0.3	Madalyn Cooper, Leah Carr	
Singapore	North West District	Sungei Buloh Besar, Kranji	5.1	10	1.2	3/05/2019	1.44966	103.7290	High slack	<1	-	-	<1	Madalyn Cooper, Leah Carr	
Singapore	North West District	Sungei Buloh Besar, Kranji	5.2	10	1.32	3/05/2019	1.44966	103.7290	High slack	<1	-	-	<1	Madalyn Cooper, Leah Carr	
Singapore	North West District	Sungei Buloh Besar, Kranji	5.3	10	1.93	3/05/2019	1.44966	103.7290	High slack	<1	-	-	<1	Madalyn Cooper, Leah Carr	
Singapore	North West District	Sungei Buloh Besar, Kranji	5.4	10	1.5	3/05/2019	1.44966	103.7290	High slack	<1	-	-	<1	Madalyn Cooper, Leah Carr	
Singapore	North West District	Sungei Buloh Besar, Kranji	5.5	10	1.32	3/05/2019	1.44966	103.7290	High slack	<1	-	-	<1	Madalyn Cooper, Leah Carr	
Singapore	North West District	Sungei Buloh Besar, Kranji	5.6	1.2	0.32	3/05/2019	1.44966	103.7290	High slack	<1	-	-	<1	Madalyn Cooper, Leah Carr	
Singapore	North West District	Sungei Buloh Besar, Kranji	5.7	1.2	0.2	3/05/2019	1.44966	103.7290	High slack	<1	-	-	<1	Madalyn Cooper, Leah Carr	
Singapore	North West District	Sungei Buloh Besar, Kranji	5.8	1.2	0.3	3/05/2019	1.44966	103.7290	High slack	<1	-	-	<1	Madalyn Cooper, Leah Carr	
Singapore	North West District	Sungei Billabong Buloh, Kranji	6.1	10	1.56	3/05/2019	1.44936	103.7269	Falling	<1	-	-	<0.3	Madalyn Cooper, Leah Carr	
Singapore	North West District	Sungei Billabong Buloh, Kranji	6.2	10	1.3	3/05/2019	1.44936	103.7269	Falling	<1	-	-	<0.3	Madalyn Cooper, Leah Carr	
Singapore	North West District	Sungei Billabong Buloh, Kranji	6.3	10	1.6	3/05/2019	1.44936	103.7269	Falling	<1	-	-	<0.3	Madalyn Cooper, Leah Carr	
Singapore	North West District	Sungei Billabong Buloh, Kranji	6.4	10	1	3/05/2019	1.44936	103.7269	Falling	<1	-	-	<0.3	Madalyn Cooper, Leah Carr	
Singapore	North West District	Sungei Billabong Buloh, Kranji	6.5	10	2.275	3/05/2019	1.44936	103.7269	Falling	<1	-	-	<0.3	Madalyn Cooper, Leah Carr	

Singapore	North West District	Sungei Billabong Buloh, Kranji	6.6	1.2	0.35	3/05/2019	1.44936	103.7269	Falling	<1	-	-	<0.3	Madalyn Cooper, Leah Carr	
Singapore	North West District	Sungei Billabong Buloh, Kranji	6.7	1.2	0.2	3/05/2019	1.44936	103.7269	Falling	<1	-	-	<0.3	Madalyn Cooper, Leah Carr	
Singapore	North West District	Sungei Billabong Buloh, Kranji	6.8	1.2	0.3	3/05/2019	1.44936	103.7269	Falling	<1	-	-	<0.3	Madalyn Cooper, Leah Carr	
Singapore	North West District	Sungei Buloh Coastal trail, Kranji	7.1	10	1.15	3/05/2019	1.44657	103.7323	Falling low	0.3	-	-	<0.3	Madalyn Cooper, Leah Carr	
Singapore	North West District	Sungei Buloh Coastal trail, Kranji	7.2	10	0.95	3/05/2019	1.44657	103.7323	Falling low	0.3	-	-	<0.3	Madalyn Cooper, Leah Carr	
Singapore	North West District	Sungei Buloh Coastal trail, Kranji	7.3	10	1.04	3/05/2019	1.44657	103.7323	Falling low	0.3	-	-	<0.3	Madalyn Cooper, Leah Carr	
Singapore	North West District	Sungei Buloh Coastal trail, Kranji	7.4	10	1.25	3/05/2019	1.44657	103.7323	Falling low	0.3	-	-	<0.3	Madalyn Cooper, Leah Carr	
Singapore	North West District	Sungei Buloh Coastal trail, Kranji	7.5	10	1.4	3/05/2019	1.44657	103.7323	Falling low	0.3	-	-	<0.3	Madalyn Cooper, Leah Carr	
Singapore	North West District	Sungei Buloh Coastal trail, Kranji	7.6	1.2	0.5	3/05/2019	1.44657	103.7323	Falling low	0.3	-	-	<0.3	Madalyn Cooper, Leah Carr	
Singapore	North West District	Sungei Buloh Coastal trail, Kranji	7.7	1.2	0.5	3/05/2019	1.44657	103.7323	Falling low	0.3	-	-	<0.3	Madalyn Cooper, Leah Carr	
Singapore	North West District	Sungei Buloh Coastal trail, Kranji	7.8	1.2	0.5	3/05/2019	1.44657	103.7323	Falling low	0.3	-	-	<0.3	Madalyn Cooper, Leah Carr	
Vietnam	Ben Tre Province	Mekong River 1, northern tributary	1.1	20	1.56	6/05/2019	10.00113	106.5666	Rising	1	-	-	<0.2	Madalyn Cooper, Leah Carr, Trần Nguyễn	
Vietnam	Ben Tre Province	Mekong River 1, northern tributary	1.2	20	1.95	6/05/2019	10.00113	106.5666	Rising	1	-	-	<0.2	Madalyn Cooper, Leah Carr, Trần Nguyễn	
Vietnam	Ben Tre Province	Mekong River 1, northern tributary	1.3	20	1.5	6/05/2019	10.00113	106.5666	Rising	1	-	-	<0.2	Madalyn Cooper, Leah Carr, Trần Nguyễn	
Vietnam	Ben Tre Province	Mekong River 1, northern tributary	1.4	20	1	6/05/2019	10.00113	106.5666	Rising	1	-	-	<0.2	Madalyn Cooper, Leah Carr, Trần Nguyễn	
Vietnam	Ben Tre Province	Mekong River 1, northern tributary	1.5	20	1.25	6/05/2019	10.00113	106.5666	Rising	1	-	-	<0.2	Madalyn Cooper, Leah Carr, Trần Nguyễn	
Vietnam	Ben Tre Province	Mekong River 1, northern tributary	1.6	1.2	0.2	6/05/2019	10.00113	106.5666	Rising	1	-	-	<0.2	Madalyn Cooper, Leah Carr, Trần Nguyễn	
Vietnam	Ben Tre Province	Mekong River 1, northern tributary	1.7	1.2	0.2	6/05/2019	10.00113	106.5666	Rising	1	-	-	<0.2	Madalyn Cooper, Leah Carr, Trần Nguyễn	
Vietnam	Ben Tre Province	Mekong River 1, northern tributary	1.8	1.2	0.2	6/05/2019	10.00113	106.5666	Rising	1	-	-	<0.2	Madalyn Cooper, Leah Carr, Trần Nguyễn	
Vietnam	Ben Tre Province	Mekong River 2, southern tributary	2.1	20	3	6/05/2019	9.97334	106.5786	Rising high	0.3	-	-	<0.3	Madalyn Cooper, Leah Carr, Trần Nguyễn	
Vietnam	Ben Tre Province	Mekong River 2, southern tributary	2.2	20	1.95	6/05/2019	9.97334	106.5786	Rising high	0.3	-	-	<0.3	Madalyn Cooper, Leah Carr, Trần Nguyễn	

Vietnam	Ben Tre Province	Mekong River 2, southern tributary	2.3	20	1.5	6/05/2019	9.97334	106.5786	Rising high	0.3	-	-	<0.3	Madalyn Cooper, Leah Carr, Trân Nguyễn	
Vietnam	Ben Tre Province	Mekong River 2, southern tributary	2.4	20	1.15	6/05/2019	9.97334	106.5786	Rising high	0.3	-	-	<0.3	Madalyn Cooper, Leah Carr, Trân Nguyễn	
Vietnam	Ben Tre Province	Mekong River 2, southern tributary	2.5	20	0.88	6/05/2019	9.97334	106.5786	Rising high	0.3	-	-	<0.3	Madalyn Cooper, Leah Carr, Trân Nguyễn	
Vietnam	Ben Tre Province	Mekong River 2, southern tributary	2.6	1.2	0.2	6/05/2019	9.97334	106.5786	Rising high	0.3	-	-	<0.3	Madalyn Cooper, Leah Carr, Trân Nguyễn	
Vietnam	Ben Tre Province	Mekong River 2, southern tributary	2.7	1.2	0.2	6/05/2019	9.97334	106.5786	Rising high	0.3	-	-	<0.3	Madalyn Cooper, Leah Carr, Trân Nguyễn	
Vietnam	Ben Tre Province	Mekong River 2, southern tributary	2.8	1.2	0.55	6/05/2019	9.97334	106.5786	Rising high	0.3	-	-	<0.3	Madalyn Cooper, Leah Carr, Trân Nguyễn	
Vietnam	Ben Tre Province	Mekong River 3	3.1	20	0.41	6/05/2019	10.01339	106.4849	Falling	2	-	-	<0.3	Madalyn Cooper, Leah Carr, Trân Nguyễn	
Vietnam	Ben Tre Province	Mekong River 3	3.2	20	1.1	6/05/2019	10.01339	106.4849	Falling	2	-	-	<0.3	Madalyn Cooper, Leah Carr, Trân Nguyễn	
Vietnam	Ben Tre Province	Mekong River 3	3.3	20	1.1	6/05/2019	10.01339	106.4849	Falling	2	-	-	<0.3	Madalyn Cooper, Leah Carr, Trân Nguyễn	
Vietnam	Ben Tre Province	Mekong River 3	3.4	20	0.52	6/05/2019	10.01339	106.4849	Falling	2	-	-	<0.3	Madalyn Cooper, Leah Carr, Trân Nguyễn	
Vietnam	Ben Tre Province	Mekong River 3	3.5	20	0.45	6/05/2019	10.01339	106.4849	Falling	2	-	-	<0.3	Madalyn Cooper, Leah Carr, Trân Nguyễn	
Vietnam	Ben Tre Province	Mekong River 3	3.6	1.2	0.25	6/05/2019	10.01339	106.4849	Falling	2	-	-	<0.3	Madalyn Cooper, Leah Carr, Trân Nguyễn	
Vietnam	Ben Tre Province	Mekong River 3	3.7	1.2	0.25	6/05/2019	10.01339	106.4849	Falling	2	-	-	<0.3	Madalyn Cooper, Leah Carr, Trân Nguyễn	
Vietnam	Ben Tre Province	Mekong River 3	3.8	1.2	0.25	6/05/2019	10.01339	106.4849	Falling	2	-	-	<0.3	Madalyn Cooper, Leah Carr, Trân Nguyễn	
Vietnam	Soc Trang Province	Mekong River 4	4.1	20	5	7/05/2019	9.54107	106.2137	Falling	<1	-	-	<0.2	Madalyn Cooper, Leah Carr, Trân Nguyễn, Son Levis	
Vietnam	Soc Trang Province	Mekong River 4	4.2	20	5	7/05/2019	9.54107	106.2137	Falling	<1	-	-	<0.2	Madalyn Cooper, Leah Carr, Trân Nguyễn, Son Levis	
Vietnam	Soc Trang Province	Mekong River 4	4.3	20	5	7/05/2019	9.54107	106.2137	Falling	<1	-	-	<0.2	Madalyn Cooper, Leah Carr, Trân Nguyễn, Son Levis	
Vietnam	Soc Trang Province	Mekong River 4	4.4	20	5	7/05/2019	9.54107	106.2137	Falling	<1	-	-	<0.2	Madalyn Cooper, Leah Carr, Trân Nguyễn, Son Levis	
Vietnam	Soc Trang Province	Mekong River 4	4.5	20	5	7/05/2019	9.54107	106.2137	Falling	<1	-	-	<0.2	Madalyn Cooper, Leah Carr, Trân Nguyễn, Son Levis	
Vietnam	Soc Trang Province	Mekong River 4	4.6	1.2	0.35	7/05/2019	9.54107	106.2137	Falling	<1	-	-	<0.2	Madalyn Cooper, Leah Carr, Trân Nguyễn, Son Levis	
Vietnam	Soc Trang Province	Mekong River 4	4.7	1.2	0.375	7/05/2019	9.54107	106.2137	Falling	<1	-	-	<0.2	Madalyn Cooper, Leah Carr, Trân Nguyễn, Son Levis	

Vietnam	Soc Trang Province	Mekong River 4	4.8	1.2	0.375	7/05/2019	9.54107	106.2137	Falling	<1	-	-	<0.2	Madalyn Cooper, Leah Carr, Trân Nguyễn, Son Levis	
Vietnam	Soc Trang Province	Mekong River 5	5.1	20	4.2	7/05/2019	9.50123	106.2118	Slack low	<0.3	-	-	<0.1	Madalyn Cooper, Leah Carr, Trân Nguyễn, Son Levis	
Vietnam	Soc Trang Province	Mekong River 5	5.2	20	2.1	7/05/2019	9.50123	106.2118	Slack low	<0.3	-	-	<0.1	Madalyn Cooper, Leah Carr, Trân Nguyễn, Son Levis	
Vietnam	Soc Trang Province	Mekong River 5	5.3	20	1.2	7/05/2019	9.50123	106.2118	Slack low	<0.3	-	-	<0.1	Madalyn Cooper, Leah Carr, Trân Nguyễn, Son Levis	
Vietnam	Soc Trang Province	Mekong River 5	5.4	20	0.3	7/05/2019	9.50123	106.2118	Slack low	<0.3	-	-	<0.1	Madalyn Cooper, Leah Carr, Trân Nguyễn, Son Levis	
Vietnam	Soc Trang Province	Mekong River 5	5.5	20	0.15	7/05/2019	9.50123	106.2118	Slack low	<0.3	-	-	<0.1	Madalyn Cooper, Leah Carr, Trân Nguyễn, Son Levis	
Vietnam	Soc Trang Province	Mekong River 5	5.6	1.2	0.3	7/05/2019	9.50123	106.2118	Slack low	<0.3	-	-	<0.1	Madalyn Cooper, Leah Carr, Trân Nguyễn, Son Levis	
Vietnam	Soc Trang Province	Mekong River 5	5.7	1.2	0.3	7/05/2019	9.50123	106.2118	Slack low	<0.3	-	-	<0.1	Madalyn Cooper, Leah Carr, Trân Nguyễn, Son Levis	
Vietnam	Soc Trang Province	Mekong River 5	5.8	1.2	0.275	7/05/2019	9.50123	106.2118	Slack low	<0.3	-	-	<0.1	Madalyn Cooper, Leah Carr, Trân Nguyễn, Son Levis	
Vietnam	Soc Trang Province	Mekong River 6	6.1	20	3.45	7/05/2019	9.50900	106.2275	RIsing	<1	-	-	<0.3	Madalyn Cooper, Leah Carr, Trân Nguyễn, Son Levis	
Vietnam	Soc Trang Province	Mekong River 6	6.2	20	1.58	7/05/2019	9.50900	106.2275	RIsing	<1	-	-	<0.3	Madalyn Cooper, Leah Carr, Trân Nguyễn, Son Levis	
Vietnam	Soc Trang Province	Mekong River 6	6.3	20	1.62	7/05/2019	9.50900	106.2275	RIsing	<1	-	-	<0.3	Madalyn Cooper, Leah Carr, Trân Nguyễn, Son Levis	
Vietnam	Soc Trang Province	Mekong River 6	6.4	20	1.6	7/05/2019	9.50900	106.2275	RIsing	<1	-	-	<0.3	Madalyn Cooper, Leah Carr, Trân Nguyễn, Son Levis	
Vietnam	Soc Trang Province	Mekong River 6	6.5	20	1	7/05/2019	9.50900	106.2275	RIsing	<1	-	-	<0.3	Madalyn Cooper, Leah Carr, Trân Nguyễn, Son Levis	
Vietnam	Soc Trang Province	Mekong River 6	6.6	1.2	0.5	7/05/2019	9.50900	106.2275	RIsing	<1	-	-	<0.3	Madalyn Cooper, Leah Carr, Trân Nguyễn, Son Levis	
Vietnam	Soc Trang Province	Mekong River 6	6.7	1.2	0.5	7/05/2019	9.50900	106.2275	RIsing	<1	-	-	<0.3	Madalyn Cooper, Leah Carr, Trân Nguyễn, Son Levis	
Vietnam	Soc Trang Province	Mekong River 6	6.8	1.2	0.5	7/05/2019	9.50900	106.2275	RIsing	<1	-	-	<0.3	Madalyn Cooper, Leah Carr, Trân Nguyễn, Son Levis	
Vietnam	Soc Trang Province	Mekong River 7	7.1	20	0.4	7/05/2019	9.51387	106.2259	Rising	1.3	-	-	<0.3	Madalyn Cooper, Leah Carr, Trân Nguyễn, Son Levis	
Vietnam	Soc Trang Province	Mekong River 7	7.2	20	0.25	7/05/2019	9.51387	106.2259	Rising	1.3	-	-	<0.3	Madalyn Cooper, Leah Carr, Trân Nguyễn, Son Levis	
Vietnam	Soc Trang Province	Mekong River 7	7.3	20	0.3	7/05/2019	9.51387	106.2259	Rising	1.3	-	-	<0.3	Madalyn Cooper, Leah Carr, Trân Nguyễn, Son Levis	
Vietnam	Soc Trang Province	Mekong River 7	7.4	20	0.65	7/05/2019	9.51387	106.2259	Rising	1.3	-	-	<0.3	Madalyn Cooper, Leah Carr, Trân Nguyễn, Son Levis	

Vietnam	Soc Trang Province	Mekong River 7	7.5	20	0.75	7/05/2019	9.51387	106.2259	Rising	1.3	-	-	<0.3	Madalyn Cooper, Leah Carr, Trân Nguyễn, Son Levis	
Vietnam	Soc Trang Province	Mekong River 7	7.6	1.2	0.3	7/05/2019	9.51387	106.2259	Rising	1.3	-	-	<0.3	Madalyn Cooper, Leah Carr, Trân Nguyễn, Son Levis	
Vietnam	Soc Trang Province	Mekong River 7	7.7	1.2	0.2	7/05/2019	9.51387	106.2259	Rising	1.3	-	-	<0.3	Madalyn Cooper, Leah Carr, Trân Nguyễn, Son Levis	
Vietnam	Soc Trang Province	Mekong River 7	7.8	1.2	0.2	7/05/2019	9.51387	106.2259	Rising	1.3	-	-	<0.3	Madalyn Cooper, Leah Carr, Trân Nguyễn, Son Levis	
Vietnam	Soc Trang Province	Mekong River 8	8.1	20	1.1	8/05/2019	9.59455	106.2622	Falling	<0.3	-	-	<0.1	Madalyn Cooper, Leah Carr, Trân Nguyễn, Son Levis	
Vietnam	Soc Trang Province	Mekong River 8	8.2	20	0.6	8/05/2019	9.59455	106.2622	Falling	<0.3	-	-	<0.1	Madalyn Cooper, Leah Carr, Trân Nguyễn, Son Levis	
Vietnam	Soc Trang Province	Mekong River 8	8.3	20	0.71	8/05/2019	9.59455	106.2622	Falling	<0.3	-	-	<0.1	Madalyn Cooper, Leah Carr, Trân Nguyễn, Son Levis	
Vietnam	Soc Trang Province	Mekong River 8	8.4	20	1.6	8/05/2019	9.59455	106.2622	Falling	<0.3	-	-	<0.1	Madalyn Cooper, Leah Carr, Trân Nguyễn, Son Levis	
Vietnam	Soc Trang Province	Mekong River 8	8.5	20	1.55	8/05/2019	9.59455	106.2622	Falling	<0.3	-	-	<0.1	Madalyn Cooper, Leah Carr, Trân Nguyễn, Son Levis	
Vietnam	Soc Trang Province	Mekong River 8	8.6	1.2	0.3	8/05/2019	9.59455	106.2622	Falling	<0.3	-	-	<0.1	Madalyn Cooper, Leah Carr, Trân Nguyễn, Son Levis	
Vietnam	Soc Trang Province	Mekong River 8	8.7	1.2	0.2	8/05/2019	9.59455	106.2622	Falling	<0.3	-	-	<0.1	Madalyn Cooper, Leah Carr, Trân Nguyễn, Son Levis	
Vietnam	Soc Trang Province	Mekong River 8	8.8	1.2	0.2	8/05/2019	9.59455	106.2622	Falling	<0.3	-	-	<0.1	Madalyn Cooper, Leah Carr, Trân Nguyễn, Son Levis	
Vietnam	Soc Trang Province	Mekong River 9	9.1	20	0.75	8/05/2019	9.57081	106.3479	Rising	0.3	-	-	<0.3	Madalyn Cooper, Leah Carr, Trân Nguyễn, Son Levis	
Vietnam	Soc Trang Province	Mekong River 9	9.2	20	0.95	8/05/2019	9.57081	106.3479	Rising	0.3	-	-	<0.3	Madalyn Cooper, Leah Carr, Trân Nguyễn, Son Levis	
Vietnam	Soc Trang Province	Mekong River 9	9.3	20	0.5	8/05/2019	9.57081	106.3479	Rising	0.3	-	-	<0.3	Madalyn Cooper, Leah Carr, Trân Nguyễn, Son Levis	
Vietnam	Soc Trang Province	Mekong River 9	9.4	20	1.02	8/05/2019	9.57081	106.3479	Rising	0.3	-	-	<0.3	Madalyn Cooper, Leah Carr, Trân Nguyễn, Son Levis	
Vietnam	Soc Trang Province	Mekong River 9	9.5	20	0.13	8/05/2019	9.57081	106.3479	Rising	0.3	-	-	<0.3	Madalyn Cooper, Leah Carr, Trân Nguyễn, Son Levis	
Vietnam	Soc Trang Province	Mekong River 9	9.6	1.2	0.2	8/05/2019	9.57081	106.3479	Rising	0.3	-	-	<0.3	Madalyn Cooper, Leah Carr, Trân Nguyễn, Son Levis	
Vietnam	Soc Trang Province	Mekong River 9	9.7	1.2	0.2	8/05/2019	9.57081	106.3479	Rising	0.3	-	-	<0.3	Madalyn Cooper, Leah Carr, Trân Nguyễn, Son Levis	
Vietnam	Soc Trang Province	Mekong River 9	9.8	1.2	0.2	8/05/2019	9.57081	106.3479	Rising	0.3	-	-	<0.3	Madalyn Cooper, Leah Carr, Trân Nguyễn, Son Levis	
Myanmar	Ayeyarwady	Bogale River, Northern tip of	1.1	20	2.42	14/05/2019	16.08638	95.3236	Rising	-	-	-	<0.3	Madie Cooper, Leah Carr, U Soe Htun, U	

		Conservation Park												Tun Tun Win, U Myat Wai Yan Oo	
Myanmar	Ayeyarwady	Bogale River, Northern tip of Conservation Park	1.2	20	2.5	14/05/2019	16.08638	95.3236	Rising	-	-	-	<0.3	Madie Cooper, Leah Carr, U Soe Htun, U Tun Tun Win, U Myat Wai Yan Oo	
Myanmar	Ayeyarwady	Bogale River, Northern tip of Conservation Park	1.3	20	4	14/05/2019	16.08638	95.3236	Rising	-	-	-	<0.3	Madie Cooper, Leah Carr, U Soe Htun, U Tun Tun Win, U Myat Wai Yan Oo	
Myanmar	Ayeyarwady	Bogale River, Northern tip of Conservation Park	1.4	20	4.8	14/05/2019	16.08638	95.3236	Rising	-	-	-	<0.3	Madie Cooper, Leah Carr, U Soe Htun, U Tun Tun Win, U Myat Wai Yan Oo	
Myanmar	Ayeyarwady	Bogale River, Northern tip of Conservation Park	1.5	20	2.5	14/05/2019	16.08638	95.3236	Rising	-	-	-	<0.3	Madie Cooper, Leah Carr, U Soe Htun, U Tun Tun Win, U Myat Wai Yan Oo	
Myanmar	Ayeyarwady	Bogale River, Northern tip of Conservation Park	1.6	1.2	0.25	14/05/2019	16.08638	95.3236	Rising	-	-	-	<0.3	Madie Cooper, Leah Carr, U Soe Htun, U Tun Tun Win, U Myat Wai Yan Oo	
Myanmar	Ayeyarwady	Bogale River, Northern tip of Conservation Park	1.7	1.2	0.25	14/05/2019	16.08638	95.3236	Rising	-	-	-	<0.3	Madie Cooper, Leah Carr, U Soe Htun, U Tun Tun Win, U Myat Wai Yan Oo	
Myanmar	Ayeyarwady	Bogale River, Northern tip of Conservation Park	1.8	1.2	0.25	14/05/2019	16.08638	95.3236	Rising	-	-	-	<0.3	Madie Cooper, Leah Carr, U Soe Htun, U Tun Tun Win, U Myat Wai Yan Oo	
Myanmar	Ayeyarwady	Bogale River	2.1	20	1.65	14/05/2019	16.04544	95.2780	Rising	<.3	-	-	-	Madie Cooper, Leah Carr, U Soe Htun, U Tun Tun Win, U Myat Wai Yan Oo	
Myanmar	Ayeyarwady	Bogale River	2.2	20	1.15	14/05/2019	16.04544	95.2780	Rising	<.3	-	-	-	Madie Cooper, Leah Carr, U Soe Htun, U Tun Tun Win, U Myat Wai Yan Oo	
Myanmar	Ayeyarwady	Bogale River	2.3	20	1.16	14/05/2019	16.04544	95.2780	Rising	<.3	-	-	-	Madie Cooper, Leah Carr, U Soe Htun, U Tun Tun Win, U Myat Wai Yan Oo	
Myanmar	Ayeyarwady	Bogale River	2.4	20	2.1	14/05/2019	16.04544	95.2780	Rising	<.3	-	-	-	Madie Cooper, Leah Carr, U Soe Htun, U Tun Tun Win, U Myat Wai Yan Oo	
Myanmar	Ayeyarwady	Bogale River	2.5	20	1.9	14/05/2019	16.04544	95.2780	Rising	<.3	-	-	-	Madie Cooper, Leah Carr, U Soe Htun, U Tun Tun Win, U Myat Wai Yan Oo	
Myanmar	Ayeyarwady	Bogale River	2.6	1.2	0.3	14/05/2019	16.04544	95.2780	Rising	<.3	-	-	-	Madie Cooper, Leah Carr, U Soe Htun, U Tun Tun Win, U Myat Wai Yan Oo	
Myanmar	Ayeyarwady	Bogale River	2.7	1.2	0.25	14/05/2019	16.04544	95.2780	Rising	<.3	-	-	-	Madie Cooper, Leah Carr, U Soe Htun, U Tun Tun Win, U Myat Wai Yan Oo	
Myanmar	Ayeyarwady	Bogale River	2.8	1.2	0.3	14/05/2019	16.04544	95.2780	Rising	<.3	-	-	-	Madie Cooper, Leah Carr, U Soe Htun, U Tun Tun Win, U Myat Wai Yan Oo	
Myanmar	Ayeyarwady	Large tributary, Bogale River	3.1	20	1.3	14/05/2019	15.95421	95.2465	Rising	<0.3	-	-	<0.3	Madie Cooper, Leah Carr, U Soe Htun, U	

														Tun Tun Win, U Myat Wai Yan Oo	
Myanmar	Ayeyarwady	Large tributary, Bogale River	3.2	20	1.6	14/05/2019	15.95421	95.2465	Rising	<0.3	-	-	<0.3	Madie Cooper, Leah Carr, U Soe Htun, U Tun Tun Win, U Myat Wai Yan Oo	
Myanmar	Ayeyarwady	Large tributary, Bogale River	3.3	20	1.4	14/05/2019	15.95421	95.2465	Rising	<0.3	-	-	<0.3	Madie Cooper, Leah Carr, U Soe Htun, U Tun Tun Win, U Myat Wai Yan Oo	
Myanmar	Ayeyarwady	Large tributary, Bogale River	3.4	20	1.3	14/05/2019	15.95421	95.2465	Rising	<0.3	-	-	<0.3	Madie Cooper, Leah Carr, U Soe Htun, U Tun Tun Win, U Myat Wai Yan Oo	
Myanmar	Ayeyarwady	Large tributary, Bogale River	3.5	20	1.1	14/05/2019	15.95421	95.2465	Rising	<0.3	-	-	<0.3	Madie Cooper, Leah Carr, U Soe Htun, U Tun Tun Win, U Myat Wai Yan Oo	
Myanmar	Ayeyarwady	Large tributary, Bogale River	3.6	1.2	0.275	14/05/2019	15.95421	95.2465	Rising	<0.3	-	-	<0.3	Madie Cooper, Leah Carr, U Soe Htun, U Tun Tun Win, U Myat Wai Yan Oo	
Myanmar	Ayeyarwady	Large tributary, Bogale River	3.7	1.2	0.25	14/05/2019	15.95421	95.2465	Rising	<0.3	-	-	<0.3	Madie Cooper, Leah Carr, U Soe Htun, U Tun Tun Win, U Myat Wai Yan Oo	
Myanmar	Ayeyarwady	Large tributary, Bogale River	3.8	1.2	0.25	14/05/2019	15.95421	95.2465	Rising	<0.3	-	-	<0.3	Madie Cooper, Leah Carr, U Soe Htun, U Tun Tun Win, U Myat Wai Yan Oo	
Myanmar	Ayeyarwady	Bogale River, Southern tip of Conservation Park	4.1	20	0.25	14/05/2019	15.88775	95.2374	Fallling	<0.3	-	-	<0.3	Madie Cooper, Leah Carr, U Soe Htun, U Tun Tun Win, U Myat Wai Yan Oo	
Myanmar	Ayeyarwady	Bogale River, Southern tip of Conservation Park	4.2	20	0.25	14/05/2019	15.88775	95.2374	Fallling	<0.3	-	-	<0.3	Madie Cooper, Leah Carr, U Soe Htun, U Tun Tun Win, U Myat Wai Yan Oo	
Myanmar	Ayeyarwady	Bogale River, Southern tip of Conservation Park	4.3	20	0.3	14/05/2019	15.88775	95.2374	Fallling	<0.3	-	-	<0.3	Madie Cooper, Leah Carr, U Soe Htun, U Tun Tun Win, U Myat Wai Yan Oo	
Myanmar	Ayeyarwady	Bogale River, Southern tip of Conservation Park	4.4	20	0.35	14/05/2019	15.88775	95.2374	Fallling	<0.3	-	-	<0.3	Madie Cooper, Leah Carr, U Soe Htun, U Tun Tun Win, U Myat Wai Yan Oo	
Myanmar	Ayeyarwady	Bogale River, Southern tip of Conservation Park	4.5	20	0.35	14/05/2019	15.88775	95.2374	Fallling	<0.3	-	-	<0.3	Madie Cooper, Leah Carr, U Soe Htun, U Tun Tun Win, U Myat Wai Yan Oo	
Myanmar	Ayeyarwady	Bogale River, Southern tip of Conservation Park	4.6	1.2	0.15	14/05/2019	15.88775	95.2374	Fallling	<0.3	-	-	<0.3	Madie Cooper, Leah Carr, U Soe Htun, U Tun Tun Win, U Myat Wai Yan Oo	
Myanmar	Ayeyarwady	Bogale River, Southern tip of Conservation Park	4.7	1.2	0.15	14/05/2019	15.88775	95.2374	Fallling	<0.3	-	-	<0.3	Madie Cooper, Leah Carr, U Soe Htun, U Tun Tun Win, U Myat Wai Yan Oo	
Myanmar	Ayeyarwady	Bogale River, Southern tip of Conservation Park	4.8	1.2	0.2	14/05/2019	15.88775	95.2374	Fallling	<0.3	-	-	<0.3	Madie Cooper, Leah Carr, U Soe Htun, U Tun Tun Win, U Myat Wai Yan Oo	
Myanmar	Tanintharyi	Auckland Bay 1, Myeik	5.1	20	1.3	17/05/2019	12.06824	98.7098	Rising high	3	-	-	<0.3	Madalyn Cooper, Leah Carr, Salai Mon	

														Nyi Nyi Lin, Khin May Chit Maung	
Myanmar	Tanintharyi	Auckland Bay 1, Myeik	5.2	20	2	17/05/2019	12.06824	98.7098	Rising high	3	-	-	<0.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin, Khin May Chit Maung	
Myanmar	Tanintharyi	Auckland Bay 1, Myeik	5.3	20	1.4	17/05/2019	12.06824	98.7098	Rising high	3	-	-	<0.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin, Khin May Chit Maung	
Myanmar	Tanintharyi	Auckland Bay 1, Myeik	5.4	20	1.2	17/05/2019	12.06824	98.7098	Rising high	3	-	-	<0.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin, Khin May Chit Maung	
Myanmar	Tanintharyi	Auckland Bay 1, Myeik	5.5	20	1.45	17/05/2019	12.06824	98.7098	Rising high	3	-	-	<0.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin, Khin May Chit Maung	
Myanmar	Tanintharyi	Auckland Bay 1, Myeik	5.6	1.2	0.5	17/05/2019	12.06824	98.7098	Rising high	3	-	-	<0.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin, Khin May Chit Maung	
Myanmar	Tanintharyi	Auckland Bay 1, Myeik	5.7	1.2	0.35	17/05/2019	12.06824	98.7098	Rising high	3	-	-	<0.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin, Khin May Chit Maung	
Myanmar	Tanintharyi	Auckland Bay 1, Myeik	5.8	1.2	0.35	17/05/2019	12.06824	98.7098	Rising high	3	-	-	<0.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin, Khin May Chit Maung	
Myanmar	Tanintharyi	Auckland Bay 2, Myeik	6.1	20	1.4	17/05/2019	11.99406	98.7424	Slack high	4.5	-	-	<0.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin, Khin May Chit Maung	
Myanmar	Tanintharyi	Auckland Bay 2, Myeik	6.2	20	1.6	17/05/2019	11.99406	98.7424	Slack high	4.5	-	-	<0.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin, Khin May Chit Maung	
Myanmar	Tanintharyi	Auckland Bay 2, Myeik	6.3	20	1.7	17/05/2019	11.99406	98.7424	Slack high	4.5	-	-	<0.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin, Khin May Chit Maung	
Myanmar	Tanintharyi	Auckland Bay 2, Myeik	6.4	20	2.92	17/05/2019	11.99406	98.7424	Slack high	4.5	-	-	<0.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin, Khin May Chit Maung	
Myanmar	Tanintharyi	Auckland Bay 2, Myeik	6.5	20	2	17/05/2019	11.99406	98.7424	Slack high	4.5	-	-	<0.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin, Khin May Chit Maung	
Myanmar	Tanintharyi	Auckland Bay 2, Myeik	6.6	1.2	0.35	17/05/2019	11.99406	98.7424	Slack high	4.5	-	-	<0.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin, Khin May Chit Maung	
Myanmar	Tanintharyi	Auckland Bay 2, Myeik	6.7	1.2	0.35	17/05/2019	11.99406	98.7424	Slack high	4.5	-	-	<0.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin, Khin May Chit Maung	
Myanmar	Tanintharyi	Auckland Bay 2, Myeik	6.8	1.2	0.35	17/05/2019	11.99406	98.7424	Slack high	4.5	-	-	<0.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin, Khin May Chit Maung	
Myanmar	Tanintharyi	Auckland Bay 3, Myeik	7.1	20	5	17/05/2019	11.96179	98.6920	Falling high	2.8	-	-	<.3	Madalyn Cooper, Leah Carr, Salai Mon	

														Nyi Nyi Lin, Khin May Chit Maung	
Myanmar	Tanintharyi	Auckland Bay 3, Myeik	7.2	20	5	17/05/2019	11.96179	98.6920	Falling high	2.8	-	-	<.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin, Khin May Chit Maung	
Myanmar	Tanintharyi	Auckland Bay 3, Myeik	7.3	20	4.1	17/05/2019	11.96179	98.6920	Falling high	2.8	-	-	<.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin, Khin May Chit Maung	
Myanmar	Tanintharyi	Auckland Bay 3, Myeik	7.4	20	5	17/05/2019	11.96179	98.6920	Falling high	2.8	-	-	<.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin, Khin May Chit Maung	
Myanmar	Tanintharyi	Auckland Bay 3, Myeik	7.5	20	5	17/05/2019	11.96179	98.6920	Falling high	2.8	-	-	<.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin, Khin May Chit Maung	
Myanmar	Tanintharyi	Auckland Bay 3, Myeik	7.6	1.2	0.5	17/05/2019	11.96179	98.6920	Falling high	2.8	-	-	<.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin, Khin May Chit Maung	
Myanmar	Tanintharyi	Auckland Bay 3, Myeik	7.7	1.2	0.5	17/05/2019	11.96179	98.6920	Falling high	2.8	-	-	<.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin, Khin May Chit Maung	
Myanmar	Tanintharyi	Auckland Bay 3, Myeik	7.8	1.2	0.5	17/05/2019	11.96179	98.6920	Falling high	2.8	-	-	<.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin, Khin May Chit Maung	
Myanmar	Tanintharyi	Auckland Bay 4, Myeik	8.1	20	1.75	17/05/2019	11.98478	98.5729	Falling	3.3	-	-	-	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin, Khin May Chit Maung	
Myanmar	Tanintharyi	Auckland Bay 4, Myeik	8.2	20	1.55	17/05/2019	11.98478	98.5729	Falling	3.3	-	-	-	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin, Khin May Chit Maung	
Myanmar	Tanintharyi	Auckland Bay 4, Myeik	8.3	20	1.5	17/05/2019	11.98478	98.5729	Falling	3.3	-	-	-	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin, Khin May Chit Maung	
Myanmar	Tanintharyi	Auckland Bay 4, Myeik	8.4	20	2.5	17/05/2019	11.98478	98.5729	Falling	3.3	-	-	-	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin, Khin May Chit Maung	
Myanmar	Tanintharyi	Auckland Bay 4, Myeik	8.5	20	1.8	17/05/2019	11.98478	98.5729	Falling	3.3	-	-	-	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin, Khin May Chit Maung	
Myanmar	Tanintharyi	Auckland Bay 4, Myeik	8.6	1.2	0.35	17/05/2019	11.98478	98.5729	Falling	3.3	-	-	-	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin, Khin May Chit Maung	
Myanmar	Tanintharyi	Auckland Bay 4, Myeik	8.7	1.2	0.35	17/05/2019	11.98478	98.5729	Falling	3.3	-	-	-	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin, Khin May Chit Maung	
Myanmar	Tanintharyi	Auckland Bay 4, Myeik	8.8	1.2	0.35	17/05/2019	11.98478	98.5729	Falling	3.3	-	-	-	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin, Khin May Chit Maung	
Myanmar	Tanintharyi	Auckland Bay 5, Myeik	9.1	20	2.9	17/05/2019	12.00667	98.5096	Falling	3.2	-	-	<0.3	Madalyn Cooper, Leah Carr, Salai Mon	

														Nyi Nyi Lin, Khin May Chit Maung	
Myanmar	Tanintharyi	Auckland Bay 5, Myeik	9.2	20	2.6	17/05/2019	12.00667	98.5096	Falling	3.2	-	-	<0.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin, Khin May Chit Maung	
Myanmar	Tanintharyi	Auckland Bay 5, Myeik	9.3	20	2.53	17/05/2019	12.00667	98.5096	Falling	3.2	-	-	<0.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin, Khin May Chit Maung	
Myanmar	Tanintharyi	Auckland Bay 5, Myeik	9.4	20	2.2	17/05/2019	12.00667	98.5096	Falling	3.2	-	-	<0.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin, Khin May Chit Maung	
Myanmar	Tanintharyi	Auckland Bay 5, Myeik	9.5	20	2.6	17/05/2019	12.00667	98.5096	Falling	3.2	-	-	<0.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin, Khin May Chit Maung	
Myanmar	Tanintharyi	Auckland Bay 5, Myeik	9.6	1.2	0.4	17/05/2019	12.00667	98.5096	Falling	3.2	-	-	<0.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin, Khin May Chit Maung	
Myanmar	Tanintharyi	Auckland Bay 5, Myeik	9.7	1.2	0.4	17/05/2019	12.00667	98.5096	Falling	3.2	-	-	<0.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin, Khin May Chit Maung	
Myanmar	Tanintharyi	Auckland Bay 5, Myeik	9.8	1.2	0.4	17/05/2019	12.00667	98.5096	Falling	3.2	-	-	<0.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin, Khin May Chit Maung	
Myanmar	Tanintharyi	Auckland Bay 6, Myeik	10.1	20	1.1	18/05/2019	12.70540	98.6260	Rising	2.6	-	-	<0.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin	
Myanmar	Tanintharyi	Auckland Bay 6, Myeik	10.2	20	1.1	18/05/2019	12.70540	98.6260	Rising	2.6	-	-	<0.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin	
Myanmar	Tanintharyi	Auckland Bay 6, Myeik	10.3	20	1	18/05/2019	12.70540	98.6260	Rising	2.6	-	-	<0.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin	
Myanmar	Tanintharyi	Auckland Bay 6, Myeik	10.4	20	1.05	18/05/2019	12.70540	98.6260	Rising	2.6	-	-	<0.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin	
Myanmar	Tanintharyi	Auckland Bay 6, Myeik	10.5	20	1.1	18/05/2019	12.70540	98.6260	Rising	2.6	-	-	<0.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin	
Myanmar	Tanintharyi	Auckland Bay 6, Myeik	10.6	1.2	0.25	18/05/2019	12.70540	98.6260	Rising	2.6	-	-	<0.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin	
Myanmar	Tanintharyi	Auckland Bay 6, Myeik	10.7	1.2	0.25	18/05/2019	12.70540	98.6260	Rising	2.6	-	-	<0.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin	
Myanmar	Tanintharyi	Auckland Bay 6, Myeik	10.8	1.2	0.25	18/05/2019	12.70540	98.6260	Rising	2.6	-	-	<0.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin	
Myanmar	Tanintharyi	Auckland Bay 7, Myeik	11.1	20	1.2	18/05/2019	12.16811	98.6487	Rising high	3.1	-	-	<0.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin	
Myanmar	Tanintharyi	Auckland Bay 7, Myeik	11.2	20	0.93	18/05/2019	12.16811	98.6487	Rising high	3.1	-	-	<0.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin	
Myanmar	Tanintharyi	Auckland Bay 7, Myeik	11.3	20	1.5	18/05/2019	12.16811	98.6487	Rising high	3.1	-	-	<0.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin	

Myanmar	Tanintharyi	Auckland Bay 7, Myeik	11.4	20	1.15	18/05/2019	12.16811	98.6487	Rising high	3.1	-	-	<0.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin	
Myanmar	Tanintharyi	Auckland Bay 7, Myeik	11.5	20	1.3	18/05/2019	12.16811	98.6487	Rising high	3.1	-	-	<0.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin	
Myanmar	Tanintharyi	Auckland Bay 7, Myeik	11.6	1.2	0.4	18/05/2019	12.16811	98.6487	Rising high	3.1	-	-	<0.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin	
Myanmar	Tanintharyi	Auckland Bay 7, Myeik	11.7	1.2	0.4	18/05/2019	12.16811	98.6487	Rising high	3.1	-	-	<0.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin	
Myanmar	Tanintharyi	Auckland Bay 7, Myeik	11.8	1.2	0.4	18/05/2019	12.16811	98.6487	Rising high	3.1	-	-	<0.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin	
Myanmar	Tanintharyi	Auckland Bay 8 (Island in Main Channel), Myeik	12.1	20	3.13	18/05/2019	12.27516	98.5228	High	2.6	-	-	<0.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin	
Myanmar	Tanintharyi	Auckland Bay 8 (Island in Main Channel), Myeik	12.2	20	2.9	18/05/2019	12.27516	98.5228	High	2.6	-	-	<0.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin	
Myanmar	Tanintharyi	Auckland Bay 8 (Island in Main Channel), Myeik	12.3	20	3	18/05/2019	12.27516	98.5228	High	2.6	-	-	<0.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin	
Myanmar	Tanintharyi	Auckland Bay 8 (Island in Main Channel), Myeik	12.4	20	3.05	18/05/2019	12.27516	98.5228	High	2.6	-	-	<0.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin	
Myanmar	Tanintharyi	Auckland Bay 8 (Island in Main Channel), Myeik	12.5	20	3.2	18/05/2019	12.27516	98.5228	High	2.6	-	-	<0.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin	
Myanmar	Tanintharyi	Auckland Bay 8 (Island in Main Channel), Myeik	12.6	1.2	0.4	18/05/2019	12.27516	98.5228	High	2.6	-	-	<0.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin	
Myanmar	Tanintharyi	Auckland Bay 8 (Island in Main Channel), Myeik	12.7	1.2	0.45	18/05/2019	12.27516	98.5228	High	2.6	-	-	<0.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin	
Myanmar	Tanintharyi	Auckland Bay 8 (Island in Main Channel), Myeik	12.8	1.2	0.5	18/05/2019	12.27516	98.5228	High	2.6	-	-	<0.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin	
Myanmar	Tanintharyi	Auckland Bay 9, Myeik	13.1	20	0.6	18/05/2019	12.34194	98.5821	Falling	6.5	-	-	<0.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin	
Myanmar	Tanintharyi	Auckland Bay 9, Myeik	13.2	20	0.7	18/05/2019	12.34194	98.5821	Falling	6.5	-	-	<0.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin	
Myanmar	Tanintharyi	Auckland Bay 9, Myeik	13.3	20	0.6	18/05/2019	12.34194	98.5821	Falling	6.5	-	-	<0.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin	
Myanmar	Tanintharyi	Auckland Bay 9, Myeik	13.4	20	0.8	18/05/2019	12.34194	98.5821	Falling	6.5	-	-	<0.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin	
Myanmar	Tanintharyi	Auckland Bay 9, Myeik	13.5	20	0.9	18/05/2019	12.34194	98.5821	Falling	6.5	-	-	<0.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin	
Myanmar	Tanintharyi	Auckland Bay 9, Myeik	13.6	1.2	0.3	18/05/2019	12.34194	98.5821	Falling	6.5	-	-	<0.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin	
Myanmar	Tanintharyi	Auckland Bay 9, Myeik	13.7	1.2	0.3	18/05/2019	12.34194	98.5821	Falling	6.5	-	-	<0.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin	
Myanmar	Tanintharyi	Auckland Bay 9, Myeik	13.8	1.2	0.3	18/05/2019	12.34194	98.5821	Falling	6.5	-	-	<0.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin	

Myanmar	Tanintharyi	Myiek Area	14.1	20	3.45	18/05/2019	12.39998	98.5586	Falling low	4.3	-	-	<0.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin	
Myanmar	Tanintharyi	Myiek Area	14.2	20	2.3	18/05/2019	12.39998	98.5586	Falling low	4.3	-	-	<0.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin	
Myanmar	Tanintharyi	Myiek Area	14.3	20	3.1	18/05/2019	12.39998	98.5586	Falling low	4.3	-	-	<0.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin	
Myanmar	Tanintharyi	Myiek Area	14.4	20	2.45	18/05/2019	12.39998	98.5586	Falling low	4.3	-	-	<0.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin	
Myanmar	Tanintharyi	Myiek Area	14.5	20	3.2	18/05/2019	12.39998	98.5586	Falling low	4.3	-	-	<0.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin	
Myanmar	Tanintharyi	Myiek Area	14.6	1.2	0.5	18/05/2019	12.39998	98.5586	Falling low	4.3	-	-	<0.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin	
Myanmar	Tanintharyi	Myiek Area	14.7	1.2	0.5	18/05/2019	12.39998	98.5586	Falling low	4.3	-	-	<0.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin	
Myanmar	Tanintharyi	Myiek Area	14.8	1.2	0.5	18/05/2019	12.39998	98.5586	Falling low	4.3	-	-	<0.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin	
Myanmar	Tanintharyi	Island Channel, Myiek	15.1	20	0.6	18/05/2019	12.37298	98.4974	Low	6.7	-	-	<0.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin	
Myanmar	Tanintharyi	Island Channel, Myiek	15.2	20	0.95	18/05/2019	12.37298	98.4974	Low	6.7	-	-	<0.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin	
Myanmar	Tanintharyi	Island Channel, Myiek	15.3	20	0.5	18/05/2019	12.37298	98.4974	Low	6.7	-	-	<0.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin	
Myanmar	Tanintharyi	Island Channel, Myiek	15.4	20	0.5	18/05/2019	12.37298	98.4974	Low	6.7	-	-	<0.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin	
Myanmar	Tanintharyi	Island Channel, Myiek	15.5	20	0.42	18/05/2019	12.37298	98.4974	Low	6.7	-	-	<0.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin	
Myanmar	Tanintharyi	Island Channel, Myiek	15.6	1.2	0.3	18/05/2019	12.37298	98.4974	Low	6.7	-	-	<0.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin	
Myanmar	Tanintharyi	Island Channel, Myiek	15.7	1.2	0.3	18/05/2019	12.37298	98.4974	Low	6.7	-	-	<0.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin	
Myanmar	Tanintharyi	Island Channel, Myiek	15.8	1.2	0.25	18/05/2019	12.37298	98.4974	Low	6.7	-	-	<0.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin	
Myanmar	Tanintharyi	Dome Island, LMMA	16.1	20	3.5	20/05/2019	12.12632	98.1263	High	2.4	-	-	<0.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin	
Myanmar	Tanintharyi	Dome Island, LMMA	16.2	20	3.2	20/05/2019	12.12632	98.1263	High	2.4	-	-	<0.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin	
Myanmar	Tanintharyi	Dome Island, LMMA	16.3	20	3.65	20/05/2019	12.12632	98.1263	High	2.4	-	-	<0.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin	
Myanmar	Tanintharyi	Dome Island, LMMA	16.4	20	3.8	20/05/2019	12.12632	98.1263	High	2.4	-	-	<0.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin	
Myanmar	Tanintharyi	Dome Island, LMMA	16.5	20	3.8	20/05/2019	12.12632	98.1263	High	2.4	-	-	<0.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin	

Myanmar	Tanintharyi	Dome Island, LMMA	16.6	1.2	0.6	20/05/2019	12.12632	98.1263	High	2.4	-	-	<0.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin	
Myanmar	Tanintharyi	Dome Island, LMMA	16.7	1.2	0.5	20/05/2019	12.12632	98.1263	High	2.4	-	-	<0.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin	
Myanmar	Tanintharyi	Dome Island, LMMA	16.8	1.2	0.5	20/05/2019	12.12632	98.1263	High	2.4	-	-	<0.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin	
Myanmar	Tanintharyi	Dome Island, estuary north	17.1	20	3.85	20/05/2019	12.10441	98.1044	Falling	2.3	-	-	<0.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin	
Myanmar	Tanintharyi	Dome Island, estuary north	17.2	20	4.2	20/05/2019	12.10441	98.1044	Falling	2.3	-	-	<0.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin	
Myanmar	Tanintharyi	Dome Island, estuary north	17.3	20	2	20/05/2019	12.10441	98.1044	Falling	2.3	-	-	<0.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin	
Myanmar	Tanintharyi	Dome Island, estuary north	17.4	20	2.65	20/05/2019	12.10441	98.1044	Falling	2.3	-	-	<0.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin	
Myanmar	Tanintharyi	Dome Island, estuary north	17.5	20	2.36	20/05/2019	12.10441	98.1044	Falling	2.3	-	-	<0.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin	
Myanmar	Tanintharyi	Dome Island, estuary north	17.6	1.2	0.45	20/05/2019	12.10441	98.1044	Falling	2.3	-	-	<0.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin	
Myanmar	Tanintharyi	Dome Island, estuary north	17.7	1.2	0.5	20/05/2019	12.10441	98.1044	Falling	2.3	-	-	<0.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin	
Myanmar	Tanintharyi	Dome Island, estuary north	17.8	1.2	0.5	20/05/2019	12.10441	98.1044	Falling	2.3	-	-	<0.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin	
Myanmar	Tanintharyi	Dome Island, estuary south	18.1	20	3.6	20/05/2019	12.11539	98.1154	Falling	1.4	-	-	<0.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin	
Myanmar	Tanintharyi	Dome Island, estuary south	18.2	20	2.6	20/05/2019	12.11539	98.1154	Falling	1.4	-	-	<0.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin	
Myanmar	Tanintharyi	Dome Island, estuary south	18.3	20	2.7	20/05/2019	12.11539	98.1154	Falling	1.4	-	-	<0.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin	
Myanmar	Tanintharyi	Dome Island, estuary south	18.4	20	2.8	20/05/2019	12.11539	98.1154	Falling	1.4	-	-	<0.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin	
Myanmar	Tanintharyi	Dome Island, estuary south	18.5	20	2.5	20/05/2019	12.11539	98.1154	Falling	1.4	-	-	<0.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin	
Myanmar	Tanintharyi	Dome Island, estuary south	18.6	1.2	0.55	20/05/2019	12.11539	98.1154	Falling	1.4	-	-	<0.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin	
Myanmar	Tanintharyi	Dome Island, estuary south	18.7	1.2	0.5	20/05/2019	12.11539	98.1154	Falling	1.4	-	-	<0.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin	
Myanmar	Tanintharyi	Dome Island, estuary south	18.8	1.2	0.5	20/05/2019	12.11539	98.1154	Falling	1.4	-	-	<0.3	Madalyn Cooper, Leah Carr, Salai Mon Nyi Nyi Lin	
Costa Rica	Northern Plains	Boca San Carlos	28.1	20	2.5	25/05/2019	10.78282	-84.20072	Non-tidal	2	29.9	NA	0.45	Jorge V, Edward, Yahaira, Edwin	
Costa Rica	Northern Plains	Boca San Carlos	28.2	20	3	25/05/2019	10.78282	-84.20072	Non-tidal	2	29.9	NA	0.45	Jorge V, Edward, Yahaira, Edwin	
Costa Rica	Northern Plains	Boca San Carlos	28.3	20	3	25/05/2019	10.78282	-84.20072	Non-tidal	2	29.9	NA	0.45	Jorge V, Edward, Yahaira, Edwin	

Costa Rica	Northern Plains	Boca San Carlos	28.4	20	3	25/05/2019	10.78282	-84.20072	Non-tidal	2	29.9	NA	0.45	Jorge V, Edward, Yahaira, Edwin	
Costa Rica	Northern Plains	Boca San Carlos	28.5	20	3	25/05/2019	10.78282	-84.20072	Non-tidal	2	29.9	NA	0.45	Jorge V, Edward, Yahaira, Edwin	
Costa Rica	Northern Plains	Boca Cureñita	29.1	20	2	26/05/2019	10.764	-84.06578	Non-tidal	2.5	28.6	NA	0.45	Jorge V, Edward, Yahaira, Edwin	
Costa Rica	Northern Plains	Boca Cureñita	29.2	20	2	26/05/2019	10.764	-84.06578	Non-tidal	2.5	28.6	NA	0.45	Jorge V, Edward, Yahaira, Edwin	
Costa Rica	Northern Plains	Boca Cureñita	29.3	20	2	26/05/2019	10.764	-84.06578	Non-tidal	2.5	28.6	NA	0.45	Jorge V, Edward, Yahaira, Edwin	
Costa Rica	Northern Plains	Boca Cureñita	29.4	20	1.5	26/05/2019	10.764	-84.06578	Non-tidal	2.5	28.6	NA	0.45	Jorge V, Edward, Yahaira, Edwin	
Costa Rica	Northern Plains	Boca Cureñita	29.5	20	2	26/05/2019	10.764	-84.06578	Non-tidal	2.5	28.6	NA	0.45	Jorge V, Edward, Yahaira, Edwin	
Costa Rica	Northern Plains	Boca Cureñita	30.1	20	1.5	26/05/2019	10.76006	-84.07448	Non-tidal	3.4	28.4	NA	0.35	Jorge V, Edward, Yahaira, Edwin	
Costa Rica	Northern Plains	Boca Cureñita	30.2	20	1.5	26/05/2019	10.76006	-84.07448	Non-tidal	3.4	28.4	NA	0.35	Jorge V, Edward, Yahaira, Edwin	
Costa Rica	Northern Plains	Boca Cureñita	30.3	20	1.5	26/05/2019	10.76006	-84.07448	Non-tidal	3.4	28.4	NA	0.35	Jorge V, Edward, Yahaira, Edwin	
Costa Rica	Northern Plains	Boca Cureñita	30.4	20	1.5	26/05/2019	10.76006	-84.07448	Non-tidal	3.4	28.4	NA	0.35	Jorge V, Edward, Yahaira, Edwin	
Costa Rica	Northern Plains	Boca Cureñita	30.5	20	2	26/05/2019	10.76006	-84.07448	Non-tidal	3.4	28.4	NA	0.35	Jorge V, Edward, Yahaira, Edwin	
Costa Rica	Northern Plains	Boca Cureñita	31.1	20	1	26/05/2019	10.76222	-84.07877	Non-tidal	1.5	28.9	NA	0.35	Jorge V, Edward, Yahaira, Edwin	
Costa Rica	Northern Plains	Boca Cureñita	31.2	20	1	26/05/2019	10.76222	-84.07877	Non-tidal	1.5	28.9	NA	0.35	Jorge V, Edward, Yahaira, Edwin	
Costa Rica	Northern Plains	Boca Cureñita	31.3	20	1	26/05/2019	10.76222	-84.07877	Non-tidal	1.5	28.9	NA	0.35	Jorge V, Edward, Yahaira, Edwin	
Costa Rica	Northern Plains	Boca Cureñita	31.4	20	1	26/05/2019	10.76222	-84.07877	Non-tidal	1.5	28.9	NA	0.35	Jorge V, Edward, Yahaira, Edwin	
Costa Rica	Northern Plains	Boca Cureñita	31.5	20	1	26/05/2019	10.76222	-84.07877	Non-tidal	1.5	28.9	NA	0.35	Jorge V, Edward, Yahaira, Edwin	
Costa Rica	Northern Plains	Boca Cureñita	32.1	20	2	27/05/2019	10.76614	-84.08883	Non-tidal	1.2	28	NA	0.4	Jorge V, Edward, Yahaira, Edwin	
Costa Rica	Northern Plains	Boca Cureñita	32.2	20	2	27/05/2019	10.76614	-84.08883	Non-tidal	1.2	28	NA	0.4	Jorge V, Edward, Yahaira, Edwin	
Costa Rica	Northern Plains	Boca Cureñita	32.3	20	2	27/05/2019	10.76614	-84.08883	Non-tidal	1.2	28	NA	0.4	Jorge V, Edward, Yahaira, Edwin	
Costa Rica	Northern Plains	Boca Cureñita	32.4	20	2	27/05/2019	10.76614	-84.08883	Non-tidal	1.2	28	NA	0.4	Jorge V, Edward, Yahaira, Edwin	
Costa Rica	Northern Plains	Boca Cureñita	32.5	20	2	27/05/2019	10.76614	-84.08883	Non-tidal	1.2	28	NA	0.4	Jorge V, Edward, Yahaira, Edwin	
Costa Rica	Northern Plains	Boca Cureñita	33.1	20	2	27/05/2019	10.77124	-84.09329	Non-tidal	1.5	28	NA	0.35	Jorge V, Edward, Yahaira, Edwin	
Costa Rica	Northern Plains	Boca Cureñita	33.2	20	2	27/05/2019	10.77124	-84.09329	Non-tidal	1.5	28	NA	0.35	Jorge V, Edward, Yahaira, Edwin	
Costa Rica	Northern Plains	Boca Cureñita	33.3	20	2	27/05/2019	10.77124	-84.09329	Non-tidal	1.5	28	NA	0.35	Jorge V, Edward, Yahaira, Edwin	
Costa Rica	Northern Plains	Boca Cureñita	33.4	20	2	27/05/2019	10.77124	-84.09329	Non-tidal	1.5	28	NA	0.35	Jorge V, Edward, Yahaira, Edwin	
Costa Rica	Northern Plains	Boca Cureñita	33.5	20	2	27/05/2019	10.77124	-84.09329	Non-tidal	1.5	28	NA	0.35	Jorge V, Edward, Yahaira, Edwin	
Costa Rica	Northern Plains	Boca Cureñita	34.1	20	2	27/05/2019	10.77096	-84.11118	Non-tidal	1.4	27.8	NA	0.3	Jorge V, Edward, Yahaira, Edwin	Largetooth and green sawfish captured and handled from same boat on the day prior
Costa Rica	Northern Plains	Boca Cureñita	34.2	20	2	27/05/2019	10.77096	-84.11118	Non-tidal	1.4	27.8	NA	0.3	Jorge V, Edward, Yahaira, Edwin	Largetooth and green sawfish captured and handled from same boat on the day prior
Costa Rica	Northern Plains	Boca Cureñita	34.3	20	2	27/05/2019	10.77096	-84.11118	Non-tidal	1.4	27.8	NA	0.3	Jorge V, Edward, Yahaira, Edwin	Largetooth and green sawfish captured and handled from same boat on the day prior

Costa Rica	Northern Plains	Boca Cureñita	34.4	20	2	27/05/2019	10.77096	-84.11118	Non-tidal	1.4	27.8	NA	0.3	Jorge V, Edward, Yahaira, Edwin	Largetooth and green sawfish captured and handled from same boat on the day prior
Costa Rica	Northern Plains	Boca Cureñita	34.5	20	2	27/05/2019	10.77096	-84.11118	Non-tidal	1.4	27.8	NA	0.3	Jorge V, Edward, Yahaira, Edwin	Largetooth and green sawfish captured and handled from same boat on the day prior
Costa Rica	Caribbean	Barra del Colorado, Estuary	35.1	20	5	31/05/2019	10.80198	-83.58608	Falling	1.5	28.2	0.1	0.5	Jorge V, Isaac C., Jesus Chaves	Largetooth and green sawfish captured and handled from same boat on the day prior
Costa Rica	Caribbean	Barra del Colorado, Estuary	35.2	10	1.5	31/05/2019	10.80198	-83.58608	Falling	1.5	28.2	0.1	0.5	Jorge V, Isaac C., Jesus Chaves	Green, dwarf, and narrow sawfish captured at this beach on the day prior
Costa Rica	Caribbean	Barra del Colorado, Estuary	35.3	20	2.6	31/05/2019	10.80198	-83.58608	Falling	1.5	28.2	0.1	0.5	Jorge V, Isaac C., Jesus Chaves	Green, dwarf, and narrow sawfish captured at this beach on the day prior
Costa Rica	Caribbean	Barra del Colorado, Estuary	35.4	20	2.5	31/05/2019	10.80198	-83.58608	Falling	1.5	28.2	0.1	0.5	Jorge V, Isaac C., Jesus Chaves	Green, dwarf, and narrow sawfish captured at this beach on the day prior
Costa Rica	Caribbean	Barra del Colorado, Estuary	35.5	20	2.8	31/05/2019	10.80198	-83.58608	Falling	1.5	28.2	0.1	0.5	Jorge V, Isaac C., Jesus Chaves	Green, dwarf, and narrow sawfish captured at this beach on the day prior
Costa Rica	Caribbean	Barra del Colorado, Estuary	36.1	20	1.5	31/05/2019	10.79511	-83.58985	-	3.4	28.4	0.1	0.5	Jorge V, Isaac C., Jesus Chaves	Green, dwarf, and narrow sawfish captured at this beach on the day prior
Costa Rica	Caribbean	Barra del Colorado, Estuary	36.2	20	1.5	31/05/2019	10.79511	-83.58985	-	3.4	28.4	0.1	0.5	Jorge V, Isaac C., Jesus Chaves	
Costa Rica	Caribbean	Barra del Colorado, Estuary	36.3	20	1.5	31/05/2019	10.79511	-83.58985	-	3.4	28.4	0.1	0.5	Jorge V, Isaac C., Jesus Chaves	
Costa Rica	Caribbean	Barra del Colorado, Estuary	36.4	20	1.3	31/05/2019	10.79511	-83.58985	-	3.4	28.4	0.1	0.5	Jorge V, Isaac C., Jesus Chaves	
Costa Rica	Caribbean	Barra del Colorado, Estuary	36.5	20	1.3	31/05/2019	10.79511	-83.58985	-	3.4	28.4	0.1	0.5	Jorge V, Isaac C., Jesus Chaves	
Costa Rica	Caribbean	Barra del Colorado, Estuary	37.1	20	1.3	31/05/2019	10.78792	-83.59003	-	4	28.5	0.1	0.5	Jorge V, Isaac C., Jesus Chaves	
Costa Rica	Caribbean	Barra del Colorado, Estuary	37.2	20	1.4	31/05/2019	10.78792	-83.59003	-	4	28.5	0.1	0.5	Jorge V, Isaac C., Jesus Chaves	
Costa Rica	Caribbean	Barra del Colorado, Estuary	37.3	20	1.2	31/05/2019	10.78792	-83.59003	-	4	28.5	0.1	0.5	Jorge V, Isaac C., Jesus Chaves	
Costa Rica	Caribbean	Barra del Colorado, Estuary	37.4	20	1.5	31/05/2019	10.78792	-83.59003	-	4	28.5	0.1	0.5	Jorge V, Isaac C., Jesus Chaves	
Costa Rica	Caribbean	Barra del Colorado, Estuary	37.5	20	1.4	31/05/2019	10.78792	-83.59003	-	4	28.5	0.1	0.5	Jorge V, Isaac C., Jesus Chaves	
Costa Rica	Caribbean	Barra del Colorado, Estuary	38.1	20	1.2	1/06/2019	10.78039	-83.59093	-	4.4	26.7	0.1	0.3	Jorge V, Isaac C., Jesus Chaves	
Costa Rica	Caribbean	Barra del Colorado, Estuary	38.2	20	1.3	1/06/2019	10.78039	-83.59093	-	4.4	26.7	0.1	0.3	Jorge V, Isaac C., Jesus Chaves	
Costa Rica	Caribbean	Barra del Colorado, Estuary	38.3	20	1.2	1/06/2019	10.78039	-83.59093	-	4.4	26.7	0.1	0.3	Jorge V, Isaac C., Jesus Chaves	
Costa Rica	Caribbean	Barra del Colorado, Estuary	38.4	20	1.3	1/06/2019	10.78039	-83.59093	-	4.4	26.7	0.1	0.3	Jorge V, Isaac C., Jesus Chaves	

Costa Rica	Caribbean	Barra del Colorado, Estuary	38.5	20	1.3	1/06/2019	10.78039	-83.59093	-	4.4	26.7	0.1	0.3	Jorge V, Isaac C., Jesus Chaves	
Costa Rica	Caribbean	Barra del Colorado, Agua Dulce Lagoon	39.1	10	5	1/06/2019	10.79294	-83.60377	-	4.8	29.8	2,6 / 14,9 surface / bottom	1.1	Jorge V, Isaac C., Jesus Chaves	
Costa Rica	Caribbean	Barra del Colorado, Agua Dulce Lagoon	39.2	5	1.6	1/06/2019	10.79294	-83.60377	-	4.8	29.8	2,6 / 14,9 surface / bottom	1.1	Jorge V, Isaac C., Jesus Chaves	
Costa Rica	Caribbean	Barra del Colorado, Agua Dulce Lagoon	39.3	5	1.5	1/06/2019	10.79294	-83.60377	-	4.8	29.8	2,6 / 14,9 surface / bottom	1.1	Jorge V, Isaac C., Jesus Chaves	
Costa Rica	Caribbean	Barra del Colorado, Agua Dulce Lagoon	39.4	5	1.6	1/06/2019	10.79294	-83.60377	-	4.8	29.8	2,6 / 14,9 surface / bottom	1.1	Jorge V, Isaac C., Jesus Chaves	
Costa Rica	Caribbean	Barra del Colorado, Agua Dulce Lagoon	39.5	5	1.7	1/06/2019	10.79294	-83.60377	-	4.8	29.8	2,6 / 14,9 surface / bottom	1.1	Jorge V, Isaac C., Jesus Chaves	
Costa Rica	Caribbean	Barra del Colorado, Back Lagoon	40.1	10	1.5	1/06/2019	10.7865	-83.60532	-	2.4	28.2	0.6	1	Jorge V, Isaac C., Jesus Chaves	
Costa Rica	Caribbean	Barra del Colorado, Back Lagoon	40.2	5	1.6	1/06/2019	10.7865	-83.60532	-	2.4	28.2	0.6	1	Jorge V, Isaac C., Jesus Chaves	
Costa Rica	Caribbean	Barra del Colorado, Back Lagoon	40.3	5	1.5	1/06/2019	10.7865	-83.60532	-	2.4	28.2	0.6	1	Jorge V, Isaac C., Jesus Chaves	
Costa Rica	Caribbean	Barra del Colorado, Back Lagoon	40.4	5	1.6	1/06/2019	10.7865	-83.60532	-	2.4	28.2	0.6	1	Jorge V, Isaac C., Jesus Chaves	
Costa Rica	Caribbean	Barra del Colorado, Back Lagoon	40.5	5	1.7	1/06/2019	10.7865	-83.60532	-	2.4	28.2	0.6	1	Jorge V, Isaac C., Jesus Chaves	
Costa Rica	Caribbean	Barra del Colorado, Back Lagoon	43.1	5	2	1/06/2019	10.7939	-83.60994	-	6.3	28.1	0,6 / 22 surface / bottom	1	Jorge V, Isaac C., Jesus Chaves	
Costa Rica	Caribbean	Barra del Colorado, Back Lagoon	43.2	5	1.6	1/06/2019	10.7939	-83.60994	-	6.3	28.1	0,6 / 22 surface / bottom	1	Jorge V, Isaac C., Jesus Chaves	
Costa Rica	Caribbean	Barra del Colorado, Back Lagoon	43.3	5	1.8	1/06/2019	10.7939	-83.60994	-	6.3	28.1	0,6 / 22 surface / bottom	1	Jorge V, Isaac C., Jesus Chaves	
Costa Rica	Caribbean	Barra del Colorado, Back Lagoon	43.4	5	2	1/06/2019	10.7939	-83.60994	-	6.3	28.1	0,6 / 22 surface / bottom	1	Jorge V, Isaac C., Jesus Chaves	
Costa Rica	Caribbean	Barra del Colorado, Back Lagoon	43.5	5	2.2	1/06/2019	10.7939	-83.60994	-	6.3	28.1	0,6 / 22 surface / bottom	1	Jorge V, Isaac C., Jesus Chaves	
Costa Rica	Caribbean	Barra del Colorado, Back Lagoon	44.1	5	1.3	1/06/2019	10.80695	-83.61305	-	7.7	27.2	0,1 / 26,7 surface / bottom	0.8	Jorge V, Isaac C., Jesus Chaves	
Costa Rica	Caribbean	Barra del Colorado, Back Lagoon	44.2	5	1.7	1/06/2019	10.80695	-83.61305	-	7.7	27.2	0,1 / 26,7 surface / bottom	0.8	Jorge V, Isaac C., Jesus Chaves	
Costa Rica	Caribbean	Barra del Colorado, Back Lagoon	44.3	5	1.2	1/06/2019	10.80695	-83.61305	-	7.7	27.2	0,1 / 26,7 surface / bottom	0.8	Jorge V, Isaac C., Jesus Chaves	
Costa Rica	Caribbean	Barra del Colorado, Back Lagoon	44.4	5	1.5	1/06/2019	10.80695	-83.61305	-	7.7	27.2	0,1 / 26,7 surface / bottom	0.8	Jorge V, Isaac C., Jesus Chaves	
Costa Rica	Caribbean	Barra del Colorado, Back Lagoon	44.5	5	1.6	1/06/2019	10.80695	-83.61305	-	7.7	27.2	0,1 / 26,7 surface / bottom	0.8	Jorge V, Isaac C., Jesus Chaves	

Costa Rica	Northern Plains	Barra del Colorado, Colorado River	45.1	20	1.9	2/06/2019	10.72151	-83.64306	-	7.7	27.8	0.1	0.3	Jorge V., Isaac C. Eduardo C., Jesús C.	
Costa Rica	Northern Plains	Barra del Colorado, Colorado River	45.2	20	2.1	2/06/2019	10.72151	-83.64306	-	7.7	27.8	0.1	0.3	Jorge V., Isaac C. Eduardo C., Jesús C.	
Costa Rica	Northern Plains	Barra del Colorado, Colorado River	45.3	20	2.5	2/06/2019	10.72151	-83.64306	-	7.7	27.8	0.1	0.3	Jorge V., Isaac C. Eduardo C., Jesús C.	
Costa Rica	Northern Plains	Barra del Colorado, Colorado River	45.4	20	2.3	2/06/2019	10.72151	-83.64306	-	7.7	27.8	0.1	0.3	Jorge V., Isaac C. Eduardo C., Jesús C.	
Costa Rica	Northern Plains	Barra del Colorado, Colorado River	45.5	20	2.3	2/06/2019	10.72151	-83.64306	-	7.7	27.8	0.1	0.3	Jorge V., Isaac C. Eduardo C., Jesús C.	
Costa Rica	Northern Plains	Barra del Colorado, Colorado River	46.1	20	1.1	2/06/2019	10.71492	-83.65375	-	3.7	28.8	0	0.25	Jorge V., Isaac C. Eduardo C., Jesús C.	
Costa Rica	Northern Plains	Barra del Colorado, Colorado River	46.2	20	1.1	2/06/2019	10.71492	-83.65375	-	3.7	28.8	0	0.25	Jorge V., Isaac C. Eduardo C., Jesús C.	
Costa Rica	Northern Plains	Barra del Colorado, Colorado River	46.3	20	1.3	2/06/2019	10.71492	-83.65375	-	3.7	28.8	0	0.25	Jorge V., Isaac C. Eduardo C., Jesús C.	
Costa Rica	Northern Plains	Barra del Colorado, Colorado River	46.4	20	1.4	2/06/2019	10.71492	-83.65375	-	3.7	28.8	0	0.25	Jorge V., Isaac C. Eduardo C., Jesús C.	
Costa Rica	Northern Plains	Barra del Colorado, Colorado River	46.5	20	1.5	2/06/2019	10.71492	-83.65375	-	3.7	28.8	0	0.25	Jorge V., Isaac C. Eduardo C., Jesús C.	
Costa Rica	Northern Plains	Barra del Colorado, Colorado River	47.1	20	0.8	2/06/2019	10.70582	-83.6539	-	2.2	29.5	0	0.25	Jorge V., Isaac C. Eduardo C., Jesús C.	
Costa Rica	Northern Plains	Barra del Colorado, Colorado River	47.2	20	1	2/06/2019	10.70582	-83.6539	-	2.2	29.5	0	0.25	Jorge V., Isaac C. Eduardo C., Jesús C.	
Costa Rica	Northern Plains	Barra del Colorado, Colorado River	47.3	20	1.2	2/06/2019	10.70582	-83.6539	-	2.2	29.5	0	0.25	Jorge V., Isaac C. Eduardo C., Jesús C.	
Costa Rica	Northern Plains	Barra del Colorado, Colorado River	47.4	20	1.2	2/06/2019	10.70582	-83.6539	-	2.2	29.5	0	0.25	Jorge V., Isaac C. Eduardo C., Jesús C.	
Costa Rica	Northern Plains	Barra del Colorado, Colorado River	47.5	20	1.3	2/06/2019	10.70582	-83.6539	-	2.2	29.5	0	0.25	Jorge V., Isaac C. Eduardo C., Jesús C.	
Costa Rica	Northern Plains	Barra del Colorado, Colorado River	48.1	20	2	2/06/2019	10.69963	-83.64599	-	4.1	29.6	0	0.35	Jorge V., Isaac C. Eduardo C., Jesús C.	
Costa Rica	Northern Plains	Barra del Colorado, Colorado River	48.2	20	2.2	2/06/2019	10.69963	-83.64599	-	4.1	29.6	0	0.35	Jorge V., Isaac C. Eduardo C., Jesús C.	
Costa Rica	Northern Plains	Barra del Colorado, Colorado River	48.3	20	2.2	2/06/2019	10.69963	-83.64599	-	4.1	29.6	0	0.35	Jorge V., Isaac C. Eduardo C., Jesús C.	
Costa Rica	Northern Plains	Barra del Colorado, Colorado River	48.4	20	2.3	2/06/2019	10.69963	-83.64599	-	4.1	29.6	0	0.35	Jorge V., Isaac C. Eduardo C., Jesús C.	
Costa Rica	Northern Plains	Barra del Colorado, Colorado River	48.5	20	2.4	2/06/2019	10.69963	-83.64599	-	4.1	29.6	0	0.35	Jorge V., Isaac C. Eduardo C., Jesús C.	
Costa Rica	Southern Pacific	Pejeperro Lagoon	49.1	5	2	12/06/2019	8.41461	-83.38315	-	1.8	28	0.6	0.85	Jorge V., Mariel M. , Esteban J.	

Costa Rica	Southern Pacific	Pejeperro Lagoon	49.2	5	2	12/06/2019	8.41461	-83.38315	-	1.8	28	0.6	0.85	Jorge V., Mariel M. , Esteban J.	
Costa Rica	Southern Pacific	Pejeperro Lagoon	49.3	5	1.5	12/06/2019	8.41461	-83.38315	-	1.8	28	0.6	0.85	Jorge V., Mariel M. , Esteban J.	
Costa Rica	Southern Pacific	Pejeperro Lagoon	49.4	5	1.5	12/06/2019	8.41461	-83.38315	-	1.8	28	0.6	0.85	Jorge V., Mariel M. , Esteban J.	
Costa Rica	Southern Pacific	Pejeperro Lagoon	49.5	5	2	12/06/2019	8.41461	-83.38315	-	1.8	28	0.6	0.85	Jorge V., Mariel M. , Esteban J.	
Costa Rica	Southern Pacific	Pejeperro Lagoon	50.1	5	1.2	12/06/2019	8.41503	-83.38882	-	1.6	28.8	0.4	0.85	Jorge V., Mariel M. , Esteban J.	
Costa Rica	Southern Pacific	Pejeperro Lagoon	50.2	10	3.8	12/06/2019	8.41503	-83.38882	-	1.6	28.8	0.4	0.85	Jorge V., Mariel M. , Esteban J.	
Costa Rica	Southern Pacific	Pejeperro Lagoon	50.3	10	3.8	12/06/2019	8.41503	-83.38882	-	1.6	28.8	0.4	0.85	Jorge V., Mariel M. , Esteban J.	
Costa Rica	Southern Pacific	Pejeperro Lagoon	50.4	10	3.3	12/06/2019	8.41503	-83.38882	-	1.6	28.8	0.4	0.85	Jorge V., Mariel M. , Esteban J.	
Costa Rica	Southern Pacific	Pejeperro Lagoon	50.5	10	4	12/06/2019	8.41503	-83.38882	-	1.6	28.8	0.4	0.85	Jorge V., Mariel M. , Esteban J.	
Costa Rica	Southern Pacific	Pejeperro Lagoon	51.1	20	5	12/06/2019	8.41865	-83.38953	-	2	28	0.2	1.1	Jorge V., Mariel M. , Esteban J.	
Costa Rica	Southern Pacific	Pejeperro Lagoon	51.2	10	4.3	12/06/2019	8.41865	-83.38953	-	2	28	0.2	1.1	Jorge V., Mariel M. , Esteban J.	
Costa Rica	Southern Pacific	Pejeperro Lagoon	51.3	10	4.3	12/06/2019	8.41865	-83.38953	-	2	28	0.2	1.1	Jorge V., Mariel M. , Esteban J.	
Costa Rica	Southern Pacific	Pejeperro Lagoon	51.4	10	4.3	12/06/2019	8.41865	-83.38953	-	2	28	0.2	1.1	Jorge V., Mariel M. , Esteban J.	
Costa Rica	Southern Pacific	Pejeperro Lagoon	51.5	10	4	12/06/2019	8.41865	-83.38953	-	2	28	0.2	1.1	Jorge V., Mariel M. , Esteban J.	
Costa Rica	Southern Pacific	Pejeperro Lagoon	52.1	20	5.5	12/06/2019	8.41741	-83.39428	-	0.7	30.4	0.8	0.7	Jorge V., Mariel M. , Esteban J.	
Costa Rica	Southern Pacific	Pejeperro Lagoon	52.2	10	2.5	12/06/2019	8.41741	-83.39428	-	0.7	30.4	0.8	0.7	Jorge V., Mariel M. , Esteban J.	
Costa Rica	Southern Pacific	Pejeperro Lagoon	52.3	10	2.5	12/06/2019	8.41741	-83.39428	-	0.7	30.4	0.8	0.7	Jorge V., Mariel M. , Esteban J.	
Costa Rica	Southern Pacific	Pejeperro Lagoon	52.4	20	5.5	12/06/2019	8.41741	-83.39428	-	0.7	30.4	0.8	0.7	Jorge V., Mariel M. , Esteban J.	
Costa Rica	Southern Pacific	Pejeperro Lagoon	52.5	20	5.7	12/06/2019	8.41741	-83.39428	-	0.7	30.4	0.8	0.7	Jorge V., Mariel M. , Esteban J.	
Costa Rica	Southern Pacific	Pejeperro Lagoon	53.1	5	2	13/06/2019	8.41936	-83.39627	-	1.2	30.5	1.2	1	Jorge V., Mariel M. , Esteban J.	
Costa Rica	Southern Pacific	Pejeperro Lagoon	53.2	10	5	13/06/2019	8.41936	-83.39627	-	1.2	30.5	1.2	1	Jorge V., Mariel M. , Esteban J.	
Costa Rica	Southern Pacific	Pejeperro Lagoon	53.3	10	5	13/06/2019	8.41936	-83.39627	-	1.2	30.5	1.2	1	Jorge V., Mariel M. , Esteban J.	
Costa Rica	Southern Pacific	Pejeperro Lagoon	53.4	10	5	13/06/2019	8.41936	-83.39627	-	1.2	30.5	1.2	1	Jorge V., Mariel M. , Esteban J.	
Costa Rica	Southern Pacific	Pejeperro Lagoon	53.5	10	5	13/06/2019	8.41936	-83.39627	-	1.2	30.5	1.2	1	Jorge V., Mariel M. , Esteban J.	
Costa Rica	Southern Pacific	Pejeperro Lagoon	53.6	1.2	0.75	13/06/2019	8.41936	-83.39627	-	1.2	30.5	1.2	1	Jorge V., Mariel M. , Esteban J.	
Costa Rica	Southern Pacific	Pejeperro Lagoon	54.1	10	5	13/06/2019	8.4222	-83.39954	-	1	30.2	1.3	1	Jorge V., Mariel M. , Esteban J.	
Costa Rica	Southern Pacific	Pejeperro Lagoon	54.2	10	5	13/06/2019	8.4222	-83.39954	-	1	30.2	1.3	1	Jorge V., Mariel M. , Esteban J.	
Costa Rica	Southern Pacific	Pejeperro Lagoon	54.3	10	5	13/06/2019	8.4222	-83.39954	-	1	30.2	1.3	1	Jorge V., Mariel M. , Esteban J.	
Costa Rica	Southern Pacific	Pejeperro Lagoon	54.4	10	5	13/06/2019	8.4222	-83.39954	-	1	30.2	1.3	1	Jorge V., Mariel M. , Esteban J.	
Costa Rica	Southern Pacific	Pejeperro Lagoon	54.5	10	5	13/06/2019	8.4222	-83.39954	-	1	30.2	1.3	1	Jorge V., Mariel M. , Esteban J.	
Costa Rica	Southern Pacific	Pejeperro Lagoon	54.6	1.2	0.8	13/06/2019	8.4222	-83.39954	-	1	30.2	1.3	1	Jorge V., Mariel M. , Esteban J.	

Costa Rica	Southern Pacific	Pejeperro Lagoon	55.1	10	5	13/06/2019	8.42577	-83.40337	-	1.1	30.8	1.1	1	Jorge V., Mariel M. , Esteban J.	
Costa Rica	Southern Pacific	Pejeperro Lagoon	55.2	10	5	13/06/2019	8.42577	-83.40337	-	1.1	30.8	1.1	1	Jorge V., Mariel M. , Esteban J.	
Costa Rica	Southern Pacific	Pejeperro Lagoon	55.3	10	5	13/06/2019	8.42577	-83.40337	-	1.1	30.8	1.1	1	Jorge V., Mariel M. , Esteban J.	
Costa Rica	Southern Pacific	Pejeperro Lagoon	55.4	10	5	13/06/2019	8.42577	-83.40337	-	1.1	30.8	1.1	1	Jorge V., Mariel M. , Esteban J.	
Costa Rica	Southern Pacific	Pejeperro Lagoon	55.5	10	5	13/06/2019	8.42577	-83.40337	-	1.1	30.8	1.1	1	Jorge V., Mariel M. , Esteban J.	
Costa Rica	Southern Pacific	Pejeperro Lagoon	55.6	1.2	0.6	13/06/2019	8.42577	-83.40337	-	1.1	30.8	1.1	1	Jorge V., Mariel M. , Esteban J.	
Costa Rica	Southern Pacific	Pejeperro Lagoon	56.1	20	4.8	14/06/2019	8.43433	-83.43294	-	1.4	30.5	2.2	1	Jorge V., Mariel M. , Esteban J.	
Costa Rica	Southern Pacific	Pejeperro Lagoon	56.2	20	5	14/06/2019	8.43433	-83.43294	-	1.4	30.5	2.2	1	Jorge V., Mariel M. , Esteban J.	
Costa Rica	Southern Pacific	Pejeperro Lagoon	56.3	20	4	14/06/2019	8.43433	-83.43294	-	1.4	30.5	2.2	1	Jorge V., Mariel M. , Esteban J.	
Costa Rica	Southern Pacific	Pejeperro Lagoon	56.4	20	3.5	14/06/2019	8.43433	-83.43294	-	1.4	30.5	2.2	1	Jorge V., Mariel M. , Esteban J.	
Costa Rica	Southern Pacific	Pejeperro Lagoon	56.5	20	3.5	14/06/2019	8.43433	-83.43294	-	1.4	30.5	2.2	1	Jorge V., Mariel M. , Esteban J.	
Costa Rica	Southern Pacific	Pejeperro Lagoon	57.1	20	4.5	14/06/2019	8.43814	-83.43549	-	0.7	31.5	2.4	0.5	Jorge V., Mariel M. , Esteban J.	
Costa Rica	Southern Pacific	Pejeperro Lagoon	57.2	20	4.5	14/06/2019	8.43814	-83.43549	-	0.7	31.5	2.4	0.5	Jorge V., Mariel M. , Esteban J.	
Costa Rica	Southern Pacific	Pejeperro Lagoon	57.3	20	5	14/06/2019	8.43814	-83.43549	-	0.7	31.5	2.4	0.5	Jorge V., Mariel M. , Esteban J.	
Costa Rica	Southern Pacific	Pejeperro Lagoon	57.4	20	4.7	14/06/2019	8.43814	-83.43549	-	0.7	31.5	2.4	0.5	Jorge V., Mariel M. , Esteban J.	
Costa Rica	Southern Pacific	Pejeperro Lagoon	57.5	20	4	14/06/2019	8.43814	-83.43549	-	0.7	31.5	2.4	0.5	Jorge V., Mariel M. , Esteban J.	
Costa Rica	Southern Pacific	Pejeperro Lagoon	58.1	20	4.5	14/06/2019	8.4397	-83.43655	-	1.1	32.1	2.5	0.5	Jorge V., Mariel M. , Esteban J.	
Costa Rica	Southern Pacific	Pejeperro Lagoon	58.2	20	4.5	14/06/2019	8.4397	-83.43655	-	1.1	32.1	2.5	0.5	Jorge V., Mariel M. , Esteban J.	
Costa Rica	Southern Pacific	Pejeperro Lagoon	58.3	20	4.5	14/06/2019	8.4397	-83.43655	-	1.1	32.1	2.5	0.5	Jorge V., Mariel M. , Esteban J.	
Costa Rica	Southern Pacific	Pejeperro Lagoon	58.4	20	5	14/06/2019	8.4397	-83.43655	-	1.1	32.1	2.5	0.5	Jorge V., Mariel M. , Esteban J.	
Costa Rica	Southern Pacific	Pejeperro Lagoon	58.5	20	5	14/06/2019	8.4397	-83.43655	-	1.1	32.1	2.5	0.5	Jorge V., Mariel M. , Esteban J.	
Costa Rica	Southern Pacific	Pejeperro Lagoon	59.1	20	4	14/06/2019	8.43933	-83.43413	-	1	32.8	2.3	0.5	Jorge V., Mariel M. , Esteban J.	
Costa Rica	Southern Pacific	Pejeperro Lagoon	59.2	20	4.5	14/06/2019	8.43933	-83.43413	-	1	32.8	2.3	0.5	Jorge V., Mariel M. , Esteban J.	
Costa Rica	Southern Pacific	Pejeperro Lagoon	59.3	20	5	14/06/2019	8.43933	-83.43413	-	1	32.8	2.3	0.5	Jorge V., Mariel M. , Esteban J.	
Costa Rica	Southern Pacific	Pejeperro Lagoon	59.4	20	4.9	14/06/2019	8.43933	-83.43413	-	1	32.8	2.3	0.5	Jorge V., Mariel M. , Esteban J.	
Costa Rica	Southern Pacific	Pejeperro Lagoon	59.5	20	4.3	14/06/2019	8.43933	-83.43413	-	1	32.8	2.3	0.5	Jorge V., Mariel M. , Esteban J.	
Costa Rica	Southern Pacific	Pejeperro Lagoon	60.1	20	5	14/06/2019	8.43569	-83.43665	-	1.2	31.5	2.4	0.5	Jorge V., Mariel M. , Esteban J.	
Costa Rica	Southern Pacific	Pejeperro Lagoon	60.2	20	4.5	14/06/2019	8.43569	-83.43665	-	1.2	31.5	2.4	0.5	Jorge V., Mariel M. , Esteban J.	
Costa Rica	Southern Pacific	Pejeperro Lagoon	60.3	20	4.5	14/06/2019	8.43569	-83.43665	-	1.2	31.5	2.4	0.5	Jorge V., Mariel M. , Esteban J.	
Costa Rica	Southern Pacific	Pejeperro Lagoon	60.4	20	4.2	14/06/2019	8.43569	-83.43665	-	1.2	31.5	2.4	0.5	Jorge V., Mariel M. , Esteban J.	
Costa Rica	Southern Pacific	Pejeperro Lagoon	60.5	20	4.5	14/06/2019	8.43569	-83.43665	-	1.2	31.5	2.4	0.5	Jorge V., Mariel M. , Esteban J.	

Costa Rica	Southern Pacific	Pejeperro Lagoon	61.1	20	3.5	14/06/2019	8.4369	-83.43921	-	1.3	31.1	2.4	0.5	Jorge V., Mariel M. , Esteban J.	
Costa Rica	Southern Pacific	Pejeperro Lagoon	61.2	20	3.4	14/06/2019	8.4369	-83.43921	-	1.3	31.1	2.4	0.5	Jorge V., Mariel M. , Esteban J.	
Costa Rica	Southern Pacific	Pejeperro Lagoon	61.3	20	3	14/06/2019	8.4369	-83.43921	-	1.3	31.1	2.4	0.5	Jorge V., Mariel M. , Esteban J.	
Costa Rica	Southern Pacific	Pejeperro Lagoon	61.4	20	3.2	14/06/2019	8.4369	-83.43921	-	1.3	31.1	2.4	0.5	Jorge V., Mariel M. , Esteban J.	
Costa Rica	Southern Pacific	Pejeperro Lagoon	61.5	20	3	14/06/2019	8.4369	-83.43921	-	1.3	31.1	2.4	0.5	Jorge V., Mariel M. , Esteban J.	
Costa Rica	Northern Pacific	Bahía Thomas	62.1	10	3.2	4/07/2019	10.92889	-85.71883	Falling	1.7	26.6	28.4	1	Jorge V., Debbie L, Keylor Alfaro	
Costa Rica	Northern Pacific	Bahía Thomas	62.2	10	3.7	4/07/2019	10.92889	-85.71883	Falling	1.7	26.6	28.4	1	Jorge V., Debbie L, Keylor Alfaro	
Costa Rica	Northern Pacific	Bahía Thomas	62.3	10	4	4/07/2019	10.92889	-85.71883	Falling	1.7	26.6	28.4	1	Jorge V., Debbie L, Keylor Alfaro	
Costa Rica	Northern Pacific	Bahía Thomas	62.4	10	4.1	4/07/2019	10.92889	-85.71883	Falling	1.7	26.6	28.4	1	Jorge V., Debbie L, Keylor Alfaro	
Costa Rica	Northern Pacific	Bahía Thomas	62.5	10	3.9	4/07/2019	10.92889	-85.71883	Falling	1.7	26.6	28.4	1	Jorge V., Debbie L, Keylor Alfaro	
Costa Rica	Northern Pacific	Bahía Thomas	63.1	10	5.1	4/07/2019	10.92658	-85.71797	Rising	1.2	27.3	31.5	1.2	Jorge V., Debbie L, Keylor Alfaro	
Costa Rica	Northern Pacific	Bahía Thomas	63.2	10	4.6	4/07/2019	10.92658	-85.71797	Rising	1.2	27.3	31.5	1.2	Jorge V., Debbie L, Keylor Alfaro	
Costa Rica	Northern Pacific	Bahía Thomas	63.3	10	4.4	4/07/2019	10.92658	-85.71797	Rising	1.2	27.3	31.5	1.2	Jorge V., Debbie L, Keylor Alfaro	
Costa Rica	Northern Pacific	Bahía Thomas	63.4	10	4.5	4/07/2019	10.92658	-85.71797	Rising	1.2	27.3	31.5	1.2	Jorge V., Debbie L, Keylor Alfaro	
Costa Rica	Northern Pacific	Bahía Thomas	63.5	10	4.8	4/07/2019	10.92658	-85.71797	Rising	1.2	27.3	31.5	1.2	Jorge V., Debbie L, Keylor Alfaro	
Costa Rica	Northern Pacific	Bahía Santa Elena	64.1	5	1.5	4/07/2019	10.91498	-85.78383	Rising	2.1	27.4	32.3	2.1	Jorge V., Debbie L, Keylor Alfaro	
Costa Rica	Northern Pacific	Bahía Santa Elena	64.2	10	4.5	4/07/2019	10.91498	-85.78383	Rising	2.1	27.4	32.3	2.1	Jorge V., Debbie L, Keylor Alfaro	
Costa Rica	Northern Pacific	Bahía Santa Elena	64.3	10	5	4/07/2019	10.91498	-85.78383	Rising	2.1	27.4	32.3	2.1	Jorge V., Debbie L, Keylor Alfaro	
Costa Rica	Northern Pacific	Bahía Santa Elena	64.4	10	4.6	4/07/2019	10.91498	-85.78383	Rising	2.1	27.4	32.3	2.1	Jorge V., Debbie L, Keylor Alfaro	
Costa Rica	Northern Pacific	Bahía Santa Elena	64.5	10	4	4/07/2019	10.91498	-85.78383	Rising	2.1	27.4	32.3	2.1	Jorge V., Debbie L, Keylor Alfaro	
Costa Rica	Northern Pacific	Bahía Santa Elena	65.1	10	4.5	4/07/2019	10.91536	-85.78587	Falling	1.9	27.5	32.3	1.9	Jorge V., Debbie L, Keylor Alfaro	
Costa Rica	Northern Pacific	Bahía Santa Elena	65.2	10	4	4/07/2019	10.91536	-85.78587	Falling	1.9	27.5	32.3	1.9	Jorge V., Debbie L, Keylor Alfaro	
Costa Rica	Northern Pacific	Bahía Santa Elena	65.3	10	4.5	4/07/2019	10.91536	-85.78587	Falling	1.9	27.5	32.3	1.9	Jorge V., Debbie L, Keylor Alfaro	
Costa Rica	Northern Pacific	Bahía Santa Elena	65.4	10	4.4	4/07/2019	10.91536	-85.78587	Falling	1.9	27.5	32.3	1.9	Jorge V., Debbie L, Keylor Alfaro	
Costa Rica	Northern Pacific	Bahía Santa Elena	65.5	10	4.5	4/07/2019	10.91536	-85.78587	Falling	1.9	27.5	32.3	1.9	Jorge V., Debbie L, Keylor Alfaro	
Costa Rica	Northern Pacific	Bahía Santa Elena	66.1	10	2.5	5/07/2019	10.91261	-85.78508	Falling	1.8	25.6	30.5	1.8	Jorge V., Debbie L, Keylor Alfaro	
Costa Rica	Northern Pacific	Bahía Santa Elena	66.2	20	3.7	5/07/2019	10.91261	-85.78508	Falling	1.8	25.6	30.5	1.8	Jorge V., Debbie L, Keylor Alfaro	
Costa Rica	Northern Pacific	Bahía Santa Elena	66.3	20	5.1	5/07/2019	10.91261	-85.78508	Falling	1.8	25.6	30.5	1.8	Jorge V., Debbie L, Keylor Alfaro	
Costa Rica	Northern Pacific	Bahía Santa Elena	66.4	20	3.5	5/07/2019	10.91261	-85.78508	Falling	1.8	25.6	30.5	1.8	Jorge V., Debbie L, Keylor Alfaro	
Costa Rica	Northern Pacific	Bahía Santa Elena	66.5	20	5.1	5/07/2019	10.91261	-85.78508	Falling	1.8	25.6	30.5	1.8	Jorge V., Debbie L, Keylor Alfaro	
Costa Rica	Northern Pacific	Bahía Santa Elena	67.1	10	4.8	5/07/2019	10.91522	-85.78911	Falling	1.1	26.3	32.5	1.1	Jorge V., Debbie L, Keylor Alfaro	

Costa Rica	Northern Pacific	Bahía Santa Elena	67.2	10	3.5	5/07/2019	10.91522	-85.78911	Falling	1.1	26.3	32.5	1.1	Jorge V., Debbie L, Keylor Alfaro	
Costa Rica	Northern Pacific	Bahía Santa Elena	67.3	10	3.9	5/07/2019	10.91522	-85.78911	Falling	1.1	26.3	32.5	1.1	Jorge V., Debbie L, Keylor Alfaro	
Costa Rica	Northern Pacific	Bahía Santa Elena	67.4	10	4	5/07/2019	10.91522	-85.78911	Falling	1.1	26.3	32.5	1.1	Jorge V., Debbie L, Keylor Alfaro	
Costa Rica	Northern Pacific	Bahía Santa Elena	67.5	10	5.1	5/07/2019	10.91522	-85.78911	Falling	1.1	26.3	32.5	1.1	Jorge V., Debbie L, Keylor Alfaro	
Costa Rica	Northern Pacific	Bahía Santa Elena	68.1	5	5	5/07/2019	10.91601	-85.79204	Low	15.6	27	32.2	5	Jorge V., Debbie L, Keylor Alfaro	
Costa Rica	Northern Pacific	Bahía Santa Elena	68.2	5	5	5/07/2019	10.91601	-85.79204	Low	15.6	27	32.2	5	Jorge V., Debbie L, Keylor Alfaro	
Costa Rica	Northern Pacific	Bahía Santa Elena	68.3	5	4.9	5/07/2019	10.91601	-85.79204	Low	15.6	27	32.2	5	Jorge V., Debbie L, Keylor Alfaro	
Costa Rica	Northern Pacific	Bahía Santa Elena	68.4	5	5.4	5/07/2019	10.91601	-85.79204	Low	15.6	27	32.2	5	Jorge V., Debbie L, Keylor Alfaro	
Costa Rica	Northern Pacific	Bahía Santa Elena	68.5	5	5.7	5/07/2019	10.91601	-85.79204	Low	15.6	27	32.2	5	Jorge V., Debbie L, Keylor Alfaro	
Costa Rica	Northern Pacific	Bahía Santa Elena	69.1	5	5.5	5/07/2019	10.91689	-85.795	Rising	17.4	27	32.2	4.5	Jorge V., Debbie L, Keylor Alfaro	
Costa Rica	Northern Pacific	Bahía Santa Elena	69.2	5	5	5/07/2019	10.91689	-85.795	Rising	17.4	27	32.2	4.5	Jorge V., Debbie L, Keylor Alfaro	
Costa Rica	Northern Pacific	Bahía Santa Elena	69.3	5	5.4	5/07/2019	10.91689	-85.795	Rising	17.4	27	32.2	4.5	Jorge V., Debbie L, Keylor Alfaro	
Costa Rica	Northern Pacific	Bahía Santa Elena	69.4	5	5	5/07/2019	10.91689	-85.795	Rising	17.4	27	32.2	4.5	Jorge V., Debbie L, Keylor Alfaro	
Costa Rica	Northern Pacific	Bahía Santa Elena	69.5	5	5	5/07/2019	10.91689	-85.795	Rising	17.4	27	32.2	4.5	Jorge V., Debbie L, Keylor Alfaro	
Costa Rica	Northern Pacific	Bahía Santa Elena	70.1	5	2.3	5/07/2019	10.91353	-85.78635	Rising	0.6	29.2	32.5	0.6	Jorge V., Debbie L, Keylor Alfaro	
Costa Rica	Northern Pacific	Bahía Santa Elena	70.2	10	4.8	5/07/2019	10.91353	-85.78635	Rising	0.6	29.2	32.5	0.6	Jorge V., Debbie L, Keylor Alfaro	
Costa Rica	Northern Pacific	Bahía Santa Elena	70.3	10	5.1	5/07/2019	10.91353	-85.78635	Rising	0.6	29.2	32.5	0.6	Jorge V., Debbie L, Keylor Alfaro	
Costa Rica	Northern Pacific	Bahía Santa Elena	70.4	10	5.2	5/07/2019	10.91353	-85.78635	Rising	0.6	29.2	32.5	0.6	Jorge V., Debbie L, Keylor Alfaro	
Costa Rica	Northern Pacific	Bahía Santa Elena	70.5	10	5	5/07/2019	10.91353	-85.78635	Rising	0.6	29.2	32.5	0.6	Jorge V., Debbie L, Keylor Alfaro	
Costa Rica	Northern Pacific	Playas Danta y Dantita	71.1	5	5.7	6/07/2019	10.479	-85.78689	Rising	3.1	30.2	31.4	3.1	Jorge V, Debbie L, Regulo Tenorio	
Costa Rica	Northern Pacific	Playas Danta y Dantita	71.2	5	5.2	6/07/2019	10.479	-85.78689	Rising	3.1	30.2	31.4	3.1	Jorge V, Debbie L, Regulo Tenorio	
Costa Rica	Northern Pacific	Playas Danta y Dantita	71.3	5	5.5	6/07/2019	10.479	-85.78689	Rising	3.1	30.2	31.4	3.1	Jorge V, Debbie L, Regulo Tenorio	
Costa Rica	Northern Pacific	Playas Danta y Dantita	71.4	5	5.4	6/07/2019	10.479	-85.78689	Rising	3.1	30.2	31.4	3.1	Jorge V, Debbie L, Regulo Tenorio	
Costa Rica	Northern Pacific	Playas Danta y Dantita	71.5	5	5.1	6/07/2019	10.479	-85.78689	Rising	3.1	30.2	31.4	3.1	Jorge V, Debbie L, Regulo Tenorio	
Costa Rica	Northern Pacific	Playas Danta y Dantita	71.6	1.2	1.9	6/07/2019	10.479	-85.78689	Rising	3.1	30.2	31.4	3.1	Jorge V, Debbie L, Regulo Tenorio	
Costa Rica	Northern Pacific	Playas Danta y Dantita	72.1	5	5.7	7/07/2019	10.48107	-85.78822	Falling	4.2	29.2	31.3	4.2	Jorge V, Debbie L, Regulo Tenorio	
Costa Rica	Northern Pacific	Playas Danta y Dantita	72.2	5	5.7	7/07/2019	10.48107	-85.78822	Falling	4.2	29.2	31.3	4.2	Jorge V, Debbie L, Regulo Tenorio	
Costa Rica	Northern Pacific	Playas Danta y Dantita	72.3	5	5.1	7/07/2019	10.48107	-85.78822	Falling	4.2	29.2	31.3	4.2	Jorge V, Debbie L, Regulo Tenorio	
Costa Rica	Northern Pacific	Playas Danta y Dantita	72.4	5	5.5	7/07/2019	10.48107	-85.78822	Falling	4.2	29.2	31.3	4.2	Jorge V, Debbie L, Regulo Tenorio	
Costa Rica	Northern Pacific	Playas Danta y Dantita	72.5	5	5.5	7/07/2019	10.48107	-85.78822	Falling	4.2	29.2	31.3	4.2	Jorge V, Debbie L, Regulo Tenorio	
Costa Rica	Northern Pacific	Playas Danta y Dantita	72.6	1.2	1.6	7/07/2019	10.48107	-85.78822	Falling	4.2	29.2	31.3	4.2	Jorge V, Debbie L, Regulo Tenorio	

Costa Rica	Northern Pacific	Playas Danta y Dantita	73.1	5	5.4	7/07/2019	10.48144	-85.79008	Falling	4.8	30.1	31.2	4.8	Jorge V, Debbie L, Regulo Tenorio	
Costa Rica	Northern Pacific	Playas Danta y Dantita	73.2	5	5.2	7/07/2019	10.48144	-85.79008	Falling	4.8	30.1	31.2	4.8	Jorge V, Debbie L, Regulo Tenorio	
Costa Rica	Northern Pacific	Playas Danta y Dantita	73.3	5	5.5	7/07/2019	10.48144	-85.79008	Falling	4.8	30.1	31.2	4.8	Jorge V, Debbie L, Regulo Tenorio	
Costa Rica	Northern Pacific	Playas Danta y Dantita	73.4	5	5	7/07/2019	10.48144	-85.79008	Falling	4.8	30.1	31.2	4.8	Jorge V, Debbie L, Regulo Tenorio	
Costa Rica	Northern Pacific	Playas Danta y Dantita	73.5	5	5.2	7/07/2019	10.48144	-85.79008	Falling	4.8	30.1	31.2	4.8	Jorge V, Debbie L, Regulo Tenorio	
Costa Rica	Northern Pacific	Playas Danta y Dantita	73.6	1.2	2.4	7/07/2019	10.48144	-85.79008	Falling	4.8	30.1	31.2	4.8	Jorge V, Debbie L, Regulo Tenorio	
Costa Rica	Northern Pacific	Playas Danta y Dantita	74.1	5	4.7	7/07/2019	10.48219	-85.79208	Low	3.1	30.7	31.1	3.1	Jorge V, Debbie L, Regulo Tenorio	
Costa Rica	Northern Pacific	Playas Danta y Dantita	74.2	5	4.6	7/07/2019	10.48219	-85.79208	Low	3.1	30.7	31.1	3.1	Jorge V, Debbie L, Regulo Tenorio	
Costa Rica	Northern Pacific	Playas Danta y Dantita	74.3	5	5.1	7/07/2019	10.48219	-85.79208	Low	3.1	30.7	31.1	3.1	Jorge V, Debbie L, Regulo Tenorio	
Costa Rica	Northern Pacific	Playas Danta y Dantita	74.4	5	5.5	7/07/2019	10.48219	-85.79208	Low	3.1	30.7	31.1	3.1	Jorge V, Debbie L, Regulo Tenorio	
Costa Rica	Northern Pacific	Playas Danta y Dantita	74.5	5	5.3	7/07/2019	10.48219	-85.79208	Low	3.1	30.7	31.1	3.1	Jorge V, Debbie L, Regulo Tenorio	
Costa Rica	Northern Pacific	Playas Danta y Dantita	74.6	1.2	2.1	7/07/2019	10.48219	-85.79208	Low	3.1	30.7	31.1	3.1	Jorge V, Debbie L, Regulo Tenorio	
Costa Rica	Northern Pacific	Playas Danta y Dantita	75.1	5	5.5	7/07/2019	10.48111	-85.79474	Rising	5.9	30.7	31.1	5.9	Jorge V, Debbie L, Regulo Tenorio	
Costa Rica	Northern Pacific	Playas Danta y Dantita	75.2	5	5.4	7/07/2019	10.48111	-85.79474	Rising	5.9	30.7	31.1	5.9	Jorge V, Debbie L, Regulo Tenorio	
Costa Rica	Northern Pacific	Playas Danta y Dantita	75.3	5	5.5	7/07/2019	10.48111	-85.79474	Rising	5.9	30.7	31.1	5.9	Jorge V, Debbie L, Regulo Tenorio	
Costa Rica	Northern Pacific	Playas Danta y Dantita	75.4	5	5.3	7/07/2019	10.48111	-85.79474	Rising	5.9	30.7	31.1	5.9	Jorge V, Debbie L, Regulo Tenorio	
Costa Rica	Northern Pacific	Playas Danta y Dantita	75.5	5	5.3	7/07/2019	10.48111	-85.79474	Rising	5.9	30.7	31.1	5.9	Jorge V, Debbie L, Regulo Tenorio	
Costa Rica	Northern Pacific	Playas Danta y Dantita	75.6	1.2	2.35	7/07/2019	10.48111	-85.79474	Rising	5.9	30.7	31.1	5.9	Jorge V, Debbie L, Regulo Tenorio	
Costa Rica	Northern Pacific	Estero Potrero Grande	76.1	5	4	23/07/2019	10.84358	-85.78929	Rising	5.3	28.4	32	5.3	Jorge V, Jorge S, Julio, Keylor	
Costa Rica	Northern Pacific	Estero Potrero Grande	76.2	5	6	23/07/2019	10.84358	-85.78929	Rising	5.3	28.4	32	5.3	Jorge V, Jorge S, Julio, Keylor	
Costa Rica	Northern Pacific	Estero Potrero Grande	76.3	5	5.25	23/07/2019	10.84358	-85.78929	Rising	5.3	28.4	32	5.3	Jorge V, Jorge S, Julio, Keylor	
Costa Rica	Northern Pacific	Estero Potrero Grande	76.4	5	5	23/07/2019	10.84358	-85.78929	Rising	5.3	28.4	32	5.3	Jorge V, Jorge S, Julio, Keylor	
Costa Rica	Northern Pacific	Estero Potrero Grande	76.5	5	5	23/07/2019	10.84358	-85.78929	Rising	5.3	28.4	32	5.3	Jorge V, Jorge S, Julio, Keylor	
Costa Rica	Northern Pacific	Estero Potrero Grande	77.1	5	5	23/07/2019	10.85008	-85.79273	Rising	4.6	28.7	31.5	4.6	Jorge V, Jorge S, Julio, Keylor	
Costa Rica	Northern Pacific	Estero Potrero Grande	77.2	5	5	23/07/2019	10.85008	-85.79273	Rising	4.6	28.7	31.5	4.6	Jorge V, Jorge S, Julio, Keylor	
Costa Rica	Northern Pacific	Estero Potrero Grande	77.3	5	5.25	23/07/2019	10.85008	-85.79273	Rising	4.6	28.7	31.5	4.6	Jorge V, Jorge S, Julio, Keylor	
Costa Rica	Northern Pacific	Estero Potrero Grande	77.4	5	5	23/07/2019	10.85008	-85.79273	Rising	4.6	28.7	31.5	4.6	Jorge V, Jorge S, Julio, Keylor	
Costa Rica	Northern Pacific	Estero Potrero Grande	77.5	5	5	23/07/2019	10.85008	-85.79273	Rising	4.6	28.7	31.5	4.6	Jorge V, Jorge S, Julio, Keylor	
Costa Rica	Northern Pacific	Estero Potrero Grande	77.6	1.2	2.15	23/07/2019	10.85008	-85.79273	Rising	4.6	28.7	31.5	4.6	Jorge V, Jorge S, Julio, Keylor	
Costa Rica	Northern Pacific	Estero Potrero Grande	78.1	10	4.8	24/07/2019	10.85492	-85.79661	Falling	1.6	28.3	31.1	1.1	Jorge V, Jorge S, Keylor	
Costa Rica	Northern Pacific	Estero Potrero Grande	78.2	10	5	24/07/2019	10.85492	-85.79661	Falling	1.6	28.3	31.1	1.1	Jorge V, Jorge S, Keylor	

Costa Rica	Northern Pacific	Estero Potrero Grande	78.3	10	5	24/07/2019	10.85492	-85.79661	Falling	1.6	28.3	31.1	1.1	Jorge V, Jorge S, Keylor	
Costa Rica	Northern Pacific	Estero Potrero Grande	78.4	10	5	24/07/2019	10.85492	-85.79661	Falling	1.6	28.3	31.1	1.1	Jorge V, Jorge S, Keylor	
Costa Rica	Northern Pacific	Estero Potrero Grande	78.5	10	5	24/07/2019	10.85492	-85.79661	Falling	1.6	28.3	31.1	1.1	Jorge V, Jorge S, Keylor	
Costa Rica	Northern Pacific	Estero Potrero Grande	79.1	10	3.5	24/07/2019	10.85365	-85.79333	Falling	0.8	30	29.7	0.8	Jorge V, Jorge S, Keylor	
Costa Rica	Northern Pacific	Estero Potrero Grande	79.2	10	4.5	24/07/2019	10.85365	-85.79333	Falling	0.8	30	29.7	0.8	Jorge V, Jorge S, Keylor	
Costa Rica	Northern Pacific	Estero Potrero Grande	79.3	10	3.5	24/07/2019	10.85365	-85.79333	Falling	0.8	30	29.7	0.8	Jorge V, Jorge S, Keylor	
Costa Rica	Northern Pacific	Estero Potrero Grande	79.4	20	2	24/07/2019	10.85365	-85.79333	Falling	0.8	30	29.7	0.8	Jorge V, Jorge S, Keylor	
Costa Rica	Northern Pacific	Estero Potrero Grande	79.5	20	2.5	24/07/2019	10.85365	-85.79333	Falling	0.8	30	29.7	0.8	Jorge V, Jorge S, Keylor	
Costa Rica	Northern Pacific	Estero Potrero Grande	80.1	20	5	24/07/2019	10.85314	-85.79021	Rising	1.3	28.5	25	0.2	Jorge V, Jorge S, Keylor	
Costa Rica	Northern Pacific	Estero Potrero Grande	80.2	20	5	24/07/2019	10.85314	-85.79021	Rising	1.3	28.5	25	0.2	Jorge V, Jorge S, Keylor	
Costa Rica	Northern Pacific	Estero Potrero Grande	80.3	20	5	24/07/2019	10.85314	-85.79021	Rising	1.3	28.5	25	0.2	Jorge V, Jorge S, Keylor	
Costa Rica	Northern Pacific	Estero Potrero Grande	80.4	20	5	24/07/2019	10.85314	-85.79021	Rising	1.3	28.5	25	0.2	Jorge V, Jorge S, Keylor	
Costa Rica	Northern Pacific	Estero Potrero Grande	80.5	20	5	24/07/2019	10.85314	-85.79021	Rising	1.3	28.5	25	0.2	Jorge V, Jorge S, Keylor	
Costa Rica	Northern Pacific	Estero Potrero Grande	81.1	20	3	24/07/2019	10.85329	-85.78761	Rising	1.3	27.3	5.9	0.25	Jorge V, Jorge S, Keylor	
Costa Rica	Northern Pacific	Estero Potrero Grande	81.2	20	3.5	24/07/2019	10.85329	-85.78761	Rising	1.3	27.3	5.9	0.25	Jorge V, Jorge S, Keylor	
Costa Rica	Northern Pacific	Estero Potrero Grande	81.3	20	3.5	24/07/2019	10.85329	-85.78761	Rising	1.3	27.3	5.9	0.25	Jorge V, Jorge S, Keylor	
Costa Rica	Northern Pacific	Estero Potrero Grande	81.4	20	4	24/07/2019	10.85329	-85.78761	Rising	1.3	27.3	5.9	0.25	Jorge V, Jorge S, Keylor	
Costa Rica	Northern Pacific	Estero Potrero Grande	81.5	20	4.25	24/07/2019	10.85329	-85.78761	Rising	1.3	27.3	5.9	0.25	Jorge V, Jorge S, Keylor	
Costa Rica	Northern Pacific	Estero Potrero Grande	82.1	10	5.5	25/07/2019	10.85143	-85.79132	Rising	0.9	28.2	31.4	0.9	Jorge V, Jorge S, Keylor	
Costa Rica	Northern Pacific	Estero Potrero Grande	82.2	10	5	25/07/2019	10.85143	-85.79132	Rising	0.9	28.2	31.4	0.9	Jorge V, Jorge S, Keylor	
Costa Rica	Northern Pacific	Estero Potrero Grande	82.3	10	5	25/07/2019	10.85143	-85.79132	Rising	0.9	28.2	31.4	0.9	Jorge V, Jorge S, Keylor	
Costa Rica	Northern Pacific	Estero Potrero Grande	82.4	10	5.25	25/07/2019	10.85143	-85.79132	Rising	0.9	28.2	31.4	0.9	Jorge V, Jorge S, Keylor	
Costa Rica	Northern Pacific	Estero Potrero Grande	82.5	10	5	25/07/2019	10.85143	-85.79132	Rising	0.9	28.2	31.4	0.9	Jorge V, Jorge S, Keylor	
Costa Rica	Northern Pacific	Estero Potrero Grande	82.6	1.2	1.625	25/07/2019	10.85143	-85.79132	Rising	0.9	28.2	31.4	0.9	Jorge V, Jorge S, Keylor	
Costa Rica	Northern Pacific	Estero Potrero Grande	82.7	1.2	1.65	25/07/2019	10.85143	-85.79132	Rising	0.9	28.2	31.4	0.9	Jorge V, Jorge S, Keylor	
Costa Rica	Northern Pacific	Estero Potrero Grande	83.1	10	3.5	25/07/2019	10.85042	-85.78737	Falling	1.1	28.7	24.4	1.1	Jorge V, Jorge S, Keylor	
Costa Rica	Northern Pacific	Estero Potrero Grande	83.2	10	4.9	25/07/2019	10.85042	-85.78737	Falling	1.1	28.7	24.4	1.1	Jorge V, Jorge S, Keylor	
Costa Rica	Northern Pacific	Estero Potrero Grande	83.3	10	4.9	25/07/2019	10.85042	-85.78737	Falling	1.1	28.7	24.4	1.1	Jorge V, Jorge S, Keylor	
Costa Rica	Northern Pacific	Estero Potrero Grande	83.4	10	5	25/07/2019	10.85042	-85.78737	Falling	1.1	28.7	24.4	1.1	Jorge V, Jorge S, Keylor	
Costa Rica	Northern Pacific	Estero Potrero Grande	83.5	10	5	25/07/2019	10.85042	-85.78737	Falling	1.1	28.7	24.4	1.1	Jorge V, Jorge S, Keylor	
Costa Rica	Northern Pacific	Estero Potrero Grande	84.1	10	3.5	25/07/2019	10.84854	-85.78851	Falling	0.9	29.5	26.7	0.9	Jorge V, Jorge S, Keylor	

Costa Rica	Northern Pacific	Estero Potrero Grande	84.2	20	5	25/07/2019	10.84854	-85.78851	Falling	0.9	29.5	26.7	0.9	Jorge V, Jorge S, Keylor	
Costa Rica	Northern Pacific	Estero Potrero Grande	84.3	20	5	25/07/2019	10.84854	-85.78851	Falling	0.9	29.5	26.7	0.9	Jorge V, Jorge S, Keylor	
Costa Rica	Northern Pacific	Estero Potrero Grande	84.4	20	5	25/07/2019	10.84854	-85.78851	Falling	0.9	29.5	26.7	0.9	Jorge V, Jorge S, Keylor	
Costa Rica	Northern Pacific	Estero Potrero Grande	84.5	20	5	25/07/2019	10.84854	-85.78851	Falling	0.9	29.5	26.7	0.9	Jorge V, Jorge S, Keylor	
Costa Rica	Northern Pacific	Estero Potrero Grande	85.1	10	5	25/07/2019	10.84629	-85.78568	Falling	0.5	29.3	24.3	0.5	Jorge V, Jorge S, Keylor	
Costa Rica	Northern Pacific	Estero Potrero Grande	85.2	10	5	25/07/2019	10.84629	-85.78568	Falling	0.5	29.3	24.3	0.5	Jorge V, Jorge S, Keylor	
Costa Rica	Northern Pacific	Estero Potrero Grande	85.3	10	5	25/07/2019	10.84629	-85.78568	Falling	0.5	29.3	24.3	0.5	Jorge V, Jorge S, Keylor	
Costa Rica	Northern Pacific	Estero Potrero Grande	85.4	10	5	25/07/2019	10.84629	-85.78568	Falling	0.5	29.3	24.3	0.5	Jorge V, Jorge S, Keylor	
Costa Rica	Northern Pacific	Estero Potrero Grande	85.5	10	5	25/07/2019	10.84629	-85.78568	Falling	0.5	29.3	24.3	0.5	Jorge V, Jorge S, Keylor	
Costa Rica	Northern Pacific	Estero Potrero Grande	85.6	1.2	2.25	25/07/2019	10.84629	-85.78568	Falling	0.5	29.3	24.3	0.5	Jorge V, Jorge S, Keylor	
Costa Rica	Northern Pacific	Estero Potrero Grande	85.7	1.2	2.4	25/07/2019	10.84629	-85.78568	Falling	0.5	29.3	24.3	0.5	Jorge V, Jorge S, Keylor	
Costa Rica	Northern Pacific	Bahía Nancite	86.1	5	5	26/07/2019	10.79166	-85.68516	Falling	7	28	31.9	5.5	Jorge V, Jorge S, Julio, Keylor	
Costa Rica	Northern Pacific	Bahía Nancite	86.2	5	5.5	26/07/2019	10.79166	-85.68516	Falling	7	28	31.9	5.5	Jorge V, Jorge S, Julio, Keylor	
Costa Rica	Northern Pacific	Bahía Nancite	86.3	5	5	26/07/2019	10.79166	-85.68516	Falling	7	28	31.9	5.5	Jorge V, Jorge S, Julio, Keylor	
Costa Rica	Northern Pacific	Bahía Nancite	86.4	5	5.1	26/07/2019	10.79166	-85.68516	Falling	7	28	31.9	5.5	Jorge V, Jorge S, Julio, Keylor	
Costa Rica	Northern Pacific	Bahía Nancite	86.5	5	5	26/07/2019	10.79166	-85.68516	Falling	7	28	31.9	5.5	Jorge V, Jorge S, Julio, Keylor	
Costa Rica	Northern Pacific	Bahía Nancite	86.6	1.2	1.66	26/07/2019	10.79166	-85.68516	Falling	7	28	31.9	5.5	Jorge V, Jorge S, Julio, Keylor	
Costa Rica	Northern Pacific	Bahía Nancite	86.7	1.2	2	26/07/2019	10.79166	-85.68516	Falling	7	28	31.9	5.5	Jorge V, Jorge S, Julio, Keylor	
Costa Rica	Northern Pacific	Bahía Nancite	87.1	5	5.1	26/07/2019	10.79306	-85.6828	Falling	8.9	27.9	32	5	Jorge V, Jorge S, Julio, Keylor	
Costa Rica	Northern Pacific	Bahía Nancite	87.2	5	5	26/07/2019	10.79306	-85.6828	Falling	8.9	27.9	32	5	Jorge V, Jorge S, Julio, Keylor	
Costa Rica	Northern Pacific	Bahía Nancite	87.3	5	5.1	26/07/2019	10.79306	-85.6828	Falling	8.9	27.9	32	5	Jorge V, Jorge S, Julio, Keylor	
Costa Rica	Northern Pacific	Bahía Nancite	87.4	5	5	26/07/2019	10.79306	-85.6828	Falling	8.9	27.9	32	5	Jorge V, Jorge S, Julio, Keylor	
Costa Rica	Northern Pacific	Bahía Nancite	87.5	5	5	26/07/2019	10.79306	-85.6828	Falling	8.9	27.9	32	5	Jorge V, Jorge S, Julio, Keylor	
Costa Rica	Northern Pacific	Bahía Nancite	87.6	1.2	2.74	26/07/2019	10.79306	-85.6828	Falling	8.9	27.9	32	5	Jorge V, Jorge S, Julio, Keylor	
Costa Rica	Northern Pacific	Bahía Nancite	87.7	1.2	2.54	26/07/2019	10.79306	-85.6828	Falling	8.9	27.9	32	5	Jorge V, Jorge S, Julio, Keylor	
Costa Rica	Northern Pacific	Bahía Nancite	88.1	5	5.1	26/07/2019	10.79437	-85.68182	Rising	5.9	27.9	32	4	Jorge V, Jorge S, Julio, Keylor	
Costa Rica	Northern Pacific	Bahía Nancite	88.2	5	5.1	26/07/2019	10.79437	-85.68182	Rising	5.9	27.9	32	4	Jorge V, Jorge S, Julio, Keylor	
Costa Rica	Northern Pacific	Bahía Nancite	88.3	5	5	26/07/2019	10.79437	-85.68182	Rising	5.9	27.9	32	4	Jorge V, Jorge S, Julio, Keylor	
Costa Rica	Northern Pacific	Bahía Nancite	88.4	5	5	26/07/2019	10.79437	-85.68182	Rising	5.9	27.9	32	4	Jorge V, Jorge S, Julio, Keylor	
Costa Rica	Northern Pacific	Bahía Nancite	88.5	5	5	26/07/2019	10.79437	-85.68182	Rising	5.9	27.9	32	4	Jorge V, Jorge S, Julio, Keylor	
Costa Rica	Northern Pacific	Estero Potrero Grande	89.1	5	5	27/07/2019	10.8513	-85.79914	Rising	5.5	27.7	31.9	3	Jorge V, Jorge S, Julio, Keylor	

Costa Rica	Northern Pacific	Estero Potrero Grande	89.2	10	5.5	27/07/2019	10.8513	-85.79914	Rising	5.5	27.7	31.9	3	Jorge V, Jorge S, Julio, Keylor	
Costa Rica	Northern Pacific	Estero Potrero Grande	89.3	10	5.5	27/07/2019	10.8513	-85.79914	Rising	5.5	27.7	31.9	3	Jorge V, Jorge S, Julio, Keylor	
Costa Rica	Northern Pacific	Estero Potrero Grande	89.4	10	5.25	27/07/2019	10.8513	-85.79914	Rising	5.5	27.7	31.9	3	Jorge V, Jorge S, Julio, Keylor	
Costa Rica	Northern Pacific	Estero Potrero Grande	89.5	10	5.5	27/07/2019	10.8513	-85.79914	Rising	5.5	27.7	31.9	3	Jorge V, Jorge S, Julio, Keylor	
Costa Rica	Northern Pacific	Estero Potrero Grande	89.6	1.2	2.36	27/07/2019	10.8513	-85.79914	Rising	5.5	27.7	31.9	3	Jorge V, Jorge S, Julio, Keylor	
Costa Rica	Northern Pacific	Estero Potrero Grande	90.1	10	5.1	27/07/2019	10.84525	-85.7832	Falling	1.9	28.4	29.2	1.9	Jorge V, Jorge S, Keylor	
Costa Rica	Northern Pacific	Estero Potrero Grande	90.2	10	5.2	27/07/2019	10.84525	-85.7832	Falling	1.9	28.4	29.2	1.9	Jorge V, Jorge S, Keylor	
Costa Rica	Northern Pacific	Estero Potrero Grande	90.3	10	5.3	27/07/2019	10.84525	-85.7832	Falling	1.9	28.4	29.2	1.9	Jorge V, Jorge S, Keylor	
Costa Rica	Northern Pacific	Estero Potrero Grande	90.4	10	5	27/07/2019	10.84525	-85.7832	Falling	1.9	28.4	29.2	1.9	Jorge V, Jorge S, Keylor	
Costa Rica	Northern Pacific	Estero Potrero Grande	90.5	10	5.2	27/07/2019	10.84525	-85.7832	Falling	1.9	28.4	29.2	1.9	Jorge V, Jorge S, Keylor	
Costa Rica	Northern Pacific	Estero Potrero Grande	90.6	1.2	2.05	27/07/2019	10.84525	-85.7832	Falling	1.9	28.4	29.2	1.9	Jorge V, Jorge S, Keylor	
Costa Rica	Caribbean	Laguna de Gandoca	41.1	10	5.5	12/08/2019	9.5903	-82.59685	-	0.2	27.27	0	0.2	Jorge V., Marie Claire, Davis	
Costa Rica	Caribbean	Laguna de Gandoca	41.2	10	5	12/08/2019	9.5903	-82.59685	-	0.2	27.27	0	0.2	Jorge V., Marie Claire, Davis	
Costa Rica	Caribbean	Laguna de Gandoca	41.3	10	5.5	12/08/2019	9.5903	-82.59685	-	0.2	27.27	0	0.2	Jorge V., Marie Claire, Davis	
Costa Rica	Caribbean	Laguna de Gandoca	41.4	10	5	12/08/2019	9.5903	-82.59685	-	0.2	27.27	0	0.2	Jorge V., Marie Claire, Davis	
Costa Rica	Caribbean	Laguna de Gandoca	41.5	10	5	12/08/2019	9.5903	-82.59685	-	0.2	27.27	0	0.2	Jorge V., Marie Claire, Davis	
Costa Rica	Caribbean	Laguna de Gandoca	42.1	10	5.5	12/08/2019	9.58899	-82.59639	-	1.5	26.42	0	0.7	Jorge V., Marie Claire, Davis	
Costa Rica	Caribbean	Laguna de Gandoca	42.2	10	5	12/08/2019	9.58899	-82.59639	-	1.5	26.42	0	0.7	Jorge V., Marie Claire, Davis	
Costa Rica	Caribbean	Laguna de Gandoca	42.3	10	5	12/08/2019	9.58899	-82.59639	-	1.5	26.42	0	0.7	Jorge V., Marie Claire, Davis	
Costa Rica	Caribbean	Laguna de Gandoca	42.4	10	5	12/08/2019	9.58899	-82.59639	-	1.5	26.42	0	0.7	Jorge V., Marie Claire, Davis	
Costa Rica	Caribbean	Laguna de Gandoca	42.5	10	5	12/08/2019	9.58899	-82.59639	-	1.5	26.42	0	0.7	Jorge V., Marie Claire, Davis	
Costa Rica	Caribbean	Río Sixaola	91.1	20	2	13/08/2019	9.57042	-82.56612	-	4.5	24.9	0	0.2	Jorge V., Marie Claire, Davis, Don Antonio	
Costa Rica	Caribbean	Río Sixaola	91.2	20	2.5	13/08/2019	9.57042	-82.56612	-	4.5	24.9	0	0.2	Jorge V., Marie Claire, Davis, Don Antonio	
Costa Rica	Caribbean	Río Sixaola	91.3	20	2.5	13/08/2019	9.57042	-82.56612	-	4.5	24.9	0	0.2	Jorge V., Marie Claire, Davis, Don Antonio	
Costa Rica	Caribbean	Río Sixaola	91.4	20	4	13/08/2019	9.57042	-82.56612	-	4.5	24.9	0	0.2	Jorge V., Marie Claire, Davis, Don Antonio	
Costa Rica	Caribbean	Río Sixaola	91.5	20	4	13/08/2019	9.57042	-82.56612	-	4.5	24.9	0	0.2	Jorge V., Marie Claire, Davis, Don Antonio	
Costa Rica	Caribbean	Río Sixaola	92.1	20	0.86	13/08/2019	9.56576	-82.56725	-	0.5	25.8	0	0.2	Jorge V., Marie Claire, Davis, Don Antonio	
Costa Rica	Caribbean	Río Sixaola	92.2	20	0.84	13/08/2019	9.56576	-82.56725	-	0.5	25.8	0	0.2	Jorge V., Marie Claire, Davis, Don Antonio	

Costa Rica	Caribbean	Río Sixaola	92.3	20	0.8	13/08/2019	9.56576	-82.56725	-	0.5	25.8	0	0.2	Jorge V., Marie Claire, Davis, Don Antonio	
Costa Rica	Caribbean	Río Sixaola	92.4	20	0.86	13/08/2019	9.56576	-82.56725	-	0.5	25.8	0	0.2	Jorge V., Marie Claire, Davis, Don Antonio	
Costa Rica	Caribbean	Río Sixaola	92.5	20	1.7	13/08/2019	9.56576	-82.56725	-	0.5	25.8	0	0.2	Jorge V., Marie Claire, Davis, Don Antonio	
Costa Rica	Caribbean	Río Sixaola	93.1	20	0.8	13/08/2019	9.56151	-82.56569	-	1.9	25.27	0	0.2	Jorge V., Marie Claire, Davis, Don Antonio	
Costa Rica	Caribbean	Río Sixaola	93.2	20	0.84	13/08/2019	9.56151	-82.56569	-	1.9	25.27	0	0.2	Jorge V., Marie Claire, Davis, Don Antonio	
Costa Rica	Caribbean	Río Sixaola	93.3	20	0.8	13/08/2019	9.56151	-82.56569	-	1.9	25.27	0	0.2	Jorge V., Marie Claire, Davis, Don Antonio	
Costa Rica	Caribbean	Río Sixaola	93.4	20	0.86	13/08/2019	9.56151	-82.56569	-	1.9	25.27	0	0.2	Jorge V., Marie Claire, Davis, Don Antonio	
Costa Rica	Caribbean	Río Sixaola	93.5	20	0.82	13/08/2019	9.56151	-82.56569	-	1.9	25.27	0	0.2	Jorge V., Marie Claire, Davis, Don Antonio	
Costa Rica	Caribbean	Laguna de Gandoca	94.1	10	5.5	14/08/2019	9.59032	-82.59988	-	3.1	25.38	0	0.3	Jorge V., Marie Claire, Davis	
Costa Rica	Caribbean	Laguna de Gandoca	94.2	10	5.5	14/08/2019	9.59032	-82.59988	-	3.1	25.38	0	0.3	Jorge V., Marie Claire, Davis	
Costa Rica	Caribbean	Laguna de Gandoca	94.3	10	5.5	14/08/2019	9.59032	-82.59988	-	3.1	25.38	0	0.3	Jorge V., Marie Claire, Davis	
Costa Rica	Caribbean	Laguna de Gandoca	94.4	10	5	14/08/2019	9.59032	-82.59988	-	3.1	25.38	0	0.3	Jorge V., Marie Claire, Davis	
Costa Rica	Caribbean	Laguna de Gandoca	94.5	10	5	14/08/2019	9.59032	-82.59988	-	3.1	25.38	0	0.3	Jorge V., Marie Claire, Davis	
Costa Rica	Caribbean	Laguna de Gandoca	94.6	1.2	0.71	14/08/2019	9.59032	-82.59988	-	3.1	25.38	0	0.3	Jorge V., Marie Claire, Davis	
Costa Rica	Caribbean	Laguna de Gandoca	94.7	1.2	1.8	14/08/2019	9.59032	-82.59988	-	3.1	25.38	0	0.3	Jorge V., Marie Claire, Davis	
Australia	Queensland	Leichardt Weir, Norman River	7.1	20	10	1/09/2019	-	-	-	-	-	-	-	Emmaline Hardy, Grace McNichols, Nicole Weller	
Australia	Queensland	Leichardt Weir, Norman River	7.2	20	12	1/09/2019	-	-	-	-	-	-	-	Emmaline Hardy, Grace McNichols, Nicole Weller	
Australia	Queensland	Leichardt Weir, Norman River	7.3	20	10	1/09/2019	-	-	-	-	-	-	-	Emmaline Hardy, Grace McNichols, Nicole Weller	
Australia	Queensland	Leichardt Weir, Norman River	7.4	20	10	1/09/2019	-	-	-	-	-	-	-	Emmaline Hardy, Grace McNichols, Nicole Weller	
Australia	Queensland	Leichardt Weir, Norman River	7.5	20	10	1/09/2019	-	-	-	-	-	-	-	Emmaline Hardy, Grace McNichols, Nicole Weller	
Australia	Queensland	Norman River	5.1	20	-	14/09/2019	-17.64433	141.0443	High	1.5	-	-		Barbara Wueringer	
Australia	Queensland	Norman River	5.2	20	8	14/09/2019	-17.64433	141.0443	High	1.5	-	-	-	Barbara Wueringer	
Australia	Queensland	Norman River	5.3	20	7	14/09/2019	-17.64433	141.0443	High	1.5	-	-	-	Barbara Wueringer	
Australia	Queensland	Norman River	5.4	20	7	14/09/2019	-17.64433	141.0443	High	1.5	-	-	-	Barbara Wueringer	
Australia	Queensland	Norman River	5.5	20	5	14/09/2019	-17.64433	141.0443	High	1.5	-	-	-	Barbara Wueringer	
Australia	Queensland	Norman River upstream	6.1	20	14	16/09/2019	-	-	-	-	-	-	-	Nicole Weller, Calvin Zhang	
Australia	Queensland	Norman River upstream	6.2	20	10	16/09/2019	-	-	-	-	-	-	-	Nicole Weller, Calvin Zhang	

Australia	Queensland	Norman River upstream	6.3	20	12	16/09/2019	-	-	-	-	-	-	-	Nicole Weller, Calvin Zhang	
Australia	Queensland	Norman River upstream	6.4	20	12	16/09/2019	-	-	-	-	-	-	-	Nicole Weller, Calvin Zhang	
Australia	Queensland	Norman River upstream	6.5	20	12	16/09/2019	-	-	-	-	-	-	-	Nicole Weller, Calvin Zhang	
Brazil	Maranhão	Itapecuru Mouth 1	6.1	20	2	2/10/2019	-2.84929	-44.2016	High	4.5	-	-	0.5	L. Feitosa & Luis Costa	
Brazil	Maranhão	Itapecuru Mouth 1	6.2	20	3	2/10/2019	-2.84929	-44.2016	High	4.5	-	-	0.5	L. Feitosa & Luis Costa	
Brazil	Maranhão	Itapecuru Mouth 1	6.3	20	2	2/10/2019	-2.84929	-44.2016	High	4.5	-	-	0.5	L. Feitosa & Luis Costa	
Brazil	Maranhão	Itapecuru Mouth 1	6.4	20	2	2/10/2019	-2.84929	-44.2016	High	4.5	-	-	0.5	L. Feitosa & Luis Costa	
Brazil	Maranhão	Itapecuru Mouth 1	6.5	20	2	2/10/2019	-2.84929	-44.2016	High	4.5	-	-	0.5	L. Feitosa & Luis Costa	
Brazil	Maranhão	Itapecuru Mouth 2	7.1	20	1	2/10/2019	-2.81118	-44.1661	High	5.5	-	-	0.5	L. Feitosa & Luis Costa	
Brazil	Maranhão	Itapecuru Mouth 2	7.2	20	1	2/10/2019	-2.81118	-44.1661	High	5.5	-	-	0.5	L. Feitosa & Luis Costa	
Brazil	Maranhão	Itapecuru Mouth 2	7.3	20	1	2/10/2019	-2.81118	-44.1661	High	5.5	-	-	0.5	L. Feitosa & Luis Costa	
Brazil	Maranhão	Itapecuru Mouth 2	7.4	20	1	2/10/2019	-2.81118	-44.1661	High	5.5	-	-	0.5	L. Feitosa & Luis Costa	
Brazil	Maranhão	Itapecuru Mouth 2	7.5	20	1	2/10/2019	-2.81118	-44.1661	High	5.5	-	-	0.5	L. Feitosa & Luis Costa	
Brazil	Maranhão	Itapecuru Mouth 2	7.6	20	1	2/10/2019	-2.81118	-44.1661	High	5.5	-	-	0.5	L. Feitosa & Luis Costa	
Brazil	Maranhão	Itapecuru Mouth 2	7.7	1.2	1	2/10/2019	-2.81118	-44.1661	High	5.5	-	-	0.5	L. Feitosa & Luis Costa	
Brazil	Maranhão	Itapecuru Mouth 2	7.8	1.2	-	2/10/2019	-2.81118	-44.1661	High	5.5	-	-	0.5	L. Feitosa & Luis Costa	
Brazil	Maranhão	Itapecuru Mouth 3	8.1	20	2	2/10/2019	-2.79306	-44.1576	Low	4	-	-	0.5	L. Feitosa & Luis Costa	
Brazil	Maranhão	Itapecuru Mouth 3	8.2	20	2	2/10/2019	-2.79306	-44.1576	Low	4	-	-	0.5	L. Feitosa & Luis Costa	
Brazil	Maranhão	Itapecuru Mouth 3	8.3	20	2	2/10/2019	-2.79306	-44.1576	Low	4	-	-	0.5	L. Feitosa & Luis Costa	
Brazil	Maranhão	Itapecuru Mouth 3	8.4	20	2	2/10/2019	-2.79306	-44.1576	Low	4	-	-	0.5	L. Feitosa & Luis Costa	
Brazil	Maranhão	Itapecuru Mouth 3	8.5	20	2	2/10/2019	-2.79306	-44.1576	Low	4	-	-	0.5	L. Feitosa & Luis Costa	
Brazil	Maranhão	Itapecuru Mouth 3	8.6	1.2	0.2	2/10/2019	-2.79306	-44.1576	Low	4	-	-	0.5	L. Feitosa & Luis Costa	
Brazil	Maranhão	Itapecuru Mouth 3	8.7	1.2	-	2/10/2019	-2.79306	-44.1576	Low	4	-	-	0.5	L. Feitosa & Luis Costa	
Brazil	Maranhão	Itapecuru Mouth 3	8.8	1.2	-	2/10/2019	-2.79306	-44.1576	Low	4	-	-	0.5	L. Feitosa & Luis Costa	
Brazil	Maranhão	Baia do Arraial	9.1	20	2	2/10/2019	-2.73022	-44.1692	Low	-	-	-	0.5	L. Feitosa & Luis Costa	
Brazil	Maranhão	Baia do Arraial	9.2	20	2	2/10/2019	-2.73022	-44.1692	Low	-	-	-	0.5	L. Feitosa & Luis Costa	
Brazil	Maranhão	Baia do Arraial	9.3	20	2	2/10/2019	-2.73022	-44.1692	Low	-	-	-	0.5	L. Feitosa & Luis Costa	
Brazil	Maranhão	Baia do Arraial	9.4	20	2	2/10/2019	-2.73022	-44.1692	Low	-	-	-	0.5	L. Feitosa & Luis Costa	
Brazil	Maranhão	Baia do Arraial	9.5	20	2	2/10/2019	-2.73022	-44.1692	Low	-	-	-	0.5	L. Feitosa & Luis Costa	
Brazil	Maranhão	Baia do Arraial	9.6	1.2	0.2	2/10/2019	-2.73022	-44.1692	Low	-	-	-	0.5	L. Feitosa & Luis Costa	
Brazil	Maranhão	Baia do Arraial	9.7	1.2	-	2/10/2019	-2.73022	-44.1692	Low	-	-	-	0.5	L. Feitosa & Luis Costa	

Brazil	Maranhão	Baia do Arraial	9.8	1.2	-	2/10/2019	-2.73022	-44.1692	Low	-	-	-	0.5	L. Feitosa & Luis Costa	
Brazil	Maranhão	Munim River 1	11.1	20	4	4/10/2019	-2.80620	-44.0706	High	6	-	Freshwater fish jumping in the water	2	L. Feitosa; C. Ramos-Jr	
Brazil	Maranhão	Munim River 1	11.2	20	5	4/10/2019	-2.80620	-44.0706	High	6	-	Freshwater fish jumping in the water	2	L. Feitosa; C. Ramos-Jr	
Brazil	Maranhão	Munim River 1	11.3	20	2	4/10/2019	-2.80620	-44.0706	High	6	-	Freshwater fish jumping in the water	2	L. Feitosa; C. Ramos-Jr	
Brazil	Maranhão	Munim River 1	11.4	20	2.4	4/10/2019	-2.80620	-44.0706	High	6	-	Freshwater fish jumping in the water	2	L. Feitosa; C. Ramos-Jr	
Brazil	Maranhão	Munim River 1	11.5	20	3	4/10/2019	-2.80620	-44.0706	High	6	-	Freshwater fish jumping in the water	2	L. Feitosa; C. Ramos-Jr	
Brazil	Maranhão	Munim River 1	11.6	1.2	0.5	4/10/2019	-2.80620	-44.0706	High	6	-	Freshwater fish jumping in the water	2	L. Feitosa; C. Ramos-Jr	
Brazil	Maranhão	Munim River 1	11.7	1.2	0.5	4/10/2019	-2.80620	-44.0706	High	6	-	Freshwater fish jumping in the water	2	L. Feitosa; C. Ramos-Jr	
Brazil	Maranhão	Munim River 1	11.8	1.2	0.5	4/10/2019	-2.80620	-44.0706	High	6	-	Freshwater fish jumping in the water	2	L. Feitosa; C. Ramos-Jr	
Brazil	Maranhão	Munim River 2	12.1	10	1	4/10/2019	-2.78438	-44.0661	High	3	-	-	2	L. Feitosa; C. Ramos-Jr	
Brazil	Maranhão	Munim River 2	12.2	10	1.5	4/10/2019	-2.78438	-44.0661	High	3	-	-	2	L. Feitosa; C. Ramos-Jr	
Brazil	Maranhão	Munim River 2	12.3	10	1	4/10/2019	-2.78438	-44.0661	High	3	-	-	2	L. Feitosa; C. Ramos-Jr	
Brazil	Maranhão	Munim River 2	12.4	10	0.5	4/10/2019	-2.78438	-44.0661	High	3	-	-	2	L. Feitosa; C. Ramos-Jr	
Brazil	Maranhão	Munim River 2	12.5	10	0.5	4/10/2019	-2.78438	-44.0661	High	3	-	-	2	L. Feitosa; C. Ramos-Jr	
Brazil	Maranhão	Munim River 2	12.6	1.2	0.2	4/10/2019	-2.78438	-44.0661	High	3	-	-	2	L. Feitosa; C. Ramos-Jr	
Brazil	Maranhão	Munim River 2	12.7	1.2	0.2	4/10/2019	-2.78438	-44.0661	High	3	-	-	2	L. Feitosa; C. Ramos-Jr	
Brazil	Maranhão	Munim River 2	12.8	1.2	0.2	4/10/2019	-2.78438	-44.0661	High	3	-	-	2	L. Feitosa; C. Ramos-Jr	
Brazil	Maranhão	Munim River 3	13.1	10	1	4/10/2019	-2.77107	-44.0685	Low	5	-	-	2	L. Feitosa; C. Ramos-Jr	
Brazil	Maranhão	Munim River 3	13.2	10	1.5	4/10/2019	-2.77107	-44.0685	Low	5	-	-	2	L. Feitosa; C. Ramos-Jr	
Brazil	Maranhão	Munim River 3	13.3	10	1	4/10/2019	-2.77107	-44.0685	Low	5	-	-	2	L. Feitosa; C. Ramos-Jr	
Brazil	Maranhão	Munim River 3	13.4	10	1	4/10/2019	-2.77107	-44.0685	Low	5	-	-	2	L. Feitosa; C. Ramos-Jr	
Brazil	Maranhão	Munim River 3	13.5	10	1.5	4/10/2019	-2.77107	-44.0685	Low	5	-	-	2	L. Feitosa; C. Ramos-Jr	
Brazil	Maranhão	Munim River 3	13.6	1.2	1.3	4/10/2019	-2.77107	-44.0685	Low	5	-	-	2	L. Feitosa; C. Ramos-Jr	

Brazil	Maranhão	Munim River 3	13.7	1.2	0.3	4/10/2019	-2.77107	-44.0685	Low	5	-	-	2	L. Feitosa; C. Ramos-Jr	
Brazil	Maranhão	Munim River 3	13.8	1.2	0.3	4/10/2019	-2.77107	-44.0685	Low	5	-	-	2	L. Feitosa; C. Ramos-Jr	
Brazil	Maranhão	Munim River 4	14.1	10	2	4/10/2019	-2.74862	-44.0783	Low	4.5	-	-	2	L. Feitosa; C. Ramos-Jr	
Brazil	Maranhão	Munim River 4	14.2	10	2	4/10/2019	-2.74862	-44.0783	Low	4.5	-	-	2	L. Feitosa; C. Ramos-Jr	
Brazil	Maranhão	Munim River 4	14.3	10	1	4/10/2019	-2.74862	-44.0783	Low	4.5	-	-	2	L. Feitosa; C. Ramos-Jr	
Brazil	Maranhão	Munim River 4	14.4	10	1	4/10/2019	-2.74862	-44.0783	Low	4.5	-	-	2	L. Feitosa; C. Ramos-Jr	
Brazil	Maranhão	Munim River 4	14.5	10	1	4/10/2019	-2.74862	-44.0783	Low	4.5	-	-	2	L. Feitosa; C. Ramos-Jr	
Brazil	Maranhão	Munim River 4	14.6	1.2	0.3	4/10/2019	-2.74862	-44.0783	Low	4.5	-	-	2	L. Feitosa; C. Ramos-Jr	
Brazil	Maranhão	Munim River 4	14.7	1.2	0.2	4/10/2019	-2.74862	-44.0783	Low	4.5	-	-	2	L. Feitosa; C. Ramos-Jr	
Brazil	Maranhão	Munim River 4	14.8	1.2	0.2	4/10/2019	-2.74862	-44.0783	Low	4.5	-	-	2	L. Feitosa; C. Ramos-Jr	
Brazil	Maranhão	Caranguejos 1	16.1	20	1	7/10/2019	-2.82972	-44.4836	Low	3	-	-	0.5	Leo Feitosa	
Brazil	Maranhão	Caranguejos 1	16.2	20	0.5	7/10/2019	-2.82972	-44.4836	Low	3	-	-	0.5	Leo Feitosa	
Brazil	Maranhão	Caranguejos 1	16.3	20	1	7/10/2019	-2.82972	-44.4836	Low	3	-	-	0.5	Leo Feitosa	
Brazil	Maranhão	Caranguejos 1	16.4	20	1	7/10/2019	-2.82972	-44.4836	Low	3	-	-	0.5	Leo Feitosa	
Brazil	Maranhão	Caranguejos 1	16.5	20	1	7/10/2019	-2.82972	-44.4836	Low	3	-	-	0.5	Leo Feitosa	
Brazil	Maranhão	Caranguejos 1	16.6	1.2	0.3	7/10/2019	-2.82972	-44.4836	Low	3	-	-	0.5	Leo Feitosa	
Brazil	Maranhão	Caranguejos 1	16.7	1.2	0.2	7/10/2019	-2.82972	-44.4836	Low	3	-	-	0.5	Leo Feitosa	
Brazil	Maranhão	Caranguejos 1	16.8	1.2	0.3	7/10/2019	-2.82972	-44.4836	Low	3	-	-	0.5	Leo Feitosa	
Brazil	Maranhão	Caranguejos 2	17.1	10	2	7/10/2019	-2.86026	-44.5002	Low	12	-	-	3	Leo Feitosa	
Brazil	Maranhão	Caranguejos 2	17.2	10	2.5	7/10/2019	-2.86026	-44.5002	Low	12	-	-	3	Leo Feitosa	
Brazil	Maranhão	Caranguejos 2	17.3	10	2.5	7/10/2019	-2.86026	-44.5002	Low	12	-	-	3	Leo Feitosa	
Brazil	Maranhão	Caranguejos 2	17.4	10	2.5	7/10/2019	-2.86026	-44.5002	Low	12	-	-	3	Leo Feitosa	
Brazil	Maranhão	Caranguejos 2	17.5	10	3	7/10/2019	-2.86026	-44.5002	Low	12	-	-	3	Leo Feitosa	
Brazil	Maranhão	Caranguejos 2	17.6	1.2	0.5	7/10/2019	-2.86026	-44.5002	Low	12	-	-	3	Leo Feitosa	
Brazil	Maranhão	Caranguejos 2	17.7	1.2	0.5	7/10/2019	-2.86026	-44.5002	Low	12	-	-	3	Leo Feitosa	
Brazil	Maranhão	Caranguejos 2	17.8	1.2	0.5	7/10/2019	-2.86026	-44.5002	Low	12	-	-	3	Leo Feitosa	
Brazil	Maranhão	Caranguejos 3	18.1	10	5	7/10/2019	-2.87727	-44.4094	Low	21	-	-	3	Leo Feitosa	
Brazil	Maranhão	Caranguejos 3	18.2	10	5	7/10/2019	-2.87727	-44.4094	Low	21	-	-	3	Leo Feitosa	
Brazil	Maranhão	Caranguejos 3	18.3	10	3	7/10/2019	-2.87727	-44.4094	Low	21	-	-	3	Leo Feitosa	
Brazil	Maranhão	Caranguejos 3	18.4	10	5	7/10/2019	-2.87727	-44.4094	Low	21	-	-	3	Leo Feitosa	
Brazil	Maranhão	Caranguejos 3	18.5	10	4	7/10/2019	-2.87727	-44.4094	Low	21	-	-	3	Leo Feitosa	
Brazil	Maranhão	Caranguejos 3	18.6	1.2	0.5	7/10/2019	-2.87727	-44.4094	Low	21	-	-	3	Leo Feitosa	
Brazil	Maranhão	Caranguejos 3	18.7	1.2	0.5	7/10/2019	-2.87727	-44.4094	Low	21	-	-	3	Leo Feitosa	
Brazil	Maranhão	Caranguejos 3	18.8	1.2	0.5	7/10/2019	-2.87727	-44.4094	Low	21	-	-	3	Leo Feitosa	
Brazil	Maranhão	Perizes 1	20.1	20	-	8/10/2019	-2.83904	-44.3402	Low	2	-	-	1	Leo Feitosa	
Brazil	Maranhão	Perizes 1	20.2	20	-	8/10/2019	-2.83904	-44.3402	Low	2	-	-	1	Leo Feitosa	
Brazil	Maranhão	Perizes 1	20.3	20	-	8/10/2019	-2.83904	-44.3402	Low	2	-	-	1	Leo Feitosa	
Brazil	Maranhão	Perizes 1	20.4	20	-	8/10/2019	-2.83904	-44.3402	Low	2	-	-	1	Leo Feitosa	
Brazil	Maranhão	Perizes 1	20.5	20	-	8/10/2019	-2.83904	-44.3402	Low	2	-	-	1	Leo Feitosa	
Brazil	Maranhão	Perizes 1	20.6	1.2	-	8/10/2019	-2.83904	-44.3402	Low	2	-	-	1	Leo Feitosa	
Brazil	Maranhão	Perizes 1	20.7	1.2	-	8/10/2019	-2.83904	-44.3402	Low	2	-	-	1	Leo Feitosa	
Brazil	Maranhão	Perizes 1	20.8	1.2	-	8/10/2019	-2.83904	-44.3402	Low	2	-	-	1	Leo Feitosa	
Brazil	Maranhão	Perizes 2	21.1	20	-	8/10/2019	-2.81062	-44.3395	Low	2	-	-	1	Leo Feitosa	
Brazil	Maranhão	Perizes 2	21.2	20	-	8/10/2019	-2.81062	-44.3395	Low	2	-	-	1	Leo Feitosa	

Brazil	Maranhão	Perizes 2	21.3	20	-	8/10/2019	-2.81062	-44.3395	Low	2	-	-	1	Leo Feitosa	
Brazil	Maranhão	Perizes 2	21.4	20	-	8/10/2019	-2.81062	-44.3395	Low	2	-	-	1	Leo Feitosa	
Brazil	Maranhão	Perizes 2	21.5	20	-	8/10/2019	-2.81062	-44.3395	Low	2	-	-	1	Leo Feitosa	
Brazil	Maranhão	Perizes 2	21.6	1.2	-	8/10/2019	-2.81062	-44.3395	Low	2	-	-	1	Leo Feitosa	
Brazil	Maranhão	Perizes 2	21.7	1.2	-	8/10/2019	-2.81062	-44.3395	Low	2	-	-	1	Leo Feitosa	
Brazil	Maranhão	Perizes 2	21.8	1.2	-	8/10/2019	-2.81062	-44.3395	Low	2	-	-	1	Leo Feitosa	
Brazil	Maranhão	Perizes 3	22.1	20	2	8/10/2019	-2.78628	-44.3369	Low	5	-	-	1	Leo Feitosa	
Brazil	Maranhão	Perizes 3	22.2	20	3	8/10/2019	-2.78628	-44.3369	Low	5	-	-	1	Leo Feitosa	
Brazil	Maranhão	Perizes 3	22.3	20	3	8/10/2019	-2.78628	-44.3369	Low	5	-	-	1	Leo Feitosa	
Brazil	Maranhão	Perizes 3	22.4	20	3	8/10/2019	-2.78628	-44.3369	Low	5	-	-	1	Leo Feitosa	
Brazil	Maranhão	Perizes 3	22.5	20	2	8/10/2019	-2.78628	-44.3369	Low	5	-	-	1	Leo Feitosa	
Brazil	Maranhão	Perizes 3	22.6	1.2	0.4	8/10/2019	-2.78628	-44.3369	Low	5	-	-	1	Leo Feitosa	
Brazil	Maranhão	Perizes 3	22.7	1.2	0.4	8/10/2019	-2.78628	-44.3369	Low	5	-	-	1	Leo Feitosa	
Brazil	Maranhão	Perizes 3	22.8	1.2	0.4	8/10/2019	-2.78628	-44.3369	Low	5	-	-	1	Leo Feitosa	
Brazil	Maranhão	Estiva	19.1	20	3	8/10/2019	-2.79983	-44.4094	High	3	-	-	0.3	Leo Feitosa	
Brazil	Maranhão	Estiva	19.2	20	0.5	8/10/2019	-2.79983	-44.4094	High	3	-	-	0.3	Leo Feitosa	
Brazil	Maranhão	Estiva	19.3	20	0.5	8/10/2019	-2.79983	-44.4094	High	3	-	-	0.3	Leo Feitosa	
Brazil	Maranhão	Estiva	19.4	20	0.5	8/10/2019	-2.79983	-44.4094	High	3	-	-	0.3	Leo Feitosa	
Brazil	Maranhão	Estiva	19.5	20	0.5	8/10/2019	-2.79983	-44.4094	High	3	-	-	0.3	Leo Feitosa	
Brazil	Maranhão	Estiva	19.6	1.2	0.1	8/10/2019	-2.79983	-44.4094	High	3	-	-	0.3	Leo Feitosa	
Brazil	Maranhão	Estiva	19.7	1.2	0.1	8/10/2019	-2.79983	-44.4094	High	3	-	-	0.3	Leo Feitosa	
Brazil	Maranhão	Estiva	19.8	1.2	0.1	8/10/2019	-2.79983	-44.4094	High	3	-	-	0.3	Leo Feitosa	
Brazil	Maranhão	Alcantara 1	23.1	20	1.5	24/10/2019	-2.43889	-44.4456	Low	7	-	-	0.3	L. Feitosa; C. Ramos-Jr	
Brazil	Maranhão	Alcantara 1	23.2	20	0.5	24/10/2019	-2.43889	-44.4456	Low	7	-	-	0.3	L. Feitosa; C. Ramos-Jr	
Brazil	Maranhão	Alcantara 1	23.3	20	1	24/10/2019	-2.43889	-44.4456	Low	7	-	-	0.3	L. Feitosa; C. Ramos-Jr	
Brazil	Maranhão	Alcantara 1	23.4	20	1	24/10/2019	-2.43889	-44.4456	Low	7	-	-	0.3	L. Feitosa; C. Ramos-Jr	
Brazil	Maranhão	Alcantara 1	23.5	20	1	24/10/2019	-2.43889	-44.4456	Low	7	-	-	0.3	L. Feitosa; C. Ramos-Jr	
Brazil	Maranhão	Alcantara 1	23.6	1.2	0.3	24/10/2019	-2.43889	-44.4456	Low	7	-	-	0.3	L. Feitosa; C. Ramos-Jr	
Brazil	Maranhão	Alcantara 1	23.7	1.2	0.3	24/10/2019	-2.43889	-44.4456	Low	7	-	-	0.3	L. Feitosa; C. Ramos-Jr	
Brazil	Maranhão	Alcantara 1	23.8	1.2	0.3	24/10/2019	-2.43889	-44.4456	Low	7	-	-	0.3	L. Feitosa; C. Ramos-Jr	
Brazil	Maranhão	Alcantara 2	24.1	20	1	24/10/2019	-2.38414	-44.5121	Low	5.5	-	-	0.3	L. Feitosa; C. Ramos-Jr	
Brazil	Maranhão	Alcantara 2	24.2	20	1.5	24/10/2019	-2.38414	-44.5121	Low	5.5	-	-	0.3	L. Feitosa; C. Ramos-Jr	
Brazil	Maranhão	Alcantara 2	24.3	20	1.3	24/10/2019	-2.38414	-44.5121	Low	5.5	-	-	0.3	L. Feitosa; C. Ramos-Jr	
Brazil	Maranhão	Alcantara 2	24.4	20	1	24/10/2019	-2.38414	-44.5121	Low	5.5	-	-	0.3	L. Feitosa; C. Ramos-Jr	
Brazil	Maranhão	Alcantara 2	24.5	20	1.5	24/10/2019	-2.38414	-44.5121	Low	5.5	-	-	0.3	L. Feitosa; C. Ramos-Jr	
Brazil	Maranhão	Alcantara 2	24.6	1.2	0.2	24/10/2019	-2.38414	-44.5121	Low	5.5	-	-	0.3	L. Feitosa; C. Ramos-Jr	
Brazil	Maranhão	Alcantara 2	24.7	1.2	0.2	24/10/2019	-2.38414	-44.5121	Low	5.5	-	-	0.3	L. Feitosa; C. Ramos-Jr	
Brazil	Maranhão	Alcantara 2	24.8	1.2	0.2	24/10/2019	-2.38414	-44.5121	Low	5.5	-	-	0.3	L. Feitosa; C. Ramos-Jr	
Brazil	Maranhão	Alcantara 3	25.1	20	1	24/10/2019	-2.41973	-44.4915	High	5	-	-	0.3	L. Feitosa; C. Ramos-Jr	

Brazil	Maranhão	Alcantara 3	25.2	20	1	24/10/2019	-2.41973	-44.4915	High	5	-	-	0.3	L. Feitosa; C. Ramos-Jr	
Brazil	Maranhão	Alcantara 3	25.3	20	1	24/10/2019	-2.41973	-44.4915	High	5	-	-	0.3	L. Feitosa; C. Ramos-Jr	
Brazil	Maranhão	Alcantara 3	25.4	20	1	24/10/2019	-2.41973	-44.4915	High	5	-	-	0.3	L. Feitosa; C. Ramos-Jr	
Brazil	Maranhão	Alcantara 3	25.5	20	1	24/10/2019	-2.41973	-44.4915	High	5	-	-	0.3	L. Feitosa; C. Ramos-Jr	
Brazil	Maranhão	Alcantara 3	25.6	1.2	0.2	24/10/2019	-2.41973	-44.4915	High	5	-	-	0.3	L. Feitosa; C. Ramos-Jr	
Brazil	Maranhão	Alcantara 3	25.7	1.2	0.2	24/10/2019	-2.41973	-44.4915	High	5	-	-	0.3	L. Feitosa; C. Ramos-Jr	
Brazil	Maranhão	Alcantara 3	25.8	1.2	0.2	24/10/2019	-2.41973	-44.4915	High	5	-	-	0.3	L. Feitosa; C. Ramos-Jr	
Brazil	Maranhão	Alcantara 4	4.1	20	1.5	24/10/2019	-2.47881	-44.5030	High	15	-	-	0.5	L. Feitosa; C. Ramos-Jr	
Brazil	Maranhão	Alcantara 4	4.2	20	1.5	24/10/2019	-2.47881	-44.5030	High	15	-	-	0.5	L. Feitosa; C. Ramos-Jr	
Brazil	Maranhão	Alcantara 4	4.3	20	2	24/10/2019	-2.47881	-44.5030	High	15	-	-	0.5	L. Feitosa; C. Ramos-Jr	
Brazil	Maranhão	Alcantara 4	4.4	20	2	24/10/2019	-2.47881	-44.5030	High	15	-	-	0.5	L. Feitosa; C. Ramos-Jr	
Brazil	Maranhão	Alcantara 4	4.5	20	2	24/10/2019	-2.47881	-44.5030	High	15	-	-	0.5	L. Feitosa; C. Ramos-Jr	
Brazil	Maranhão	Alcantara 4	4.6	1.2	0.5	24/10/2019	-2.47881	-44.5030	High	15	-	-	0.5	L. Feitosa; C. Ramos-Jr	
Brazil	Maranhão	Alcantara 4	4.7	1.2	0.5	24/10/2019	-2.47881	-44.5030	High	15	-	-	0.5	L. Feitosa; C. Ramos-Jr	
Brazil	Maranhão	Alcantara 4	4.8	1.2	0.3	24/10/2019	-2.47881	-44.5030	High	15	-	-	0.5	L. Feitosa; C. Ramos-Jr	
Australia	Queensland	Skardon River	26.1	20	4	30/11/2019	-11.81143	142.0942	High	2.8	-	-	0.6	BW, Ritchie Hansen, Glen Woodrow	
Australia	Queensland	Skardon River	26.2	20	3	30/11/2019	-11.81143	142.0942	High	2.8	-	-	0.6	BW, Ritchie Hansen, Glen Woodrow	
Australia	Queensland	Skardon River	26.3	20	4	30/11/2019	-11.81143	142.0942	High	2.8	-	-	0.6	BW, Ritchie Hansen, Glen Woodrow	
Australia	Queensland	Skardon River	26.4	20	5	30/11/2019	-11.81143	142.0942	High	2.8	-	-	0.6	BW, Ritchie Hansen, Glen Woodrow	
Australia	Queensland	Skardon River	26.5	20	5	30/11/2019	-11.81143	142.0942	High	2.8	-	-	0.6	BW, Ritchie Hansen, Glen Woodrow	
Australia	Queensland	Skardon River	27.1	20	6	2/12/2019			Incoming	4.5	29.8	31.3	1.5	BW, Ritchie Hansen, Glen Woodrow	
Australia	Queensland	Skardon River	27.2	20	6	2/12/2019			Incoming	4.5	29.8	31.3	1.5	BW, Ritchie Hansen, Glen Woodrow	
Australia	Queensland	Skardon River	27.3	20	6	2/12/2019			Incoming	4.5	29.8	31.3	1.5	BW, Ritchie Hansen, Glen Woodrow	
Australia	Queensland	Skardon River	27.4	20	6	2/12/2019			Incoming	4.5	29.8	31.3	1.5	BW, Ritchie Hansen, Glen Woodrow	
Australia	Queensland	Skardon River	27.5	20	6	2/12/2019			Incoming	4.5	29.8	31.3	1.5	BW, Ritchie Hansen, Glen Woodrow	
Australia	Queensland	Namaleta Creek mouth, Port Musgrave	28.1	20	4	2/12/2019	-11.97446	141.9491	Incoming	0.8	-	-	1.5	BW, Ritchie Hansen, Glen Woodrow	
Australia	Queensland	Namaleta Creek mouth, Port Musgrave	28.2	20	4	2/12/2019	-11.97446	141.9491	Incoming	0.8	-	-	1.5	BW, Ritchie Hansen, Glen Woodrow	
Australia	Queensland	Namaleta Creek mouth, Port Musgrave	28.3	20	4	2/12/2019	-11.97446	141.9491	Incoming	0.8	-	-	1.5	BW, Ritchie Hansen, Glen Woodrow	
Australia	Queensland	Namaleta Creek mouth, Port Musgrave	28.4	20	4	2/12/2019	-11.97446	141.9491	Incoming	0.8	-	-	1.5	BW, Ritchie Hansen, Glen Woodrow	

Australia	Queensland	Namaleta Creek mouth, Port Musgrave	28.5	20	4	2/12/2019	-11.97446	141.9491	Incoming	0.8	-	-	1.5	BW, Ritchie Hansen, Glen Woodrow	
Australia	Queensland	Walsh River - Timble crossing	21.1	10	7	24/07/2020	-16.54633	143.7856	Non-tidal	1.1	22.6	135	2	Barbara Wueringer, Daniela Mattheus-Holland	
Australia	Queensland	Walsh River - Timble crossing	21.2	10	5	24/07/2020	-16.54633	143.7856	Non-tidal	1.1	22.6	135	2	Barbara Wueringer, Daniela Mattheus-Holland	
Australia	Queensland	Walsh River - Timble crossing	21.3	10	5	24/07/2020	-16.54633	143.7856	Non-tidal	1.1	22.6	135	2	Barbara Wueringer, Daniela Mattheus-Holland	
Australia	Queensland	Walsh River - Timble crossing	21.4	10	2.5	24/07/2020	-16.54633	143.7856	Non-tidal	1.1	22.6	135	2	Barbara Wueringer, Daniela Mattheus-Holland	
Australia	Queensland	Walsh River - Timble crossing	21.5	10	2.5	24/07/2020	-16.54633	143.7856	Non-tidal	1.1	22.6	135	2	Barbara Wueringer, Daniela Mattheus-Holland	
Australia	Queensland	Highbury Station, Mitchell River	22.1	10	10	24/07/2020	-16.34625	143.0602	Non-tidal	1.5	24.7	51.1	1.5	Elissa Mastroianni, DMH	
Australia	Queensland	Highbury Station, Mitchell River	22.2	10	10	24/07/2020	-16.34625	143.0602	Non-tidal	1.5	24.7	51.1	1.5	Elissa Mastroianni, DMH	
Australia	Queensland	Highbury Station, Mitchell River	22.3	10	5	24/07/2020	-16.34625	143.0602	Non-tidal	1.5	24.7	51.1	1.5	Elissa Mastroianni, DMH	
Australia	Queensland	Highbury Station, Mitchell River	22.4	10	2.5	24/07/2020	-16.34625	143.0602	Non-tidal	1.5	24.7	51.1	1.5	Elissa Mastroianni, DMH	
Australia	Queensland	Highbury Station, Mitchell River	22.5	10	5	24/07/2020	-16.34625	143.0602	Non-tidal	1.5	24.7	51.1	1.5	Elissa Mastroianni, DMH	
Australia	Queensland	Shark Hole, Koolatah Station, Mitchell River	23.1	10	11	26/07/2020	-15.66451	142.1060	Non-tidal	-	-	-	1.2	Alex Barber, DMH	
Australia	Queensland	Shark Hole, Koolatah Station, Mitchell River	23.2	10	5	26/07/2020	-15.66451	142.1060	Non-tidal	-	-	-	1.2	Alex Barber, DMH	
Australia	Queensland	Shark Hole, Koolatah Station, Mitchell River	23.3	20	14	26/07/2020	-15.66451	142.1060	Non-tidal	-	-	-	1.2	Alex Barber, DMH	
Australia	Queensland	Shark Hole, Koolatah Station, Mitchell River	23.4	20	10	26/07/2020	-15.66451	142.1060	Non-tidal	-	-	-	1.2	Alex Barber, DMH	
Australia	Queensland	Shark Hole, Koolatah Station, Mitchell River	23.5	20	10	26/07/2020	-15.66451	142.1060	Non-tidal	-	-	-	1.2	Alex Barber, DMH	
Australia	Queensland	Surprise creek, Camp 2.5, Mitchell River	24.1	20	10	28/07/2020	-15.26123	141.7811	-	-	-	-	-	Shane Ross, James Donaldson	
Australia	Queensland	Surprise creek, Camp 2.5, Mitchell River	24.2	20	8	28/07/2020	-15.26123	141.7811	-	-	-	-	-	Shane Ross, James Donaldson	
Australia	Queensland	Surprise creek, Camp 2.5, Mitchell River	24.3	20	2	28/07/2020	-15.26123	141.7811	-	-	-	-	-	Shane Ross, James Donaldson	

Australia	Queensland	Surprise creek, Camp 2.5, Mitchell River	24.4	20	10	28/07/2020	-15.26123	141.7811	-	-	-	-	-	Shane Ross, James Donaldson	
Australia	Queensland	Surprise creek, Camp 2.5, Mitchell River	24.5	20	10	28/07/2020	-15.26123	141.7811	-	-	-	-	-	Shane Ross, James Donaldson	
Australia	Queensland	Suprise creek, Sawfish Heaven, Mitchell River	25.1	20	10	2/08/2020	-15.25752	141.7754	Ebb	0.4	23	4.77		Helen Penrose, BW	
Australia	Queensland	Suprise creek, Sawfish Heaven, Mitchell River	25.2	20	10	2/08/2020	-15.25752	141.7754	Ebb	0.4	23	4.77		Helen Penrose, BW	
Australia	Queensland	Suprise creek, Sawfish Heaven, Mitchell River	25.3	20	10	2/08/2020	-15.25752	141.7754	Ebb	0.4	23	4.77		Helen Penrose, BW	
Australia	Queensland	Suprise creek, Sawfish Heaven, Mitchell River	25.4	20	10	2/08/2020	-15.25752	141.7754	Ebb	0.4	23	4.77		Helen Penrose, BW	
Australia	Queensland	Suprise creek, Sawfish Heaven, Mitchell River	25.5	20	10	2/08/2020	-15.25752	141.7754	Ebb	0.4	23	4.77		Helen Penrose, BW	

Table A2. Summary of environmental DNA sample metadata for samples with positive qPCR detection of sawfishes. Data are sorted by date. Dash indicates missing data. Asterisks indicates samples with evidence of contamination.

Country	Region/Province	Site Name	Field replicate no.	Pore size (µm)	Filtrate vol. (L)	Date	Latitude	Longitude	Field Comment	Positive detection (Y/N)	Species Detected
Australia	Exmouth Gulf, Western Australia	Tent Island	TI5	10	2	10/05/2017	-22.01161	114.5395		Y	*Pristis zijsron*
Australia	Exmouth Gulf, Western Australia	Sandalwood Landing Creek	SLC6	10	2	11/05/2017	-22.46796	114.2248		Y	*Pristis zijsron*
Papua New Guinea	East Sepik Province, Momase	Ramu River Mouth	R1	10	-	16/09/2017	-4.01859	144.6669		Y	*Anoxypristis cuspidata*
Papua New Guinea	East Sepik Province, Momase	Ramu River Mouth	R2	10	-	16/09/2017	-4.01892	144.6521		Y	*Anoxypristis cuspidata*
Papua New Guinea	East Sepik Province, Momase	Ramu River Mouth	R3	10	-	16/09/2017	-4.01892	144.6521		Y	*Anoxypristis cuspidata*
Papua New Guinea	East Sepik Province, Momase	Ramu River Mouth	R5	10	-	16/09/2017	-4.01892	144.6521		Y	*Anoxypristis cuspidata*
Papua New Guinea	East Sepik Province, Momase	Murik Lakes	M1	10	-	17/09/2017	-3.78389	144.2681		Y	*Anoxypristis cuspidata*
Papua New Guinea	East Sepik Province, Momase	Murik Lakes	M2	10	-	17/09/2017	-3.78411	144.2673		Y	*Anoxypristis cuspidata*
Papua New Guinea	East Sepik Province, Momase	Murik Lakes	M3	10	-	17/09/2017	-3.78424	144.2682		Y	*Anoxypristis cuspidata*
Papua New Guinea	East Sepik Province, Momase	Murik Lakes	M4	10	-	17/09/2017	-3.78450	144.2685		Y	*Anoxypristis cuspidata*
Australia	Darwin, Northern Territory	Mickets Creek - Shoal Bay	5.1	5	1	12/12/2017	-12.33960	130.9449		Y	*Pristis clavata*
Australia	Darwin, Northern Territory	Mickets Creek - Shoal Bay	5.2	5	1	12/12/2017	-12.33789	130.9473		Y	*Pristis clavata*
Australia	Kakadu, Northern Territory	Brooke's Creek	13.1	20	1.5	14/12/2017	-12.20428	132.4150		Y	*Pristis clavata*
Australia	Kakadu, Northern Territory	Brooke's Creek	13.2	20	5	14/12/2017	-12.21007	132.4156		Y	*Pristis clavata*
Australia	Kakadu, Northern Territory	West Alligator River Head	17.3	20	6	15/12/2017	-12.1859	132.2646		Y	*Pristis clavata*
United States	Tampa Bay, Florida	Apollo Beach Nature Park	2.4	5	2	27/03/2018	27.79249	-82.41884		Y	*Pristis pectinata*
United States	Caloosahatchee River, Florida	Cape Coral Yacht Club boat ramp, Caloosahatchee River	7.2	5	2	28/03/2018	26.54255	-81.95244		Y	*Pristis pectinata*
United States	Caloosahatchee River, Florida	Cape Coral Yacht Club boat ramp, Caloosahatchee River	7.4	5	2	28/03/2018	26.54255	-81.95244		Y	*Pristis pectinata*
United States	Ten Thousand Islands National Wildlife Reserve, Florida	Stop Key	16.4	1.2	0.5	1/04/2018	25.81497	-81.45647		Y	*Pristis pectinata*
United States	Chokoloskee Bay, Florida	Causeway, Chokoloskee Island	18.1	5	0.5	1/04/2018	25.82748	-81.36375	20+ juvenile smalltooth sawfish within sight of sampling location	Y	*Pristis pectinata*
United States	Chokoloskee Bay, Florida	Causeway, Chokoloskee Island	18.2	10	2	1/04/2018	25.82748	-81.36375	20+ juvenile smalltooth sawfish within sight of sampling location	Y	*Pristis pectinata*

United States	Chokoloskee Bay, Florida	Causeway, Chokoloskee Island	18.3	10	2	1/04/2018	25.82748	-81.36375	20+ juvenile smalltooth sawfish within sight of sampling location	Y	*Pristis pectinata*
United States	Chokoloskee Bay, Florida	Causeway, Chokoloskee Island	18.4	10	2	1/04/2018	25.82748	-81.36375	20+ juvenile smalltooth sawfish within sight of sampling location	Y	*Pristis pectinata*
United States	Chokoloskee Bay, Florida	Causeway, Chokoloskee Island	18.5	1.2	1	1/04/2018	25.82748	-81.36375	20+ juvenile smalltooth sawfish within sight of sampling location	Y	*Pristis pectinata*
United States	Chokoloskee Bay, Florida	Causeway, Chokoloskee Island	18.6	10	1.5	1/04/2018	25.82748	-81.36375	20+ juvenile smalltooth sawfish within sight of sampling location	Y	*Pristis pectinata*
United States	Chokoloskee Bay, Florida	Causeway, Chokoloskee Island	18.7	10	1.5	1/04/2018	25.82748	-81.36375	20+ juvenile smalltooth sawfish within sight of sampling location	Y	*Pristis pectinata*
United States	Chokoloskee Bay, Florida	Causeway, Chokoloskee Island	18.8	20	2	1/04/2018	25.82748	-81.36375	20+ juvenile smalltooth sawfish within sight of sampling location	Y	*Pristis pectinata*
United States	Chokoloskee Bay, Florida	Causeway, Chokoloskee Island	19.1	10	1.5	1/04/2018	25.82748	-81.36375	20+ juvenile smalltooth sawfish within sight of sampling location	Y	*Pristis pectinata*
United States	Chokoloskee Bay, Florida	Causeway, Chokoloskee Island	19.2	10	3	1/04/2018	25.82748	-81.36375	20+ juvenile smalltooth sawfish within sight of sampling location	Y	*Pristis pectinata*
United States	Chokoloskee Bay, Florida	Causeway, Chokoloskee Island	19.3	10	3	1/04/2018	25.82748	-81.36375	20+ juvenile smalltooth sawfish within sight of sampling location	Y	*Pristis pectinata*
United States	Chokoloskee Bay, Florida	Causeway, Chokoloskee Island	19.4	10	3	1/04/2018	25.82748	-81.36375	20+ juvenile smalltooth sawfish within sight of sampling location	Y	*Pristis pectinata*
United States	Chokoloskee Bay, Florida	Causeway, Chokoloskee Island	19.6	10	4	1/04/2018	25.82748	-81.36375	20+ juvenile smalltooth sawfish within sight of sampling location	Y	*Pristis pectinata*
United States	Chokoloskee Bay, Florida	Causeway, Chokoloskee Island	19.5	1.2	1	2/04/2018	25.82748	-81.36375	20+ juvenile smalltooth sawfish within sight of sampling location	Y	*Pristis pectinata*
Australia	Townsville, Queensland	Toolakea Beach	3.4	10	1	8/11/2018	-19.14569	146.5753		Y	*Anoxypristis cuspidata*
Papua New Guinea	Western Province	Oxbow 181	9.4	20	5	25/02/2019	-7.81170	141.5786		Y	Pristis pristis
Costa Rica	Northern Plains	Boca San Carlos	15.1	20	3.2	4/03/2019	10.78982	-84.1917		Y	*Pristis pristis*
Costa Rica	Northern Plains	Boca San Carlos	15.2	20	3.25	4/03/2019	10.78982	-84.1917		Y	*Pristis pristis*
Costa Rica	Northern Plains	Boca San Carlos	15.3	20	3.2	4/03/2019	10.78982	-84.1917		Y	*Pristis pristis*
Costa Rica	Northern Plains	Boca San Carlos	17.2	20	3	4/03/2019	10.78543	-84.1972		Y	*Pristis pristis*
Costa Rica	Northern Plains	Boca San Carlos	17.3	20	3	4/03/2019	10.78543	-84.1972		Y	*Pristis pristis*
Costa Rica	Northern Plains	Boca San Carlos	17.5	20	3.5	4/03/2019	10.78543	-84.1972		Y	*Pristis pristis*
Costa Rica	Northern Plains	Boca Cureñita	18.1	20	3.7	5/03/2019	10.76593	-84.0625		Y	*Pristis pristis*

Australia	Queensland	Mapoon/Trout Beach	12.2	10	5	11/04/2019	-11.64433	141.8914	Green, dwarf, and narrow sawfish captured at this beach on the day prior	Y*	*(1) Pristis clavata;* (2) *Pristis zijsron*
Australia	Queensland	Mapoon/Trout Beach	12.4	10	5	11/04/2019	-11.64433	141.8914	Green, dwarf, and narrow sawfish captured at this beach on the day prior	Y*	*Pristis zijsron*
Australia	Queensland	Darn Flats, Port Musgrave	14.4	20	5	14/04/2019	-	-	Largetooth and green sawfish captured and handled from same boat on the day prior	Y*	*(1) Pristis pristis, (2) Pristis zijsron*
Australia	Queensland	Darn Flats, Port Musgrave	14.5	20	5	14/04/2019	-	-	Largetooth and green sawfish captured and handled from same boat on the day prior	Y*	*Pristis pristis*
Australia	Queensland	Darn Flats, Port Musgrave	14.6	1.2	1	14/04/2019	-	-	Largetooth and green sawfish captured and handled from same boat on the day prior	Y*	*Pristis pristis*
Costa Rica	Northern Plains	Boca San Carlos	28.3	20	3	25/05/2019	10.78282	-84.2007		Y	*Pristis pristis*
Costa Rica	Northern Plains	Boca San Carlos	28.4	20	3	25/05/2019	10.78282	-84.2007		Y	*Pristis pristis*
Costa Rica	Northern Plains	Boca Cureñita	30.4	20	1.5	26/05/2019	10.76006	-84.0745		Y	*Pristis pristis*
Costa Rica	Northern Plains	Boca Cureñita	32.1	20	2	27/05/2019	10.76614	-84.0888		Y	*Pristis pristis*
Costa Rica	Northern Plains	Boca Cureñita	33.4	20	2	27/05/2019	10.77124	-84.0933		Y	*Pristis pristis*
Costa Rica	Northern Plains	Boca Cureñita	34.4	20	2	27/05/2019	10.77096	-84.1112		Y	*Pristis pristis*
Costa Rica	Caribbean	Barra del Colorado, Estuary	35.5	20	2.8	31/05/2019	10.80198	-83.5861		Y	*Pristis pristis*
Costa Rica	Caribbean	Barra del Colorado, Estuary	36.4	20	1.3	31/05/2019	10.79511	-83.5899		Y	*Pristis pristis*
Australia	Queensland	Norman River upstream	6.2	20	10	16/09/2019	-	-		Y	*Pristis pristis*
Australia	Queensland	Skardon River	26.2	20	3	30/11/2019	-11.81143	142.0942		Y*	*Pristis pristis*
Australia	Queensland	Highbury Station, Mitchell River	22.4	10	2.5	24/07/2020	-16.34625	143.0602		Y	*Pristis pristis*
Australia	Queensland	Shark Hole, Koolatah Station, Mitchell River	23.1	10	11	26/07/2020	-15.66451	142.1060		Y	*Pristis pristis*
Australia	Queensland	Shark Hole, Koolatah Station, Mitchell River	23.3	20	14	26/07/2020	-15.66451	142.1060		Y	*Pristis pristis*
Australia	Queensland	Surprise creek, Camp 2.5, Mitchell River	24.4	20	10	28/07/2020	-15.26123	141.7811		Y	*Pristis pristis*
Australia	Queensland	Surprise creek, Camp 2.5, Mitchell River	24.5	20	10	28/07/2020	-15.26123	141.7811		Y	*Pristis pristis*
Australia	Queensland	Suprirse creek, Sawfish Heaven, Mitchell River	25.1	20	10	2/08/2020	-15.25752	141.7754		Y	*Pristis pristis*
Australia	Queensland	Baffle group Islands, Norman River	1.4	20	5	21/11/2018	-17.62372	141.0040		Y	*Pristis pristis*
Australia	Queensland	Baffle group Islands, Norman River	1.5	20	5	21/11/2018	-17.62372	141.0040		Y	*Pristis pristis*

Table A3. Summary of sawfish eDNA detection and amplicon sequence data. Grey highlighted nucleotide indicates a T↔C transition mutation site in the *12S* gene fragment that was sequenced from eDNA samples. At this nucleotide position in largetooth sawfish *Pristis pristis*, cytosine (C) is only found in Costa Rica and thymine (T) in Australia and Papua New Guinea.

	Country	Field replicate no.	Species Detected	No. positive technical replicates	Amplicon sequence (5' - 3')	Match to reference (% Pairwise identity)
1	Australia	TI5	*Pristis zijsron*	1/6	GGAGCCTGTTCTATAACCGATAATCCCCGTTAAACCCCACCACTTCTTGCTATCAACTGCCTATATACCGCCGTCGTCAGCTCACC CCATGAGGGGTTAAAAGTAAGCAAAATGA	100
2	Australia	SLC6	*Pristis zijsron*	1/6	GGAGCCTGTTCTATAACCGATAATCCCCGTTAAMCCCMCCACTTCTTGCTATCAACTGCCTATATACCGCCGTCGTCAGCTCACC CCATGAGGGGTTAAAAGTAAGCAAAATGA	98.3
3	Papua New Guinea	R1	*Anoxypristis cuspidata*	11/12	GGAGCCTGTTCTATAACCGATAATCCCCGTTAAACCTCACCACTTCTTGCCACTAACCGCCTATATACCGCCGTCGTCAGCTCACC CCATGAGGGAACAAAAGTAAGCAAAATG	100
4	Papua New Guinea	R2	*Anoxypristis cuspidata*	12/12	GGAGCCTGTTCTATAACCGATAATCCCCGTTAAACCTCACCACTTCTTGCCACTAACCGCCTATATACCGCCGTCGTCAGCTCACC CCATGAGGGAACAAAAGTAAGCAAAATG	100
5	Papua New Guinea	R3	*Anoxypristis cuspidata*	6/12	GGAGCCTGTTCTATAACCGATAATCCCCGTTAAACCTCACCACTTCTTGCCACTAACCGCCTATATACCGCCGTCGTCAGCTCACC CCATGAGGGAACAAAAGTAAGCAAAATG	100
6	Papua New Guinea	R5	*Anoxypristis cuspidata*	9/12	GGAGCCTGTTCTATAACCGATAATCCCCGTTAAACCTCACCACTTCTTGCCACTAACCGCCTATATACCGCCGTCGTCAGCTCACC CCATGAGGGAACAAAAGTAAGCAAAATG	100
7	Papua New Guinea	M1	*Anoxypristis cuspidata*	12/12	GGAGCCTGTTCTATAACCGATAATCCCCGTTAAACCTCACCACTTCTTGCCACTAACCGCCTATATACCGCCGTCGTCAGCTCACC CCATGAGGGAACAAAAGTAAGCAAAATG	100
8	Papua New Guinea	M2	*Anoxypristis cuspidata*	12/12	GGAGCCTGTTCTATAACCGATAATCCCCGTTAAACCTCACCACTTCTTGCCACTAACCGCCTATATACCGCCGTCGTCAGCTCACC CCATGAGGGAACAAAAGTAAGCAAAATG	100
9	Papua New Guinea	M3	*Anoxypristis cuspidata*	12/12	GGAGCCTGTTCTATAACCGATAATCCCCGTTAAACCTCACCACTTCTTGCCACTAACCGCCTATATACCGCCGTCGTCAGCTCACC CCATGAGGGAACAAAAGTAAGCAAAATG	100
10	Papua New Guinea	M4	*Anoxypristis cuspidata*	12/12	GGAGCCTGTTCTATAACCGATAATCCCCGTTAAACCTCACCACTTCTTGCCACTAACCGCCTATATACCGCCGTCGTCAGCTCACC CCATGAGGGAACAAAAGTAAGCAAAATG	100
11	Australia	3.4	*Anoxypristis cuspidata*	1/12	CTCACCACTTCTTGCCACTAACCGCCTATATACCGCCGTCGTCAGCTCACCCCATGAGGGAACAAAAGTAA	100
12	Australia	5.1	*Pristis clavata*	1/6	GAGCCTGTTCTATAACCGATAATCCCCGTTAAACCTCACCATTTCTTGCTATCAACCGCCTATATACCGCCGTCGTCAGCTCACCC CATGAGGGAACAAAAGTAAGCAAAA	100
13	Australia	5.2	*Pristis clavata*	2/6	GAGCCTGTTCTATAACCGATAATCCCCGTTAAACCTCACCATTTCTTGCTATCAACCGCCTATATACCGCCGTCGTCAGCTCACCC CATGAGGGAACAAAAGTAAGCAAAA	100
14	Australia	13.1	*Pristis clavata*	1/6	GAGCCTGTTCTATAACCGATAATCCCCGTTAAACCTCACCATTTCTTGCTATCAACCGCCTATATACCGCCGTCGTCAGCTCACCC CATGAGGGAACAAAAGTAAGCAAAA	100
15	Australia	13.2	*Pristis clavata*	2/6	GAGCCTGTTCTATAACCGATAATCCCCGTTAAACCTCACCATTTCTTGCTATCAACCGCCTATATACCGCCGTCGTCAGCTCACCC CATGAGGGAACAAAAGTAAGCAAAA	100
16	Australia	17.3	*Pristis clavata*	1/6	GAGCCTGTTCTATAACCGATAATCCCCGTTAAACCTCACCATTTCTTGCTATCAACCGCCTATATACCGCCGTCGTCAGCTCACCC CATGAGGGAACAAAAGTAAGCAAAA	100
17	United States	2.4	*Pristis pectinata*	1/30	AGGAGCCTGTTCTATAACCGATAATCCCCGTTAAACCTCACCATTCCTTGCTATTAACCGCCTATATACCGCCGTCGTCAGCTCAC CCCATGAGGGAGTAAAAGTAAGCAAAATGGACT	100
18	United States	7.2	*Pristis pectinata*	1/30	AGGAGCCTGTTCTATAACCGATAATCCCCGTTAAACCTCACCATTCCTTGCTATTAACCGCCTATATACCGCCGTCGTCAGCTCAC CCCATGAGGGAGTAAAAGTAAGCAAAATGGACT	100
19	United States	7.4	*Pristis pectinata*	5/30	AGGAGCCTGTTCTATAACCGATAATCCCCGTTAAACCTCACCATTCCTTGCTATTAACCGCCTATATACCGCCGTCGTCAGCTCAC CCCATGAGGGAGTAAAAGTAAGCAAAATGGACT	100
20	United States	16.4	*Pristis pectinata*	1/30	AGGAGCCTGTTCTATAACCGATAATCCCCGTTAAACCTCACCATTCCTTGCTATTAACCGCCTATATACCGCCGTCGTCAGCTCAC CCCATGAGGGAGTAAAAGTAAGCAAAATGGACT	100
21	United States	18.1	*Pristis pectinata*	30/30	AGGAGCCTGTTCTATAACCGATAATCCCCGTTAAACCTCACCATTCCTTGCTATTAACCGCCTATATACCGCCGTCGTCAGCTCAC CCCATGAGGGAGTAAAAGTAAGCAAAATGGACT	100
22	United States	18.2	*Pristis pectinata*	28/30	AGGAGCCTGTTCTATAACCGATAATCCCCGTTAAACCTCACCATTCCTTGCTATTAACCGCCTATATACCGCCGTCGTCAGCTCA	100

23	United States	18.3	*Pristis pectinata*	27/30	AGGAGCCTGTTCTATAACCGATAATCCCCGTTAAACCTCACCWTTCCTTGCTATTAACCGCCTATATACCGCCGTCGTCAGCTCAC CCCATGAGGGAGTAAAAGTAAGCAAAATGGACT	99.6
24	United States	18.4	*Pristis pectinata*	30/30	AGGAGCCTGTTCTATAACCGATAATCCCCGTTAAACCTCACCATTCCTTGCTATTAACCGCCTATATACCGCCGTCGTCAGCTCAC CCCATGAGGGAGTAAAAGTAAGCAAAATGGACT	100
25	United States	18.5	*Pristis pectinata*	30/30	TTCCTTGCTATTAACCGCCTATATACCGCCGTCGTCASCTCWCCCCATGAGGGAGTAAAAGTAAGCAAAATGGACT	98.7
26	United States	18.6	*Pristis pectinata*	29/30	AGGAGCCTGTTCTATAACCGATAATCCCCGTTAAACCTCACCATTCCTTGCTATTAACCG	100
27	United States	18.7	*Pristis pectinata*	27/30	AGGAGCCTGTTCTATAACCGATAATCCCCGTTAAACCTCACCATTCCTTGCTATTAACCGCCTATATACCGCCGTCGTCAGCTCAC CCCATGAGGGAGTAAAAGTAAGCAAAATGGACT	100
28	United States	18.8	*Pristis pectinata*	30/30	AGGAGCCTGTTCTATAACCGATAATCCCCGTTAAACMTCRCCATTCCTTGCTATTAACCGCCTATATACCGCCGTCGTCMSCTYAC CCCATGAGGGAGTAAAAGTAAGCAAAATGGACT	97.9
29	United States	19.1	*Pristis pectinata*	30/30	AGGAGCCTGTTCTATAACCGATAATCCCCGTTAAACCTCACCATTCCTTGCTATTAACCGCCTATATACCGCCGTCGTCAGCTCA	100
30	United States	19.2	*Pristis pectinata*	30/30	AGGAGCCTGTTCTATAACCGATAATCCCCGTTAAACCTCACCATTCCTTGCTATTAACCGCCTATATACCGCCGTCGTCAGCTCAC CCCATGAGGGAGTAAAAGTAAGCAAAATGGACT	100
31	United States	19.3	*Pristis pectinata*	30/30	AGGAGCCTGTTCTATAACCGATAATCCCCGTTAAAMCTSACCATTCCTTGCTATTAACCGCCTATATACCGCCGTCGTCAGCTCAC CCCATGAGGGAGTAAAAGTAAGCAAAATGGACT	99.2
32	United States	19.4	*Pristis pectinata*	30/30	AGGAGCCTGTTCTATAACCGATAATCCCCGTTAAACCTCACCATTCCTTGCTATTAACCGCCTATATACCGCCGTCGTCAGCTCAC CCCATGAGGGAGTAAAAGTAAGCAAAATGGACT	100
33	United States	19.6	*Pristis pectinata*	26/30	AGGAGCCTGTTCTATAACCGATAATCCCCGTTAAACCTCACCWTTCCTTGCTATTAACCGCCTATATACCGCCGTCGTCAGCTCAC CCC	99.4
34	United States	19.5	*Pristis pectinata*	30/30	AGGAGCCTGTTCTATAACCGATAATCCCCGTTAAACCTCACCATTCCTTGCTATTAACCGCCTATATACCGCCGTCGTCAGCTCAC CCCATGAGGGAGTAAAAGTAAGCAAAATGGACT	100
35	Papua New Guinea	9.4	*Pristis pristis*	1/12	CTTCTTGCCATCAACCGCCTATATACCGCCGTCGTCAGCTCACCCCATGAGGGAACAAAAGTAAGCAAAATGAACTAACCTTCAA**T** ACGTCAGGTCGAGGTGTAGCGAATGAAGTGGAAAGAAATGGGCTACATTTT	100
36	Costa Rica	15.1	*Pristis pristis*	6/12	AACCTCACCACTTCTTGCCATCAACCGCCTATATACCGCCGTCGTCAGCTCACCCCATGAGGGAACAAAAGTAAGCAAAATGAAC TAACCTTCAA**C**ACGTCAGGTCGAGGTGTAGCGAATGAAGTGGAAAGAAATGGGCTACATTTTCTCCTAAGAAAAAACGAACAGTAT	99.4
37	Costa Rica	15.2	*Pristis pristis*	1/12	NA	
38	Costa Rica	15.3	*Pristis pristis*	1/12	NA	
39	Costa Rica	17.2	*Pristis pristis*	2/12	TGCCTCAGACCCACCTAGAGGAGCCTGTTCTATAACCGATAATCCCCGTTAAACCTCACCACTTCTTGCCATCAACCGCCTATATA CCGCCGTCGTCAGCTCACCCCATGAGGGAACAAAAGTAAGCAAAATGAACTAACCTTCAA**C**ACGTCAGGTCGAGGTGTAGCGAA TGAAGTGGAAAGAAATGGGCTACATTTTCTCCTAAGAAAAAACGAACAGTATGATG	99.6
40	Costa Rica	17.3	*Pristis pristis*	3/12	GCCTCAGACCCACCTAGAGGAGCCTGTTCTATAACCGATAATCCCCGTTAAACCTCACCACTTCTTGCCATCAACCGCCTATATAC CGCCGTCGTCAGCTCACCCCATGAGGGAACAAAAGTAAGCAAAATGAACTAACCTTCAA**C**ACGTCAGGTCGAGGTGTAGCGAAT GAAGTGGAAAGAAATGGGCTACATTTTCTCCTAAGAAAAAACGAACAGTATG	99.5
41	Costa Rica	17.5	*Pristis pristis*	5/12	GCCTCAGACCCACCTAGAGGAGCCTGTTCTATAACCGATAATCCCCGTTAAACCTCACCACTTCTTGCCATCAACCGCCTATATAC CGCCGTCGTCAGCTCACCCCATGAGGGAACAAAAGTAAGCAAAATGAACTAACCTTCAA**C**ACGTCAGGTCGAGGTGTAGCGAAT GAAGTGGAAAGAAATGGGCTACATTTTCTCCTAAGAAAAAACGAACAGTAT	99.5
42	Costa Rica	18.1	*Pristis pristis*	1/12	TGCCTCAGACCCACCTAGAGGAGCCTGTTCTATAACCGATAATCCCCGTTAAACCTCACCACTTCTTGCCATCAACCGCCTATATA CCGCCGTCGTCAGCTCACCCCATGAGGGAACAAAAGTAAGCAAAATGAACTAACCTTCAA**C**ACGTCAGGTCGAGGTGTAGCGAA TGAAGTGGAAAGAAATGGGCTACATTTTCTCCTAAGAAAAAAC	99.5
43	Costa Rica	28.3	*Pristis pristis*	1/12	NA	
44	Costa Rica	28.4	*Pristis pristis*	2/12	TGCCTCAGACCCACCTAGAGGAGCCTGTTCTATAACCGATAATCCCCGTTAAACCTCACCACTTCTTGCCATCAACCGCCTATATA CCGCCGTCGTCAGCTCACCCCATGAGGGAACAAAAGTAAGCAAAATGAACTAACCTTCAA**C**ACGTCAGGTCGAGGTGTAGCGAA TGAAGTGGAAAGAAATGGGCTACATTTTCTCCTAAGAAAAA	99.5
45	Costa Rica	30.4	*Pristis pristis*	1/12	NA	-
46	Costa Rica	32.1	*Pristis pristis*	1/12	TGCCTCAGACCCACCTAGAGGAGCCTGTTCTATAACCGATAATCCCCGTTAAACCTCACCACTTCTTGCCATCAACCGCCTATATA CCGCCGTCGTCAGCTCACCCCATGAGGGAACAAAAGTAAGCAAAATGAACTAACCTTCAA**C**ACGTCAGGTCGAGGTGTAGCGAA TGAAGTGGAAAGAAATGGGCTACATTTTCTCCTAAGAAAAAACGAACAGTAT	99.5

47	Costa Rica	33.4	*Pristis pristis*	1/12	GTGCCTCAGACCCACCTAGAGGAGCCTGTTCTATAACCGATAATCCCCGTTAAACCTCACCACTTCTTGCCATCAACCGCCTATAT ACCGCCGTCGTCAGCTCACCCCATGAGGGAACAAAAGTAAGCAAAATGAACTAACCTTCAA**C**ACGTCAGGTCGAGGTGTAGCGA ATGAAGTGGAAAGAAATGGGCTACATTTTCTCCTAAGAAAAAACGAACAGTAT	99.6
48	Costa Rica	34.4	*Pristis pristis*	1/12	NA	-
49	Costa Rica	35.5	*Pristis pristis*	1/12	GTCAGCTCACCCCATGAGGGAACAAAAGTAAGCAAAATGAACTAACCTTCAA**C**ACGTCAGGTCGAGGTGTAGCGAATGAAGTGGA AAGAAATGGGCTACATTTTCTCCTAAGAAAAAACGAACAGTATGATG	99.2
50	Costa Rica	36.4	*Pristis pristis*	1/12	GGAGCCTGTTCTATAACCGATAATCCCCGTTAAACCTCACCACTTCTTGCCATCAACCGCCTATATACCGCCGTCGTCAGCTCACC CCATGAGGGAACAAAAGTAAGCAAAATGAACTAACCTTCAA**C**ACGTCAGGTCGAGGTGTAGCGAATGAAGTGGAAAGAAATGGG CTACATTTTCTCCTAAGAAAAA	99.2
51	Australia	6.2	*Pristis pristis*	1/12	GGAGCCTGTTCTATAACCGATAATCCCCGTTAAACCTCACCACTTCTTGCCATCAACCGCCTATATACCGCCGTCGTCAGCTCACC CCATGAGGGAACAAAAGTAAGCAAAATGAACTAACCTTCAA**T**ACGTCAGGTCGAGGTGTAGCGAATGAAGTGGAAAGAAATGGGC TACATTTT	100
52	Australia	22.4	*Pristis pristis*	1/12	GGAGCCTGTTCTATAACCGATAATCCCCGTTAAACCTCACCACTTCTTGCCATCAACCGCCTATATACCGCCGTCGTCAGCTCACC CCATGAGGGAACAAAAGTAAGCAAAATGAACTAACCTTCAA**T**ACGTCAGGTCGAGGTGTAGCGAATGAAGTGGAAAGAAATGGGC TACATTTT	100
53	Australia	23.1	*Pristis pristis*	1/12	GGAGCCTGTTCTATAACCGATAATCCCCGTTAAACCTCACCACTTCTTGCCATCAACCGCCTATATACCGCCGTCGTCAGCTCACC CCATGAGGGAACAAAAGTAAGCAAAATGAACTAACCTTCAA**T**ACGTCAGGTCGAGGTGTAGCGAATGAAGTGGAAAGAAATGGGC TACATTTT	100
54	Australia	23.3	*Pristis pristis*	2/12	GGAGCCTGTTCTATAACCGATAATCCCCGTTAAACCTCACCACTTCTTGCCATCAACCGCCTATATACCGCCGTCGTCAGCTCACC CCATGAGGGAACAAAAGTAAGCAAAATGAACTAACCTTCAA**T**ACGTCAGGTCGAGGTGTAGCGAATGAAGTGGAAAGAAATGGGC TACATTTT	100
55	Australia	24.4	*Pristis pristis*	6/12	GGAGCCTGTTCTATAACCGATAATCCCCGTTAAACCTCACCACTTCTTGCCATCAACCGCCTATATACCGCCGTCGTCAGCTCACC CCATGAGGGAACAAAAGTAAGCAAAATGAACTAACCTTCAA**T**ACGTCAGGTCGAGGTGTAGCGAATGAAGTGGAAAGAAATGGGC TACATTTT	100
56	Australia	24.5	*Pristis pristis*	1/12	GGAGCCTGTTCTATAACCGATAATCCCCGTTAAACCTCACCACTTCTTGCCATCAACCGCCTATATACCGCCGTCGTCAGCTCACC CCATGAGGGAACAAAAGTAAGCAAAATGAACTAACCTTCAA**T**ACGTCAGGTCGAGGTGTAGCGAATGAAGTGGAAAGAAATGGGC TACATTTT	100
57	Australia	25.1	*Pristis pristis*	1/12	GGAGCCTGTTCTATAACCGATAATCCCCGTTAAACCTCACCACTTCTTGCCATCAACCGCCTATATACCGCCGTCGTCAGCTCACC CCATGAGGGAACAAAAGTAAGCAAAATGAACTAACCTTCAA**T**ACGTCAGGTCGAGGTGTAGCGAATGAAGTGGAAAGAAATGGGC TACATTTT	100
58	Australia	1.4	*Pristis pristis*	1/14	GGAGCCTGTTCTATAACCGATAATCCCCGTTAAACCTCACCACTTCTTGCCATCAACCGCCTATATACC-GCCGTCGTCAGCTCACCCCATGAGGGAACAAAAGTAAGCAAAATGAACTAACCTTCAA**T**ACGTCAGGTCGAGGTGTAGCGAATGA AGTGGAAAGAAATGGGCTACATTTT	100
59	Australia	1.5	*Pristis pristis*	14/14	GGAGCCTGTTCTATAACCGATAATCCCCGTTAAACCTCACCACTTCTTGCCATCAACCGCCTATATACC-GCCGTCGTCAGCTCACCCCATGAGGGAACAAAAGTAAGCAAAATGAACTAACCTTCAA**T**ACGTCAGGTCGAGGTGTAGCGAATGA AGTGGAAAGAAATGGGCTACATTTT	100

Data A1. Global Sawfish Search eDNA sample collection manual

Protocol for collecting and packaging eDNA samples

Prepared by:
Madalyn K. Cooper

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Here we provide the details of a sampling protocol designed for detecting sawfish. This manual accompanies a 'Global Sawfish Search' eDNA sampling kit, which contains a pump and all materials necessary to properly collect and package eDNA samples. There is flexibility in some of the equipment used and there may be instances where the collaborator will have to purchase/replenish some of the consumables (e.g. bleach, gloves, rubbish bags, distilled water) used in this kit. If this is not possible, please contact Madie (madie.cooper@my.jcu.edu.au) or Colin (colin.simpfendorfer@jcu.edu.au).

Basic information

Avoiding contamination

Due to the *extremely high sensitivity* of eDNA analyses, care must be taken to avoid contamination. Primary sources of contamination are anything that has been exposed to the target species or its DNA, including hands, clothes, used equipment, water samples and the field vehicle/vessel. If you suspect that any forceps, filter housing, samples, or anything else have become contaminated, stop sampling and start over (carry spares for these circumstances). Change gloves as frequently as needed.

Throughout this document, we refer to items as being either "dirty" or "clean".

"Dirty" examples: equipment, gloves, or clothing that became wet and contaminated with DNA at the previous site
"Clean" examples: equipment and consumables that are clean/sterile and inside a clean bag or box. They should only ever be touched with clean/new glove.

It can be easier to carry out sampling if you have a team of 2 or more people to designate "clean" and "dirty" tasks, and to scribe.

Wearing gloves

Your hands will become wet and dirty while in the field. Use gloves to minimize the risk of contaminating your eDNA sample. Specifically:

- Wear new gloves when pulling sampling kits from the green nally bin
- Wear new gloves when removing a filter with eDNA on it and placing in preservative tube
- You do not need to wear gloves when handling the outside of the pump or hosing, as these are downstream of the filter (that is, they are below the filter and do not come into contact with sample water before it is filtered), but for simplicity you may choose to wear gloves and continue to change them
- You do not need to wear gloves when handling "dirty" items or the box they are to be placed in, but for simplicity you may choose to wear gloves and continue to change them

Boat-based sampling

It is imperative that the boat used for sampling is not contaminated with target species DNA. You must:

- Clean the work area within the boat by wiping with 10% bleach solution before commencing sampling at a new location
- Clean the boat by washing/rinsing with freshwater before and after use

- Do not transport fishing gear on the boat during eDNA field sampling

Field trip preparation

Pre-departure preparation

You will require:

- GPS-equipped device to record sampling positions
- 10 L bucket (to measure filtrate amount)
- Access to an electrical connection to ensure batteries are fully charged at the start of each day (batteries are shipped with <30% charge)
- Clean clothes for each day of sampling
- Depth sounder (or a means to measure depth)
- Bleach tablets or liquid for cleaning
- Clean water (250 mL of water per sampling site for equipment control)
- Clipboard for data sheets
- Water temperature gauge
- Secchi disc

Cleaning and packing equipment

If equipment has been used complete 1 and 2:

1. Decontaminate used equipment such as forceps, containers and hosing with 10% bleach solution in a bucket. Ensure the equipment is submerged in bleach solution for 10 minutes, then rinse with freshwater (important to remove residual bleach) and dry in a clean environment.
   <i> *Caution when flushing used bleach down the drain* <i>

2. Decontaminate surface of boxes and pump by wiping surface with 10% bleach. Ensure the bleach solution is left for 10 minutes then wipe/rinse with freshwater (important to remove residual bleach) and dry in a clean environment

3. Cut off two pieces of the provided hosing to the desired length (i.e. if boat-based sampling, the hose must be long enough the reach over the side of the boat and into the water)
   - Input hose – length to reach water body
   - Output hose – length to reach from pump to bucket
   - If you are collecting water using a bottle (see video: part II) cut input hose into approx. 1.5-inch length

4. Organise all equipment needed for field sampling; this will include:
   - Black carry case
   - Pump
   - Hosing
   - Tube box with 2 mL tubes containing preservative
   - Gloves
   - Sampling equipment (filter housing, forceps, filter papers) - enough for each location/site you plan to sample

- 250mL clean water per site – we have provided 1 L bottles that you can put drinking/tap water into
- Paper towel
- Datasheet and marker pen/pencil
- Rinse bottles
- Bleach tablets
- You will need to provide a bucket and a means to keep the clean and dirty equipment from mingling

Using bleach tablets

One bleach tablet dissolved per 1 L water. Stir/shake vigorously for immediate use.

In the field use the wash bottles provided to clean as you go. If you are covering more than one site per day, you can use the same bottle of bleach to clean the pump at every site.

Site selection

- Distribution of eDNA is heterogeneous in aquatic systems, so site selection can be important for detection
- Samples should be collected in sites likely to be used by sawfish, such as mangroves, mud flats or sand banks
- Please describe the habitat type the field data sheet
- When sampling multiple sites in the same river system, always begin sampling at the site that is furthest downstream and sample sequentially as you move upstream i.e. fixed, periodic intervals of hundreds of metres up to one kilometre depending on location size
- It is best to samples as many different sites as possible to get a representative snapshot of presence or absence for this rare species

Location = River system

Site = Sample site within location (these are numbered sequentially for each country)

For example, samples sites (blue pins) at Bahia Potrero Grande, Costa Rica along a transect from tributaries through the river main stem and out the mouth of the river

Image courtesy of Jorge Valerio and Mario Espinoza

Sample collection

Data collection

Upon arriving at sample site, please fill in *Site information* on the *eDNA Sampling Datasheet.*

Make a note of any information that could be of interest (e.g. sawfish caught in fishing nets X days/weeks prior).

Pump assembly

The person designated to perform "dirty" tasks can start the following:

1. Put on gloves
2. Take out pump and place in clean, dry working position
3. Grab input and output hosing and forcibly push into the respective input and output fittings of the pump; approximately 1.2 cm of the hose can be pushed into the fitting

The person designated to perform "clean" tasks can start the following:

4. With clean gloves, grab sampling equipment (filter housing, thick hose, forceps, filter paper) and 250 mL bottle of clean water and place within the clean black work box
5. Attach the thick short hose to the white side of the filter housing
6. <i>*Important to use clean gloves*<i> place a filter paper inside the filter housing
7. Ensure filter housing is firmly closed, then attach black fitting into the end of input hose

Water filtration

Record what pore size you are using on the data sheet

Equipment control to test for field contamination:

8. Designated "clean" person:
9. Turn pump on
10. Pour 250 mL of clean water into the filter housing while holding upright
11. One finished, turn the pump off
12. Remove the white top from the filter housing and place somewhere clean
13. Using one hand and forceps, roll up the filter paper, fold in half and place inside 2 mL tube of preservative
14. Cap 2 mL tube firmly and note tube label on data sheet

15. **Collecting a field sample**: "Clean" person, put on new gloves, grab a new filter paper and place inside the filter housing
16. Immediately replace lid on filter paper container
17. "Dirty" person, close filter housing and perform the following:
18. Reach the input hosing over the side of the boat and put the thick hose of the filter housing into the water body
19. Do not submerge filter housing. *In rough conditions this will be difficult*

20. Place the output hose in a bucket
21. Turn on the pump and monitor filtrate amount (5 L is desirable)
22. When target volume has been reached, invert the filter housing upwards out of the water and filter the remaining water inside the housing
23. Turn the pump off
24. Remove the white top with thick tube still attached and place in round container on work bench
25. Carefully discard filtrate into water body, but not directly where you are sampling from
26. "Clean" person, with new gloves:
27. Open 2 mL tube with preservative labelled X.1 (labelled sequentially for replicates)
28. Using one hand and forceps, roll and fold the filter paper and place into tube
29. Close 2 mL tube firmly
30. Remove gloves
31. Record filtration data on the *eDNA Sampling Datasheet*
32. Repeat steps 14-29 for a total of five times (e.g. 5 replicates per site)

33. Repeat steps 14-29 an additional three times for filter papers with pore size 1.2 µm, filtering as much water as possible (this may only be up to few hundred mL's of water)

Decontamination between sites while in field

34. After filtering, put all used materials away from clean equipment
35. Prepare 10% bleach solution with 500 mL of water ½ bleach tablet in rinse bottle label '10% bleach'. Shake mixture vigorously
36. Decontaminate the surfaces if the workbench, pump, hosing, containers, bucket and storage bin by wiping with paper towel saturated in 10% bleach solution
37. Leave to stand for a minimum of 5 minutes, or while you are moving to the next site
38. You can leave the pump and hosing inside the bucket for this step
39. Wipe with clean water and then dry the workbench surface using paper towel
40. Used equipment and paper towel should be put inside a bag or box

Troubleshooting

Turbid water – a filter paper with a small pore size can be easily blocked in turbid water. Likewise, if the filter paper is blocked, the clear hosing can collapse under high pressure. If the filtration rate begins to slow, the hosing collapses or you cannot filter more than 1 L of water, complete the following:

"Dirty" person:

1. Lift filter housing from water, and pump through remaining water
2. Turn pump off and open filter housing

"Clean" person:

3. Remove and discard the filter paper in garbage bag
4. Change gloves, locate spare filter papers in green box, and replace the filter paper with one that has a larger pore size (e.g. 20 µm)
5. Make note of this on data sheet
6. Proceed sampling as normal

If you continue to have problems filtering turbid water, filter the maximum amount possible and make note of this problem on the data sheet

Bubbles in output hosing, or leaks in fittings – there is air getting into the pump. You must firmly push the hosing in to the pump to correctly seal, ~1.2 cm of hosing should fit inside the fitting

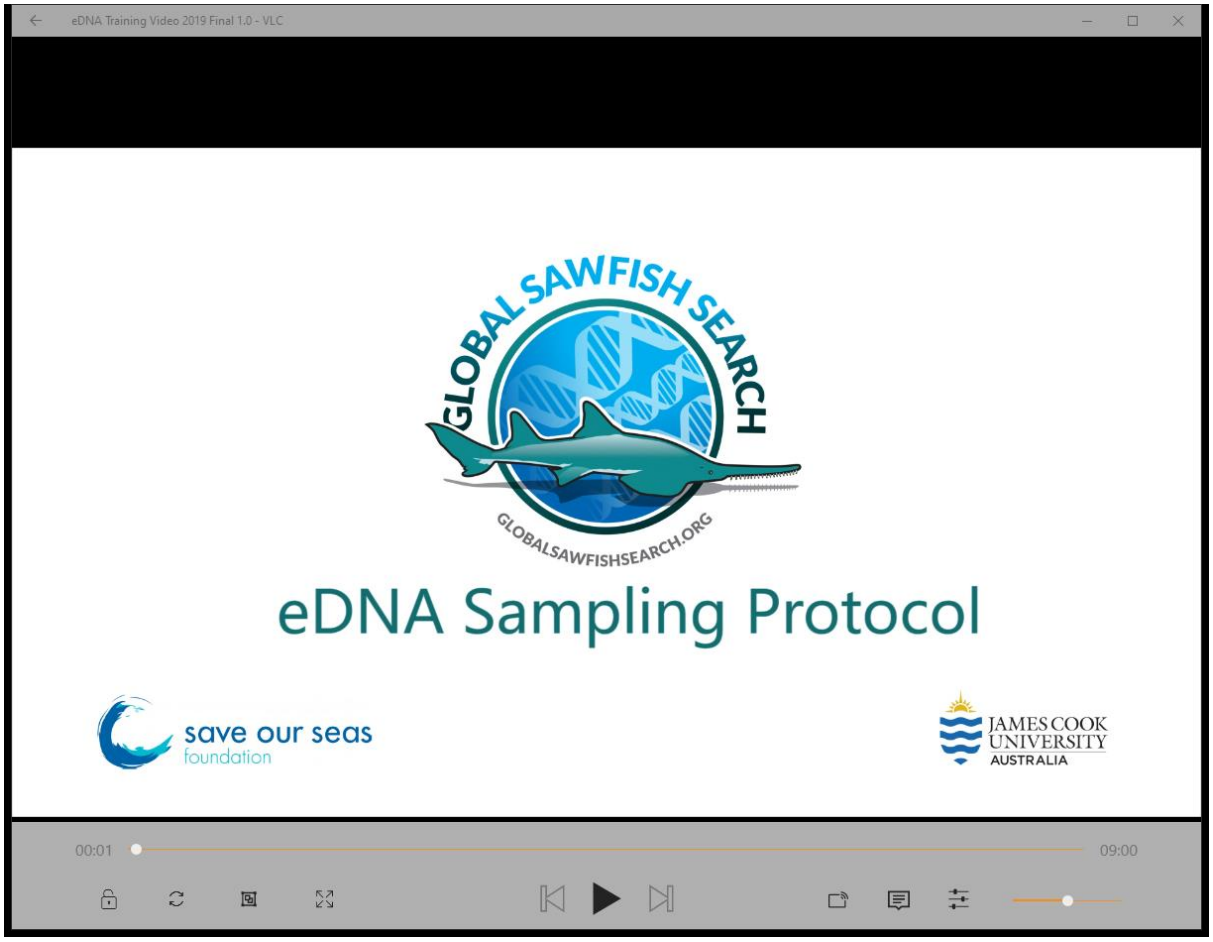
Pump 'on' but not pumping water – there is an air bubble in the diaphragm. Hold hoses upright while still attached to pump, fill ½ with water, turn pump on and then firmly and carefully hit the pump against a hard surface to shift the air bubble. You will know it is working with air bubbles rise through water in hosing and the pump starts to pump water

Pump not turning on – charge batteries. If you have been using the pump in direct sunlight, it or the batteries may have over heated. In this case, swap to the spare pump.

This protocol is adapted from JCU eDNA Complete Laboratory & Field Manual Protocol Version 18 19th April 2017 (Centre for Sustainable Tropical Fisheries & TropWater, James Cook University), with reference to online eDNA protocols by Goldberg, C. & Strickler, K. (2017, eDNA Protocol Sample Collection, Washington State University) and Carim, L., McKelvey, K., Young, M., Wilcox, T. & Schwartz, M. (2016, A protocol for collection environmental DNA samples from streams, U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station).

Data A2. Global Sawfish Search Training Video

Link here



APPENDIX B: RESEARCH OUTPUTS AND ACTIVITIES

I.I Publications associated with PhD research

Peer-reviewed publications:

Cooper, M.K.*, Villacorta-Rath, C.*, Burrows, D., Jerry, D. R., Carr, L., Barnett, A., Huveneers, C., & Simpfendorfer, C. A. (2022). Practical eDNA sampling methods inferred from particle size distribution and comparison of capture techniques for a Critically Endangered elasmobranch. *Environmental DNA*, 4(5), 1011-1023. https://doi.org/10.1002/edn3.279 *co-first authors

Cooper, M. K., Huerlimann, R., Edmunds, R. C., Budd, A. M., Le Port, A., Kyne, P. M., Jerry, D. R., & Simpfendorfer, C. A. (2021). Improved detection sensitivity using an optimal eDNA preservation and extraction workflow and its application to threatened sawfishes. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 31(8), 2131–2148. https://doi.org/10.1002/aqc.3591

Huerlimann, R.*, **Cooper, M. K.***, Edmunds, R. C.*, Villacorta-Rath, C., Le Port, A., Robson, H. L. A., Strugnell, J. M., Burrows, D., Jerry, D. R. (2020). Enhancing tropical conservation and ecology research with aquatic environmental DNA methods: an introduction for non-environmental DNA specialists. *Animal Conservation*, 23(6), 632–645. https://doi.org/10.1111/acv.12583 *co-first authors

Book chapter:

Le Port, A., Bakker, J., **Cooper, M. K.**, Huerlimann, R., & Mariani, S. (2018). Environmental DNA (eDNA): A valuable tool for ecological inference and management of sharks and their relatives. In J. C. Carrier, M. R. Heithaus, & C. A. Simpfendorfer (Eds.), *Shark Research: Emerging Technologies and Applications for the Field and Laboratory* (pp. 255–283). CRC Press, Boca Raton, Florida, United States. https://doi.org/10.1201/B21842-21

I.II Additional research outputs during PhD candidature

Peer-reviewed publications:

De Brauwer M., Chariton A., Clarke L.J., **Cooper M.K.,** DiBattista J., Furlan E., Giblot-Ducray D., Gleeson D., Harford A., Herbert S., MacDonald A.J., Miller A., Montgomery K., Mooney T., Noble L.M., Rourke M., Sherman C.D.H., Stat M., Suter L., West K.M., White N., Villacorta-Rath C., Zaiko A., Trujillo-Gonzalez A. Best practice guidelines for environmental DNA biomonitoring in Australia and New Zealand. *Environmental DNA*. Accepted.

Budd, A. M., Schils, T., **Cooper, M. K.**, Port, Lyons, M. B., Mills, M. S., Deinhart, M. E., Le Port, A., Huerlimann, R., & Strugnell, J. M. (2023) Monitoring threatened species with environmental DNA and open ecological data: local distribution and habitat preferences of scalloped hammerhead sharks (*Sphyrna lewini*). *Biological Conservation*, *278*, 109881, https://doi.org/10.1016/j.biocon.2022.109881

Budd, A. M., **Cooper, M. K.**, Port, A. Le, Schils, T., Mills, M. S., Deinhart, M. E., Huerlimann, R., & Strugnell, J. M. (2021). First detection of critically endangered scalloped hammerhead sharks (*Sphyrna lewini*) in Guam, Micronesia, in five decades using environmental DNA. *Ecological Indicators*, *127*, 107649. https://doi.org/10.1016/j.ecolind.2021.107649

Publication in review:

Valerio-Vargas, J. A., **Cooper, M. K.**, Simpfendorfer C. A., Espinoza, M. Identifying potential freshwater hotspots of the critically endangered largetooth sawfish in Central America using environmental DNA.

Technical reports:

De Brauwer M., Chariton A., Clarke L.J., **Cooper M.K.,** DiBattista J., Furlan E., Giblot-Ducray D., Gleeson D., Harford A., Herbert S., MacDonald A.J., Miller A., Montgomery K., Mooney T., Noble L.M., Rourke M., Sherman C.D.H., Stat M., Suter L., West K.M., White N., Villacorta-Rath C., Zaiko A., Trujillo-Gonzalez A. (2022). Environmental DNA test validation guidelines. National eDNA Reference Centre, Canberra.

De Brauwer M., Chariton A., Clarke L.J., **Cooper M.K.,** DiBattista J., Furlan E., Giblot-Ducray D., Gleeson D., Harford A., Herbert S., MacDonald A.J., Miller A., Montgomery K., Mooney T., Noble L.M., Rourke M., Sherman C.D.H., Stat M., Suter L., West K.M., White N., Villacorta-Rath C., Zaiko A., Trujillo-Gonzalez A. (2022). Environmental DNA protocol development guide for biomonitoring. National eDNA Reference Centre, Canberra.

Budd A.M., **Cooper M.K.**, Le Port A., Schils T., Mills M.S., Deinhart M.E., Huerlimann R. & Strugnell J. (2020). Detection of Scalloped hammerhead sharks in Apra Harbor and adjacent waters, Guam, using environmental DNA. Prepared by Marine Laboratory, University of Guam, for Naval Facilities Engineering Command Marianas. 131 pp.

Villacorta-Rath, C., **Cooper, M.**, & Burrows, D. (2020). Environmental DNA (eDNA) survey of largetooth sawfish in south central Arnhem Land. Report 20/40, Centre for tropical Water and Aquatic Ecosystem Research (TropWATER), James Cook University, Townsville.

Villacorta-Rath, C., **Cooper, M.**, & Burrows, D. (2020). Sawfish environmental DNA (eDNA) survey in Groote Eylandt. Report 20/39, Centre for Tropical Water and Aquatic Ecosystem Research (TropWATER), James Cook University, Townsville.

Edmunds, R.C., **Cooper, M.**, Huerlimann, R., Robson, H., and Burrows, D. (2019). Environmental DNA Survey of Eureka Creek, Upper Mitchell, and Walsh River for Invasive *Oreochromis mossambicus* and *Tilapia mariae* (November 2017). Report19/06, Centre for Tropical Water and Aquatic Ecosystem Research (TropWATER), James Cook University, Townsville.

I.II Conference presentation and invited talk

2019 – Invited talk, '*Environmental DNA for conservation: finding the World's most endangered marine fish*', Can Tho University

2019 – Conference presentation and poster, '*eDNA methods matter for tropical conservation: A review and case study from threatened sawfishes*', Fisheries Society of the British Isles (FSBI) Symposium: Advances in eDNA-based Approaches to Fish Ecology and Management, Hull, UK

I.III Awards and Scholarship

2017-2020 – Australian Government Research Training Program Scholarship (RTPS)

2017-2020 – James Cook University Prestige Research Scholarship

2017-2020 – National Environmental Science Program (NESP) Top-Up Scholarship

2019 – Student Research Grant, Oceania Chondrichthyan Society (OCS)

2018 – James Cook University College of Science and Engineering Postgraduate Student Travel Grant

2018 – Student Award, 2nd place, Fisheries Society of the British Isles (FSBI) Symposium: Advances in eDNA-based Approaches to Fish Ecology and Management, Hull, UK

I.IV Outreach activities

2019 – Radio interview, '*eDNA research in the tropics*', ABC North Queensland morning news

2019 – Bog post, '*Are Myeik's thriving mangroves a safe haven for sawfish?*', Save Our Seas Foundation

2018 – TV interview, '*International Sawfish Day*', Nine News Townsville evening news

2018 – Science fair, Dauphin Island Sea Lab science education and outreach event, Alabama, U.S.

2018 – Blog post, '*A probe into sawfish occurrence: in the laboratory of Global Sawfish Search*', Save Our Seas Foundation

I.V Professional service

2021-current – Conference organising committee, 1st Australian & New Zealand Environmental DNA (eDNA) Conference: Innovation & Application

2020-current – General member & Working group member, Southern eDNA (seDNA) Society

2017-current – Oceania Chondrichthyan Society (OCS) general member

2017-2022 – Oceania Chondrichthyan Society (OCS) committee member

2019 – Student representative, Centre for Sustainable Tropical Fisheries & Aquaculture, James Cook University

2020 – Student representative, Molecular Ecology & Evolution Lab, Australian Tropical Sciences and Innovation Precinct (ATSIP), James Cook University

2018-2020 – Laboratory mentor, Molecular Ecology & Evolution Lab, Australian Tropical Sciences and Innovation Precinct (ATSIP), James Cook University

Ongoing – Reviewer for Conservation Genetics Resources, Science of the Total Environment, Diversity

I.V Other professional contributions and activities

Teaching:

2017-2020 – Tutor & Marking, BS1001 *Introduction to Biological Processes*

2020 – Tutor & Marking, BS1007 *Introduction to biodiversity*

2020 – Tutor, BS2470 *Evolution*

2020 – Teaching assistant, creation of online course content, Diploma BS1001 *Introduction to Biological Processes*

Other projects & roles:

2020-current – Senior Researcher, OceanOmics program, Flourishing Oceans Initiative, Minderoo Foundation, Australia

2017-2021 – Associate Researcher, Project '*Detection of scalloped hammerhead sharks in Apra Harbor and adjacent waters, Guam, using environmental DNA'*, Joint project between James Cook University and University of Guam for Naval Facilities Command Marianas, Department of the Navy

2018-2020 – Principal Investigator, Project *'Nanopore genome-skimming of the critically endangered Largetooth sawfish*', Joint project with members of Centre for Tropical Bioinformatics and Molecular Biology, James Cook University

2020 – Associate Researcher, Engagement and advise on emergency response plan to Cid Harbour shark incidents, Joint Workshop with Fish & Fisheries Lab JCU, Reef Ecologic, Whitsundays tourism operators, and Queensland Government

2019-2020 – Programme Partner, Conservation genetics specialist, SharkSearch Indo-Pacific

2019-2020 – Advisor, Masters Student, Project 'A beacon of hope: distribution and current status of largetooth sawfish in Costa Rica', University of Costa Rica

2018-2020 – Associate Researcher, Project '*The Northern Australia eDNA Program: Revolutionising Aquatic Monitoring and Field Surveys in Tropical Waters*', Centre for Tropical Water and Aquatic Ecosystem Research (TropWATER), James Cook University

2018-2020 – Associate Researcher, Project '*Detecting sawfish refuges using eDNA in FNQ*', Sharks and Rays Australia, Queensland, Australia

2018 – Laboratory assistant, Project '*Guidelines for development and validation of assays for marine pests'*, Aquaculture Research Group, James Cook University

2017-2020 – Field assistant, various research projects, Fish & Fisheries Lab, James Cook University

2017-2018 – Manuscript editor, Australian Awards Scholarship Tutorial Assistance Program, James Cook University