ENVIRONMENTAL DNA – FORENSIC DNA DETECTION OF AQUATIC SPECIES AND PROFILING OF ENTIRE BIOLOGICAL COMMUNITIES

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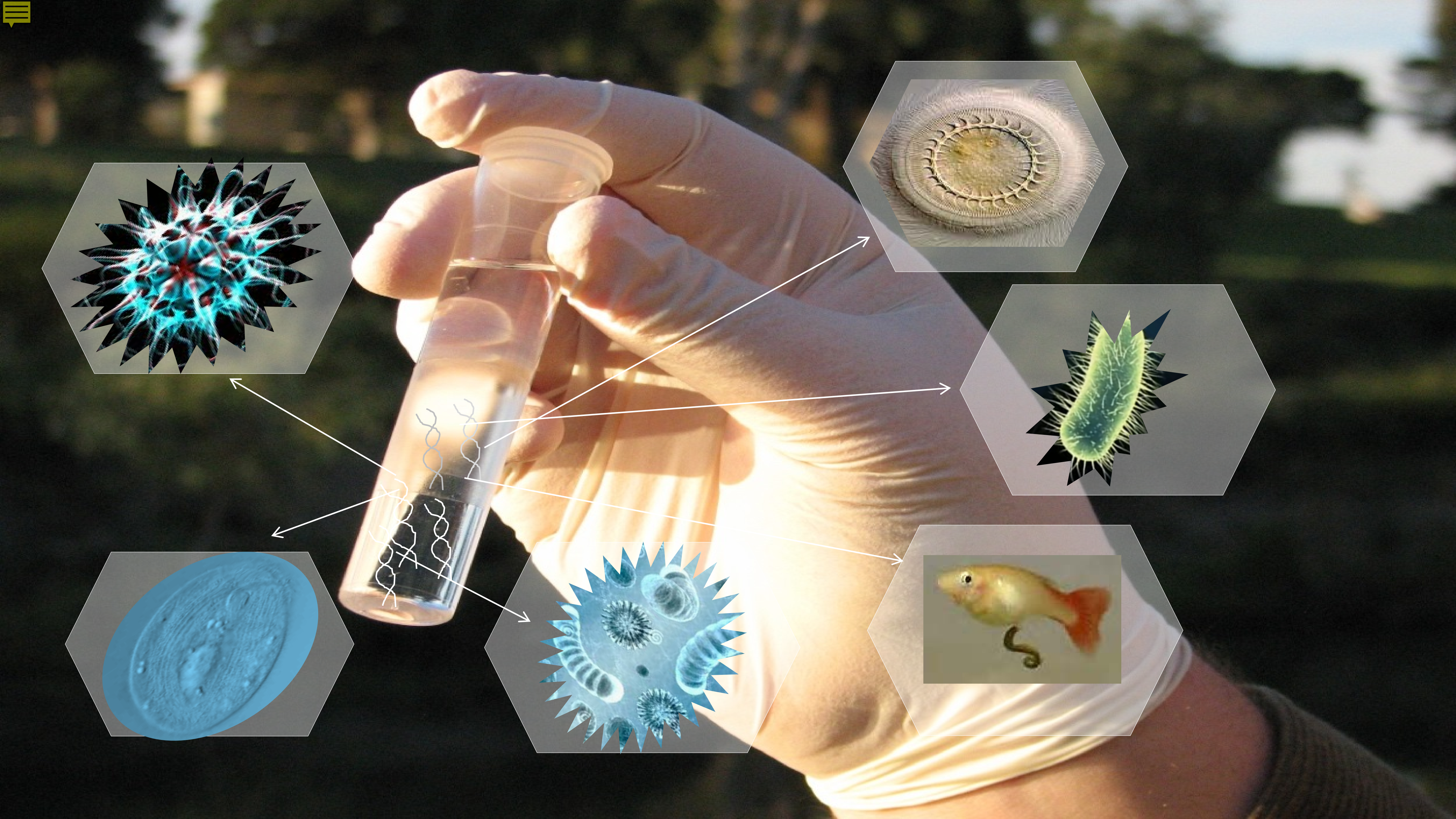
CLASSICAL AQUATIC BIODIVERSITY SURVEY TECHNIQUES

- RELY ON PHYSICAL CAPTURE OR VISUALISATION
- HAVE DIFFERENT EFFICIENCIES
  - SPECIES DIFFICULT TO FIND
  - LOW ABUNDANCE
  - SITE ACCESS

AND OF COURSE OTHER PERILS

HOWEVER, EVEN IF YOU CAN'T CATCH THEM….

- ALL LIVING ORGANISMS, REGARDLESS OF THEIR SIZE, LEAVE BEHIND TRACES OF DNA
- DNA LEFT BEHIND IS CALLED ENVIRONMENTAL DNA "eDNA"
- ENTERS THE ENVIRONMENT THROUGH:
  - FAECES, URINE, EGGS/SPERM, MUCUS, OR EVEN DEAD ANIMAL
- DNA IS BOTH EXTRACELLULAR & INTRACELLULAR



eDNA – HOW DOES IT WORK?

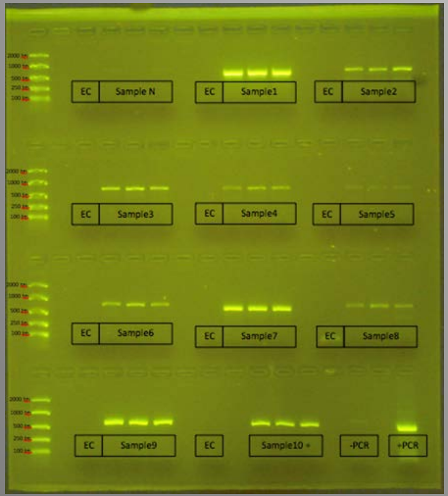
Skin cells

Faeces Urine

Eggs sperm

Mucous

Species diagnostic primers



Presence/ absence

eDNA - APPLICATIONS

- INVASIVE PEST DETECTION
- RARE SPECIES/LOW ABUNDANCE DETECTION
- PATHOGEN DETECTION
- MOVEMENT/BARRIERS
- PRIORITIZING FIELD EFFORT
- CRYPTIC SPECIES
- SPECIES DISTRIBUTION DETERMINATION
- REMOTE SAMPLINGS
- ANY AQUATIC SPECIES TURTLES, FROGS/TOADS, INVERTEBRATES

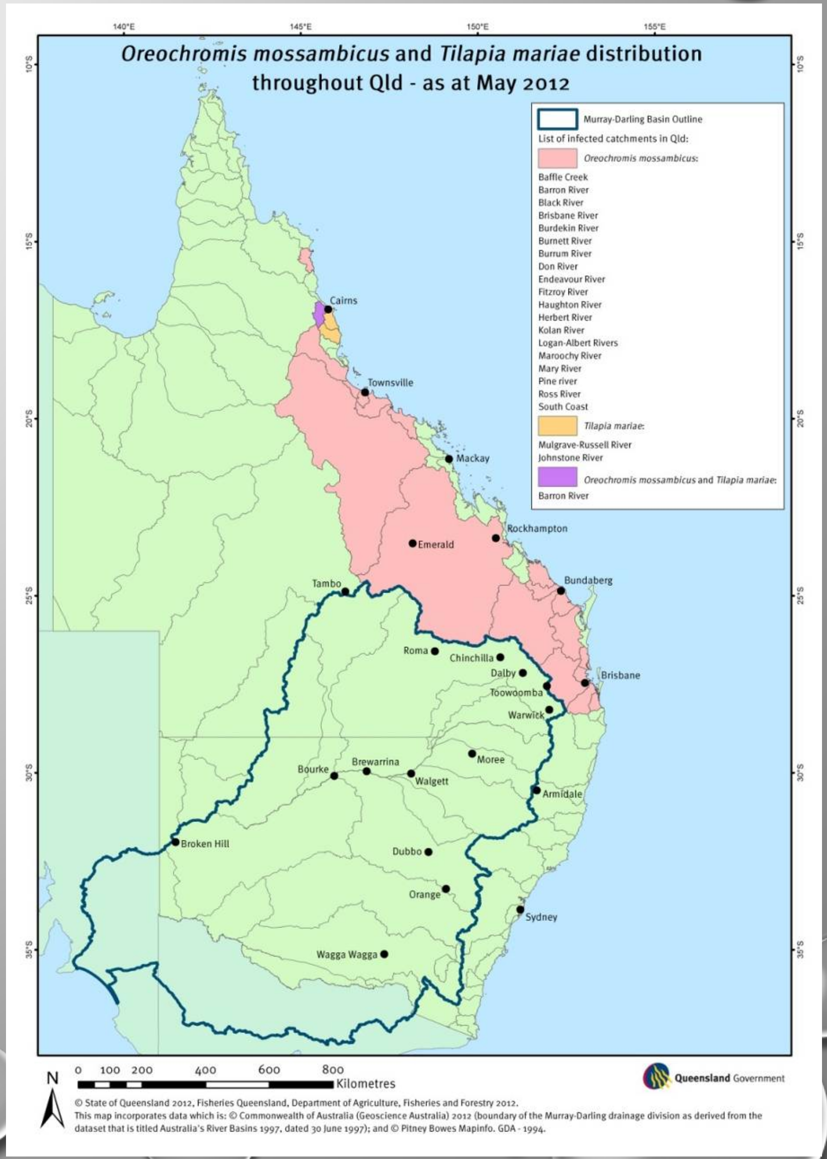
ARE FISH MOVING ABOVE BARRIERS

CRYPTIC SPECIES

PRIORITISING EFFORT

eDNA - INVASIVE SPECIES DETECTION

- MOZAMBIQUE AND SPOTTED TILAPIA ARE HIGHLY INVASIVE
- ONE OF ONLY 8 FISH SPECIES LISTED IN THE TOP 100 PESTS IN THE WORLD – IUCN GLOBAL INVASIVE SPECIES PROGRAM (2004)
- JCU DEVELOPED eDNA DETECTION FOR TILAPIA



Queensland Government
Department of Agriculture and Fisheries

Invasive Animals Cooperative Research Centre

eDNA - INVASIVE SPECIES DETECTION

- FITZROY BASIN AUTHORITY AND REEF CATCHMENTS
- 14 LOCATIONS IN LOWER FITZROY CATCHMENT
- 5 SAMPLES OF 2 LITRES
- ELECTROFISHING AT SAME SITES
- COMPARED AGAINST CATCHES AND SIGHTINGS RECORD

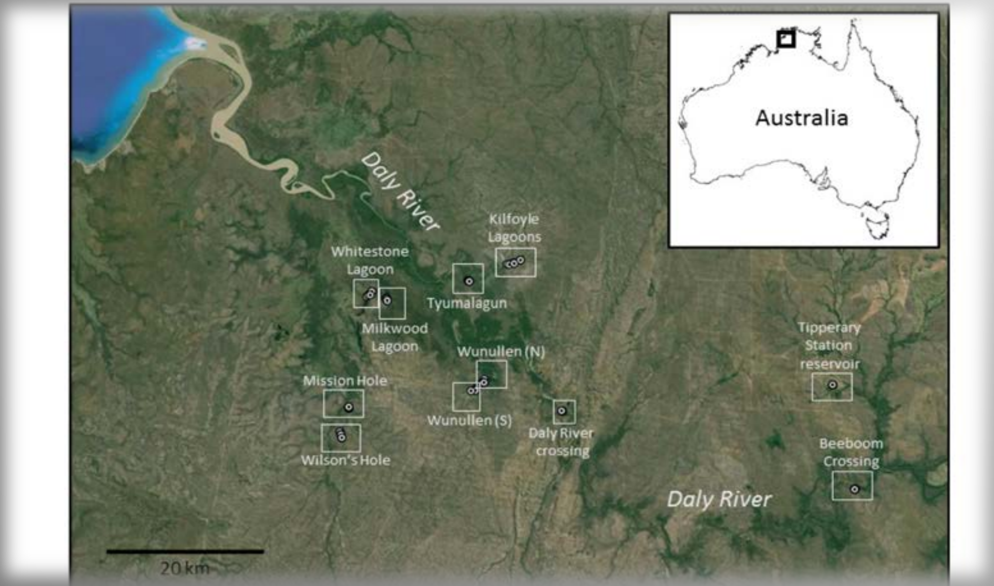
eDNA VS ELECTROFISHING!

eDNA VS ELECTROFISHING – AND THE WINNER IS….

Site	Number of tilapia caught (electrofishing)		Tilapia detected (eDNA)	Tilapia sightings
Nankin Creek Lagoon	0		Yes	No
Moores Creek	0		Yes	Yes
River Road Lagoon 1	0		No	Yes
Raglan Creek	0		Yes	No
Bajool Weir Pool	0		Yes	Yes
Gracemere Lagoon	0		No	No
Yeppen Lagoon	2		Yes	Yes
Lion Creek	40		Yes	Yes
Splitters Creek	20		Yes	Yes
Belmont Creek	0		n/a	Yes
Alligator Creek	0		n/a	Yes
Rossmoya Rd Creek	0		Yes	No
Hedlow Creek	0		n/a	No
Eden Bann Weir	0		n/a	No

eDNA – RARE SPECIES DETECTION

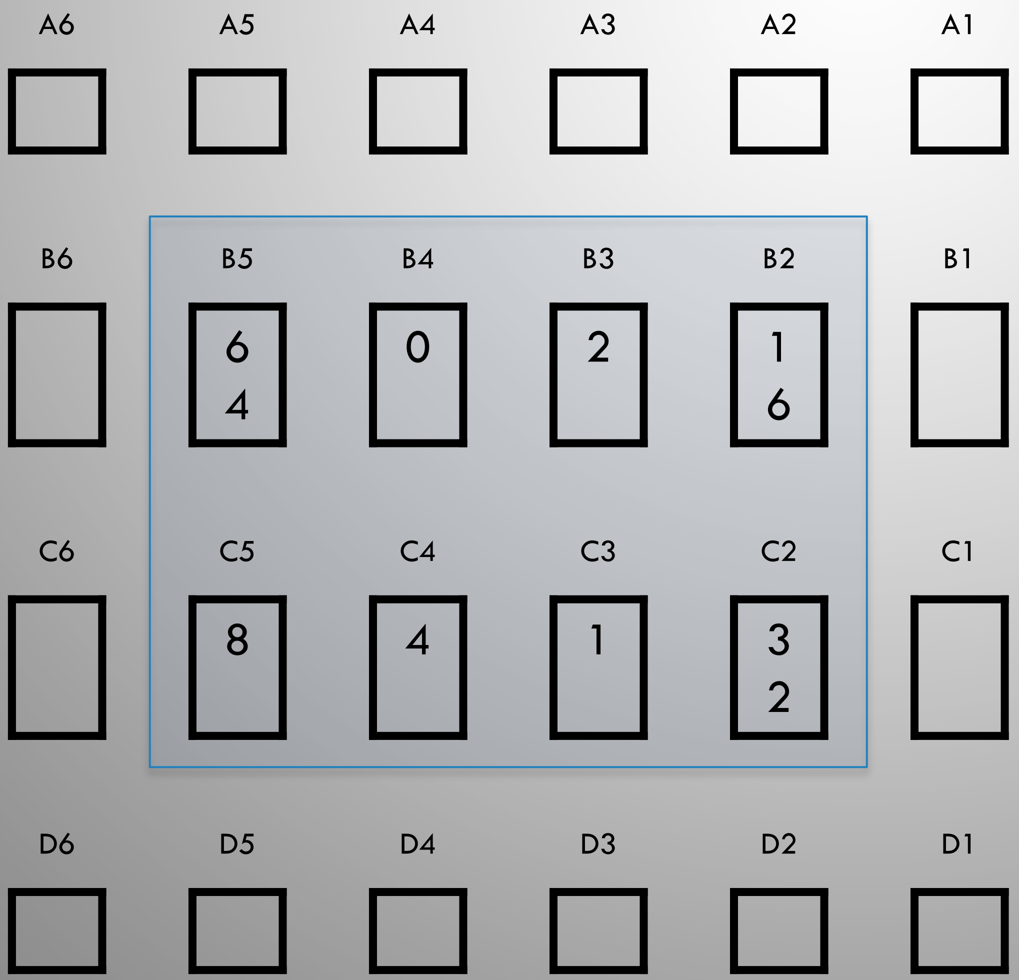
- LARGETOOTH SAWFISH ON ICUN REDLIST
- *PRISTIS PRISTIS* mtDNA COI PRIMERS (145 BP)
- AQUARIA TRIALS (REEFHQ – 700,000L)
- FIELD SURVEY DALY RIVER, NT



eDNA – RARE SPECIES DETECTION

Site	Site type	*Pristis pristis* records	eDNA results (positive)
Mission Hole	Floodplain	None	✓
Wilson's Hole	Floodplain	None	✓
Milkwood Lagoon	Floodplain	None	✓
Whitestone Lagoon	Floodplain	None	✓
Daly River Crossing	River main channel	Gillnet survey	
Kilfoyle Lagoons	Floodplain	Traditional Know	
Tyumalagun	Floodplain	Traditional Know	✓
Wunullen (north)	Floodplain	Gillnet survey	✓
Wunullen (south)	Floodplain	Gillnet survey	✓

eDNA – HOW SENSITIVE IS IT REALLY?



PHASE 1- ACCUMULATION

- RAN FOR 10 DAYS-8 PONDS (0.4 ML EACH)
- ALTERNATING DAYS
  - SAMPLE & FILTER
  - EXTRACT

PHASE 2-"FLOW" EXPERIMENT

- RAN FOR 4 DAYS-4 PONDS
- LET WATER RUN THROUGH THE SYSTEM TO CREATE A "RIVER" EFFECT
- IT ALSO HAPPENED TO RAIN EVERY DAY OF THE FLOW EXPERIMENT!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eDNA – HOW SENSITIVE

ACCUMULATION & DETECTION

- EARLY DETECTION CAN BE VARIABLE
- IN HIGH DENSITY PONDS DETECTION AT 48HRS
- COULD DETECT 1 FISH IN 0.4 ML AFTER 4 DAYS
- EDNA IS VERY POWERFUL TO DETECT INVASION FRONTS

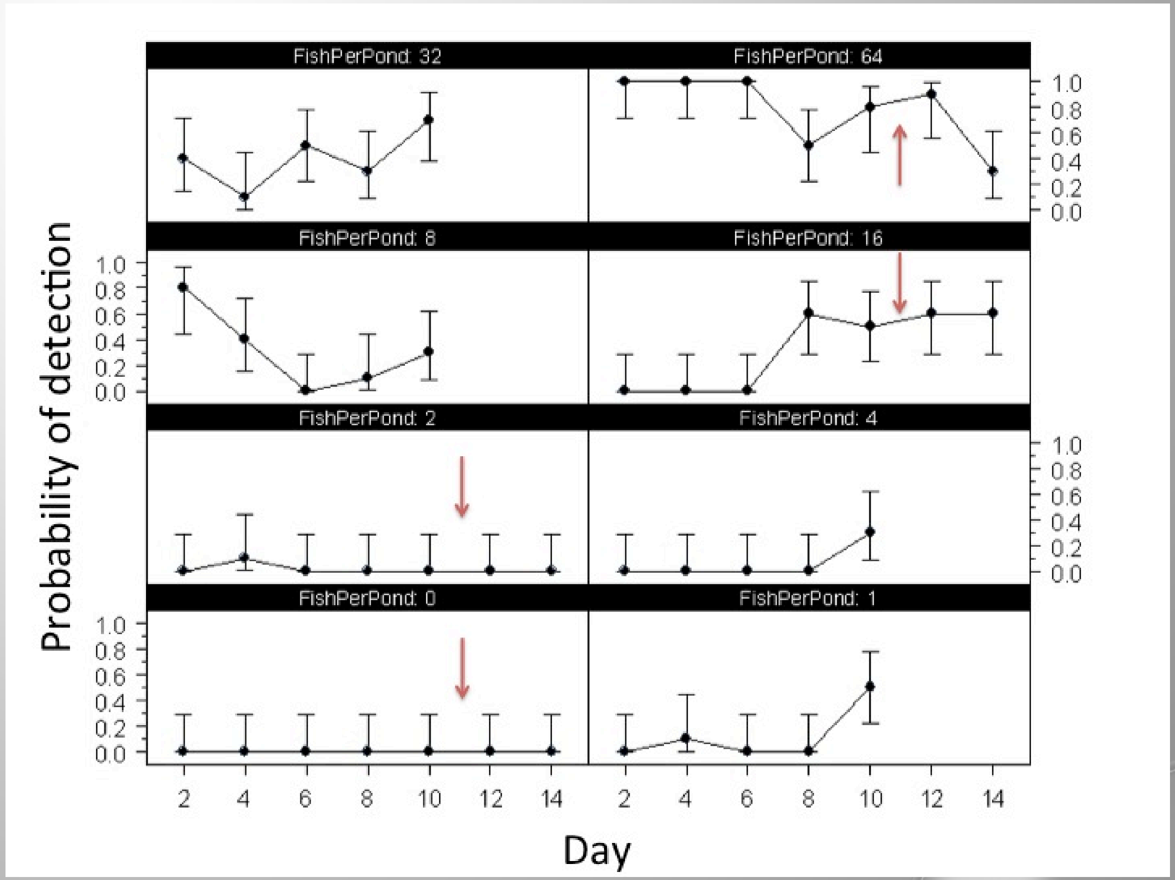


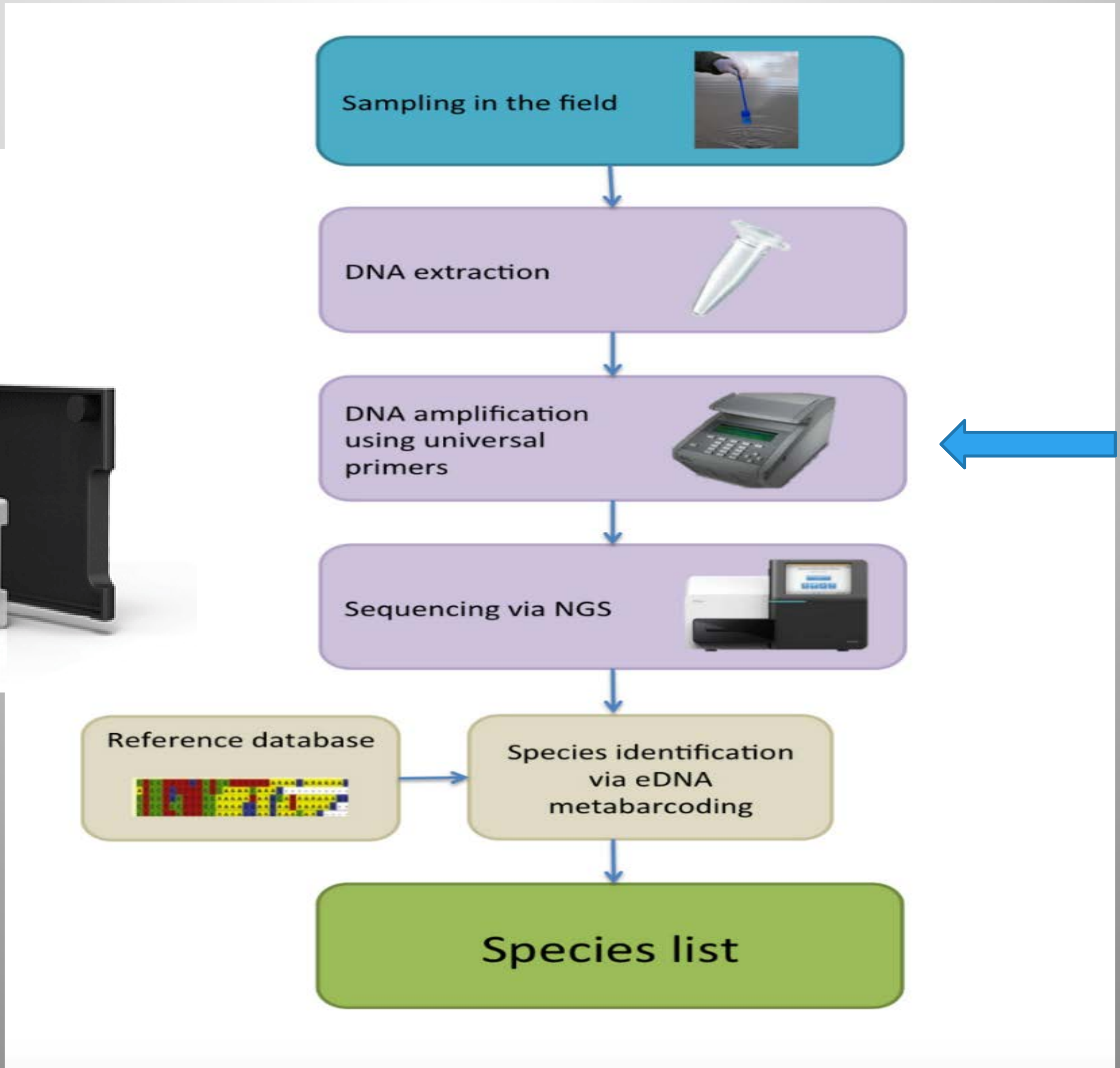
Figure 3. The relationship between tilapia density and time (post fish introduction) on the probability of detecting eDNA in water samples (+/- 95% confidence limits for each treatment pond). The red arrows indicate the time “water flow” commenced in four of the treatment ponds.

FROM SINGLE SPECIES TO WHOLE COMMUNITIES

to

DNA METABARCODING – HOW IT WORKS

Illumina MiSeq – 25 million 300bp reads

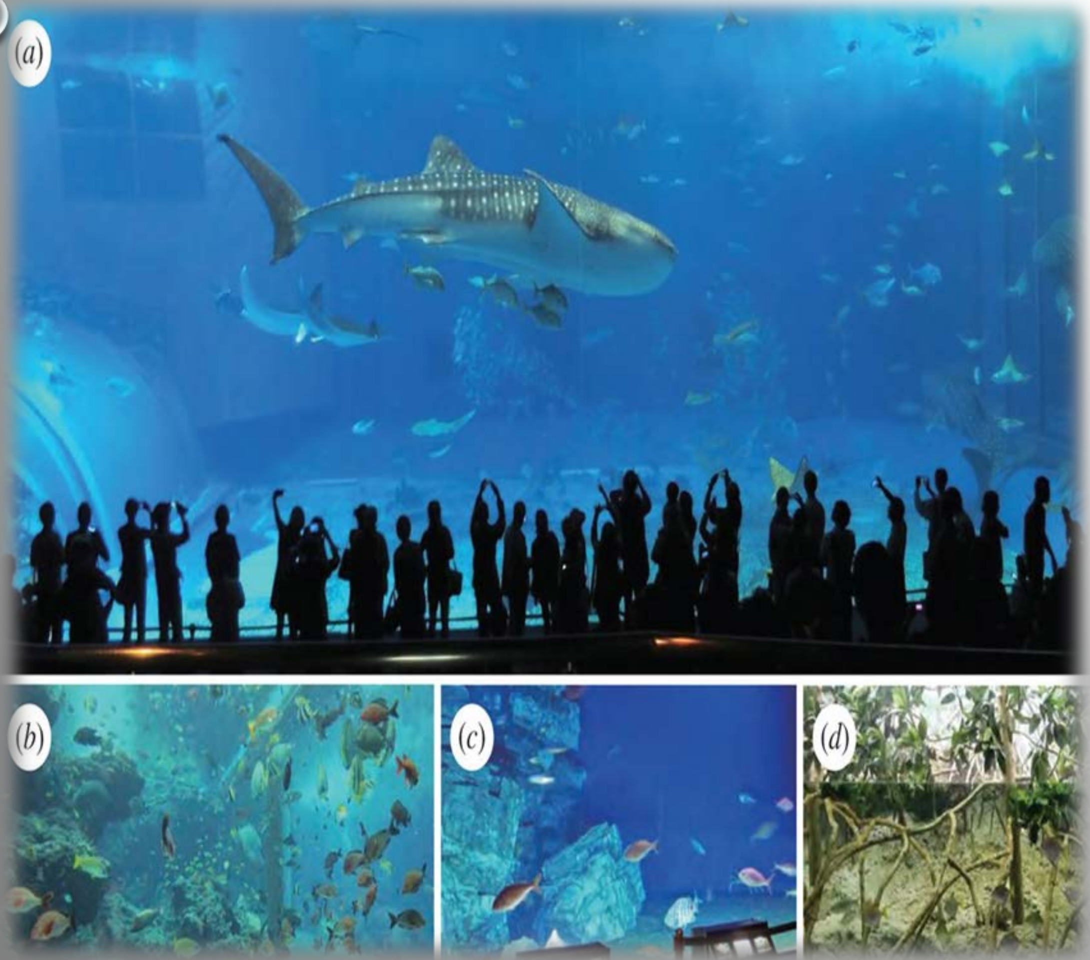


12S rRNA primers

METABARCODING – WHAT IT OFFERS

- RAPID COMMUNITY SURVEYING
- COMMUNITY MONITORING (CHANGES BEFORE/AFTER)
- PEST MONITORING
- DIETARY ANALYSES/GUT CONTENTS
- SOIL MESCO/MICROCOSMS
- LOGISTICALLY DIFFICULT LOCATIONS
- WATERBODY ACCESS/USAGE
- COMMUNITY SCIENCE

METABARCODING – CASE STUDY



(*a–d*) Four tanks used for water sampling in the Okinawa Churaumi Aquarium

(a) Kuroshio (water volume =7,500,000 L)
(b) tropical fish (700,000 L)
(c) deep-sea (230,000 L)
(d) mangrove (35,600 L)

M. Miya et al. R. Soc. open sci. 2015;2:150088

METABARCODING – CASE STUDY

Tank	Kuroshio	Tropical fish	Deep-sea	Mangrove	Total
# tank species	75	159	15	8	249
# species with reference sequences	63	105	13	8	180
# tank species detected	61 (96.8%)	95 (90.5%)	13 (100%)	13 (100%)	168 (93.3%)

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eDNA AND SPECIES ABUNDANCE

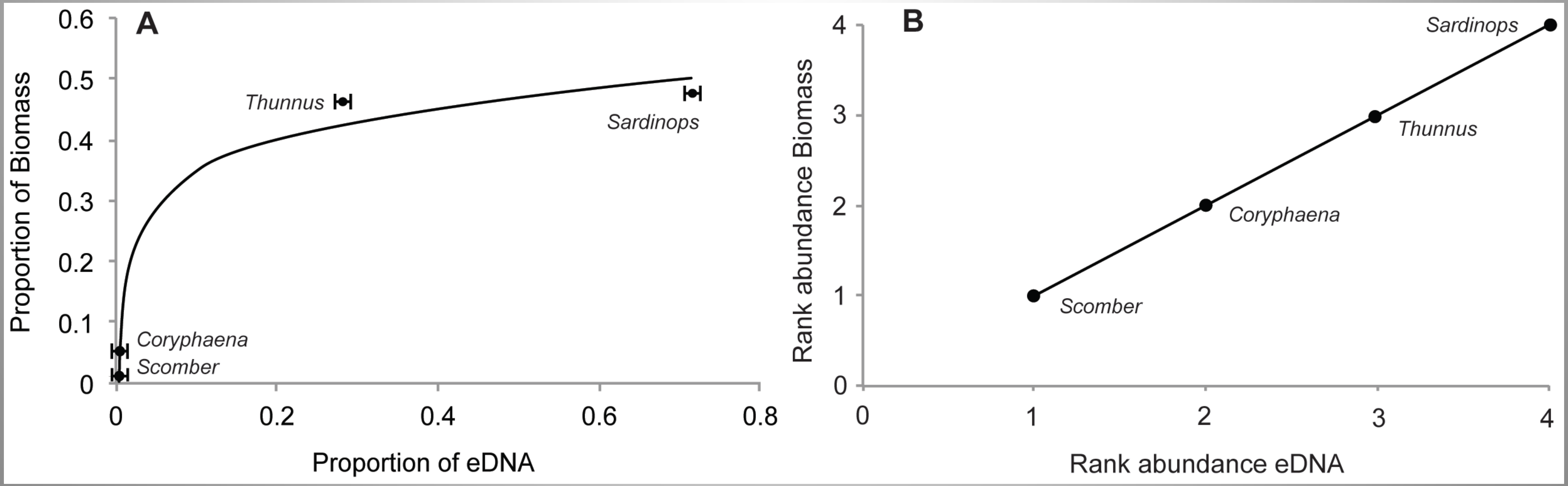
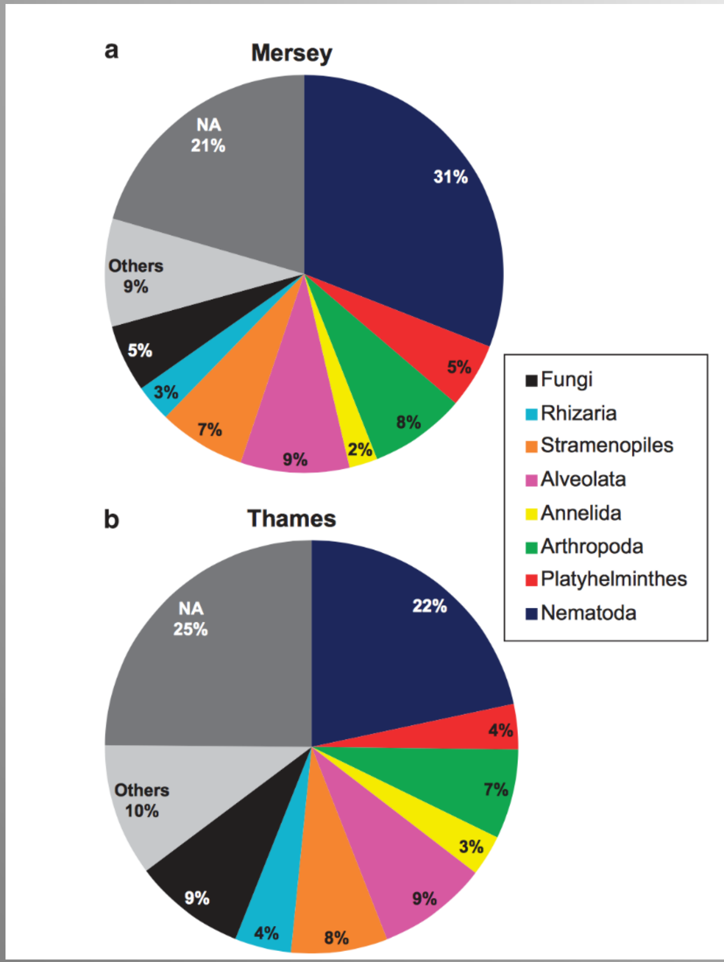
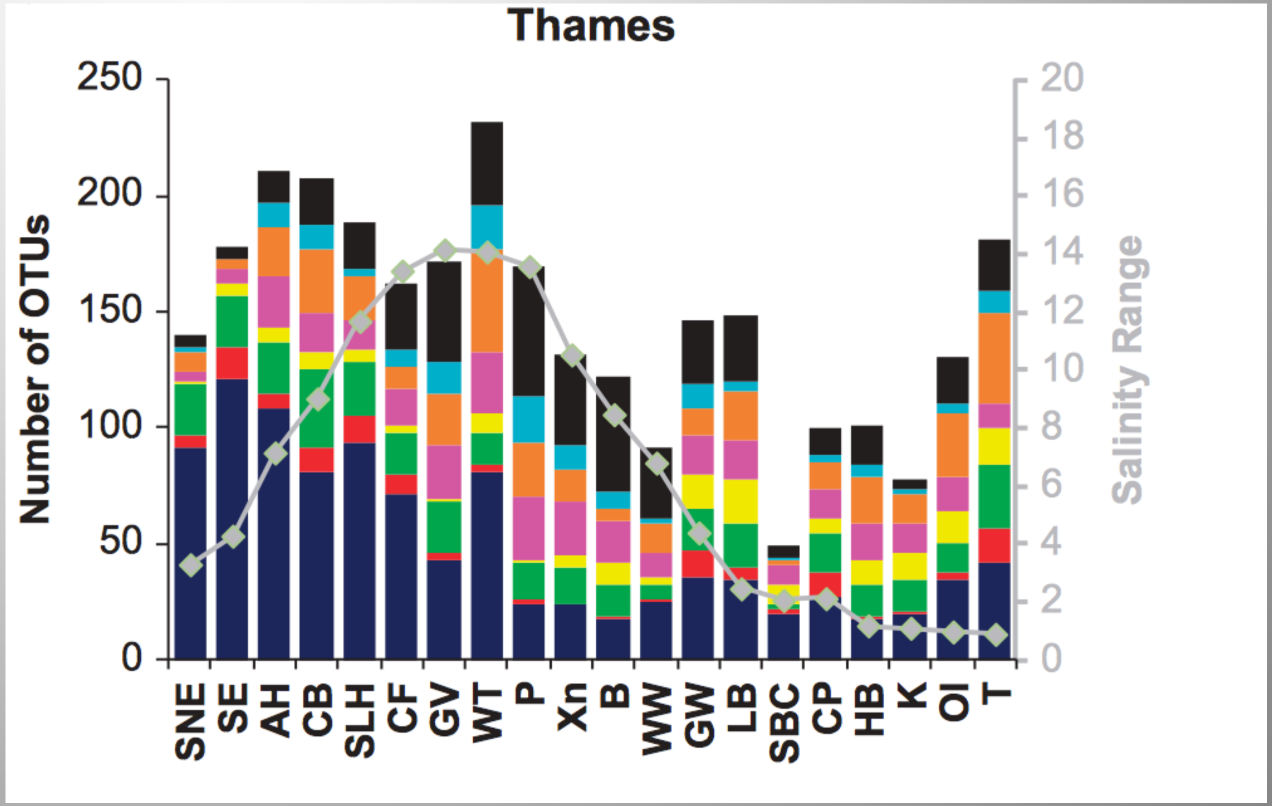


Figure 3. Comparison of the proportion of eDNA sequences recovered to estimated species biomass in the 1-L tank sample. (A) Relationship between the proportion of eDNA sequences and proportion of biomass in the tank (Best fit line = $y = 0.0759*\ln(x)+0.5257$) and (B) the rank abundances of these proportions for the four tank exhibit genera detected. The error bars represent the standard deviation of the three individual PCR replicates for the 1-L tank sample. Kelly et al 2014 doi:10.1371/journal.pone.0086175.g003

eDNA – CHARACTERISING COMMUNITIES AND CHANGE





eDNA METABARCODING VS THE CLASSICS

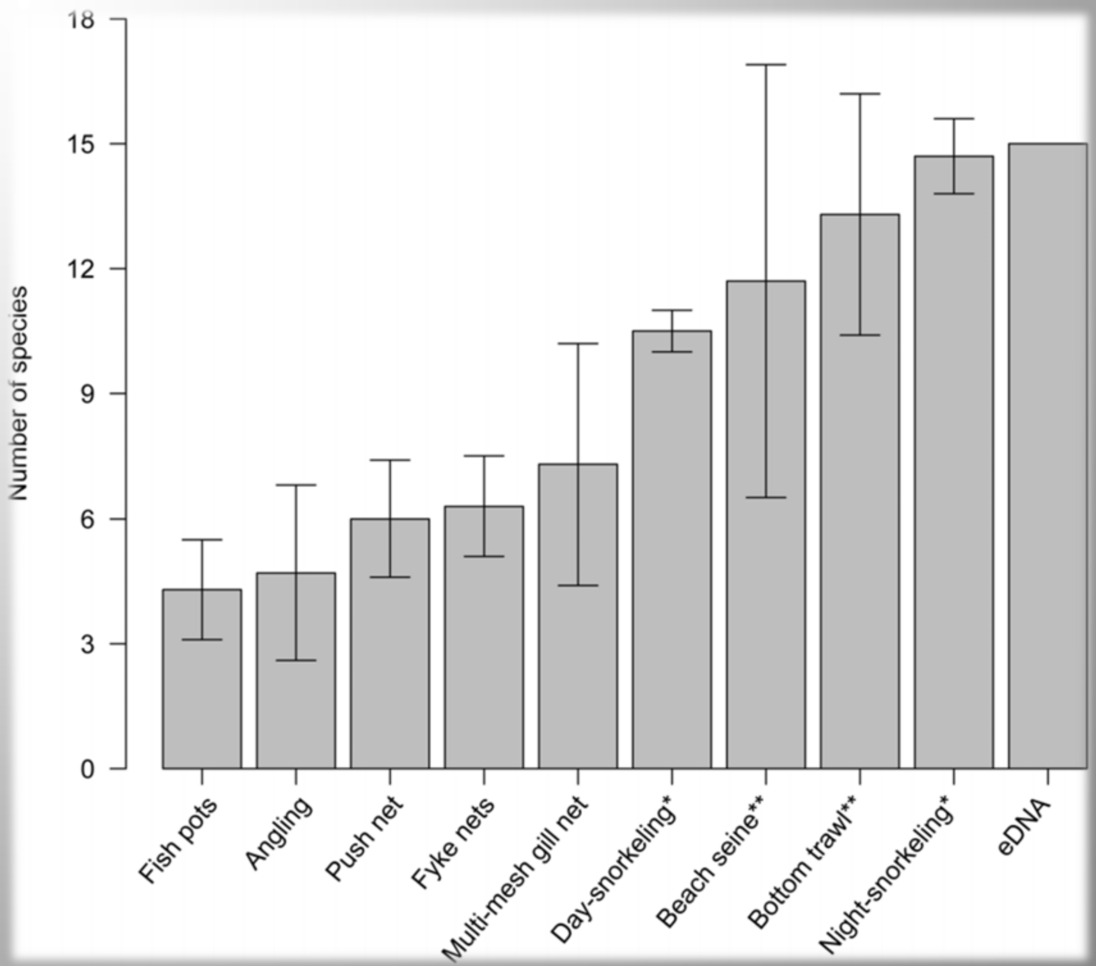


Figure 2. Number of fish species recorded by 9 different conventional survey methods and eDNA at The Sound of Elsinore, Denmark. Bars show mean number of fish species caught across surveys in 2009, 2010 and 2011 and error bars represent the standard deviation (Thomsen et al 2014 Plos1 doi:10.1371/journal.pone.0041732.g002)

HOW CAN eDNA MEASURE ENVIRONMENTAL DRIVERS ETC

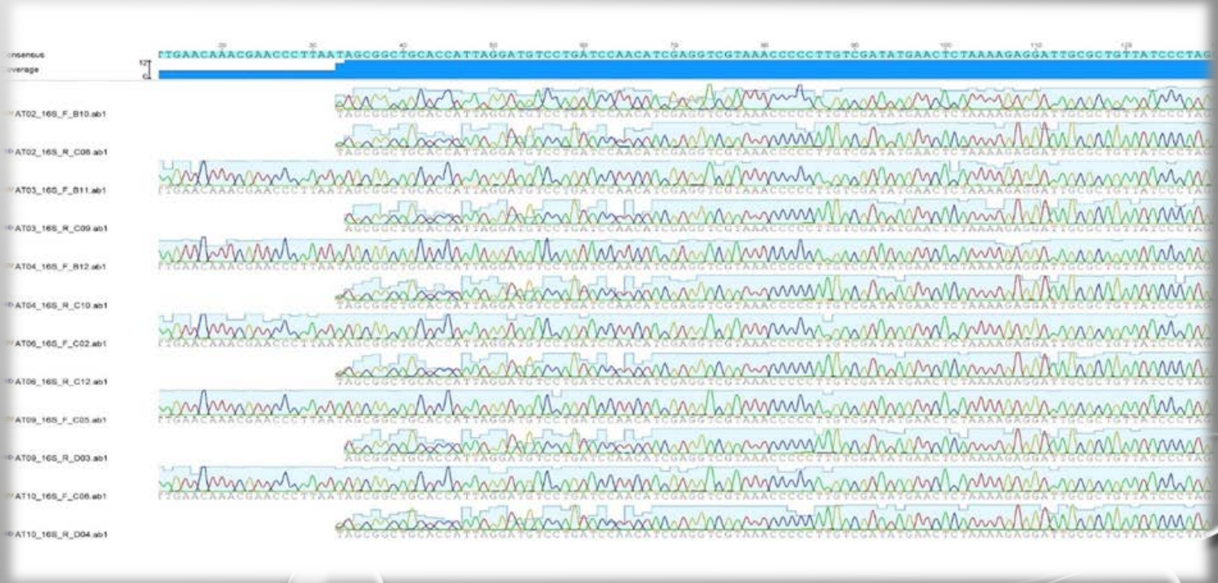
- METABARCODING - DETERMINE WHOLE-OF-COMMUNITY BIODIVERSITY CHANGES
  - CLIMATE PERTURBATIONS
  - MINING
  - LAND CHANGES
  - PESTS/DISEASES
- SPECIES-SPECIFIC DNA
  - MINUTELY EXAMINE HABITAT USE
  - PRESENCE/ABSENCE BEFORE/AFTER DISTURBANCES
  - MOVEMENT PATTERNS (NATIVES/INTRODUCED)

WHAT ARE THE CONSTRAINTS?

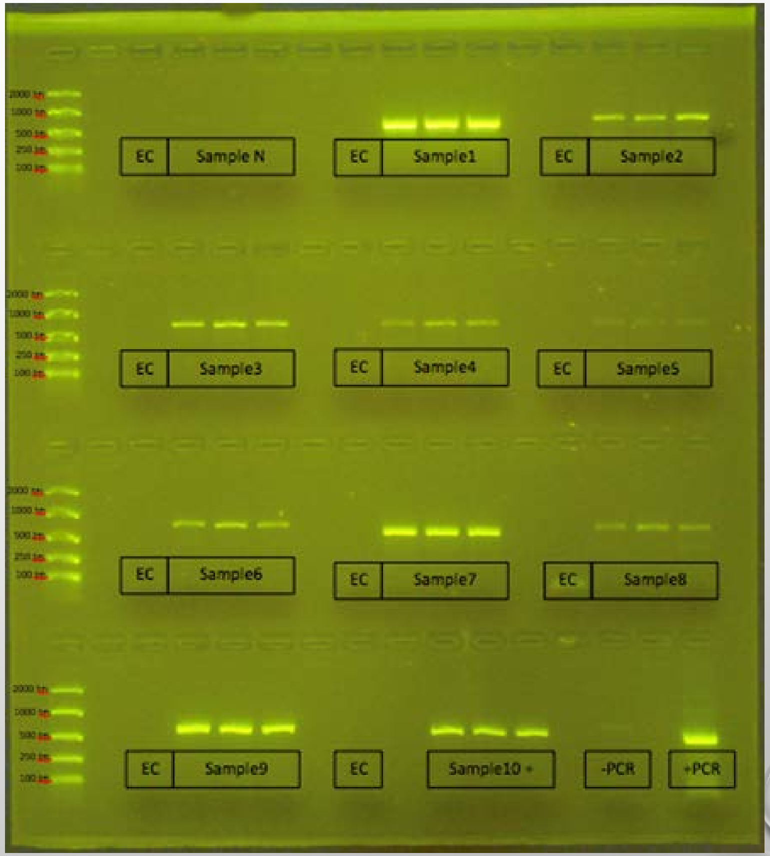
- LIMITED DNA PROBES FOR AUSTRALIAN TROPICAL SPECIES

FISH → REPTILES → INVERTEBRATES

- METABARCODING CONSTRAINED BY LACK OF SEQUENCE
- FILTERING AND PRESERVATION IN REMOTE REGIONS



THANK YOU AND QUESTIONS



METABARCODING – OUR EXPERIENCES – ALICE RIVER 16S

Organism	Common name	% Pairwise identity	% Coverage	Fragment length	E-value
Gambusia affinis	Mosquitofish	99.1%	100.0%	225	1.65E-109
Hypseleotris compressa	Empire Gudgeon	100.0%	100.0%	218	5.93E-109
Melanotaenia parkinsoni	Rainbowfish	99.1%	100.0%	213	2.78E-102
Oreochromis sp.	Tilapia	100.0%	100.0%	232	9.79E-117
Homo sapiens	Human	99.6%	100.0%	242	1.26E-120
Canis lupus	Dingo	99.6%	100.0%	239	5.85E-119
Litoria aurea	Green and golden bell frog	97.5%	100.0%	200	1.31E-90
Chaetonotus schultzei	Lophotrochozoa	99.5%	94.2%	212	5.97E-104
Dero furcata	Annelid	98.5%	91.0%	204	1.30E-95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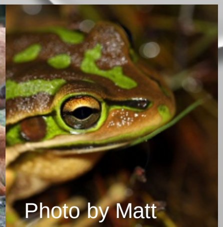
Photo by Matt

METABARCODING – OUR EXPERIENCES WITH AN ARTIFICIAL SAMPLE

Scientific Name	16S PCR band	16S Sequence	16S ID DF 10^2	COI PCR band	COI Sequence	COI ID	12S PCR band	12S Sequence	12S ID
Ambassis agassizii	Y	N	Genus	Y	Y	Species	Y	Y	NF
Amblygaster sirm	Y	Y	Species	Y	Y	Species	Y	N	NF
Amniataba percoides	Y	N	NF	Y	Y	NF	Y	Y	NF
Anabas testudineus	Y	Y	Species	Y	Y	Species	Y	Y	Species
Bagrus bayad	Y	N	NF	Y	Y	Species	Y	N	Family
Bagrus docmak	Y	N	NF	Y	Y	Species	Y	N	Family
Craterocephalus stercusmuscarum	Y	N	NF	Y	Y	Genus	Y	Y	NF
Epinephelus taurina	Y	N	Genus	Y	N	Genus	Y	N	Genus
Giuris margaritacea	Y	N	NF	Y	Y	Species	Y	Y	NF
Guyu wujalwujalensis	Y	N	NF	Y	N	NF	Y	N	NF
Hephaestus fuliginosus	Y	N	NF	Y	N	NF	Y	N	NF
Herklotsichthys lippa	Y	N	Genus	N	N	NF	Y	N	NF
Hypseleotris compressa	Y	Y	Species	Y	Y	Species	Y	Y	Species
Hypseleotris galii	Y	N	NF	Y	Y	NF	Y	Y	Species
Kuhlia rupestris	Y	Y	Species	N	Y	Genus	Y	Y	Species
Lates calcarifer	Y	Y	Species	Y	Y	Species	Y	Y	Species
Lutjanus johnii	Y	Y	Species	Y	Y	Species	Y	Y	Species
Maccullochella peelii	Y	Y	Species	Y	Y	Species	Y	Y	Species
Nematalosa erebi	Y	Y	Genus	Y	Y	Species	Y	N	Genus
Neosilurus ater	Y	N	NF	Y	N	NF	Y	N	NF
Oreochromis mossambicus	Y	Y	Genus	Y	Y	Species	Y	Y	Species
Philypnodon grandiceps	Y	Y	Species	Y	Y	Species	Y	Y	Species
Philypnodon macrostomus	Y	N	NF	Y	Y	Species	Y	N	Genus
Porochilus obbesi	Y	N	NF	Y	N	NF	Y	N	NF
Protonibea diacanthus	Y	Y	Species	Y	Y	Species	Y	Y	Species
Rastrelliger kanagurta	Y	Y	Species	Y	Y	Species	Y	Y	Species
Sardinella albella	Y	Y	Species	N	Y	NF	Y	Y	Species
Sardinella gibbosa	Y	Y	Genus	Y	Y	Species	Y	N	Genus
Scomberomorus commerson	Y	Y	Genus	Y	Y	Species	Y	Y	Species
Scomberomorus munroi	Y	Y	Species	Y	Y	Species	Y	Y	Species
Scomberomorus semifasciatus	Y	Y	Genus	Y	Y	Genus	Y	Y	Genus
Selar boops	Y	N	Genus	Y	Y	Species	Y	N	Genus
Stolephorus indicus	Y	N	NF	N	Y	NF	Y	N	NF
Tandanus tandanus	Y	N	NF	N	Y	Species	Y	Y	NF
Toxotes chatareus	Y	Y	Species	Y	Y	Genus	Y	Y	Species

HOW FAST DOES eDNA DEGRADE IN THE TROPICS

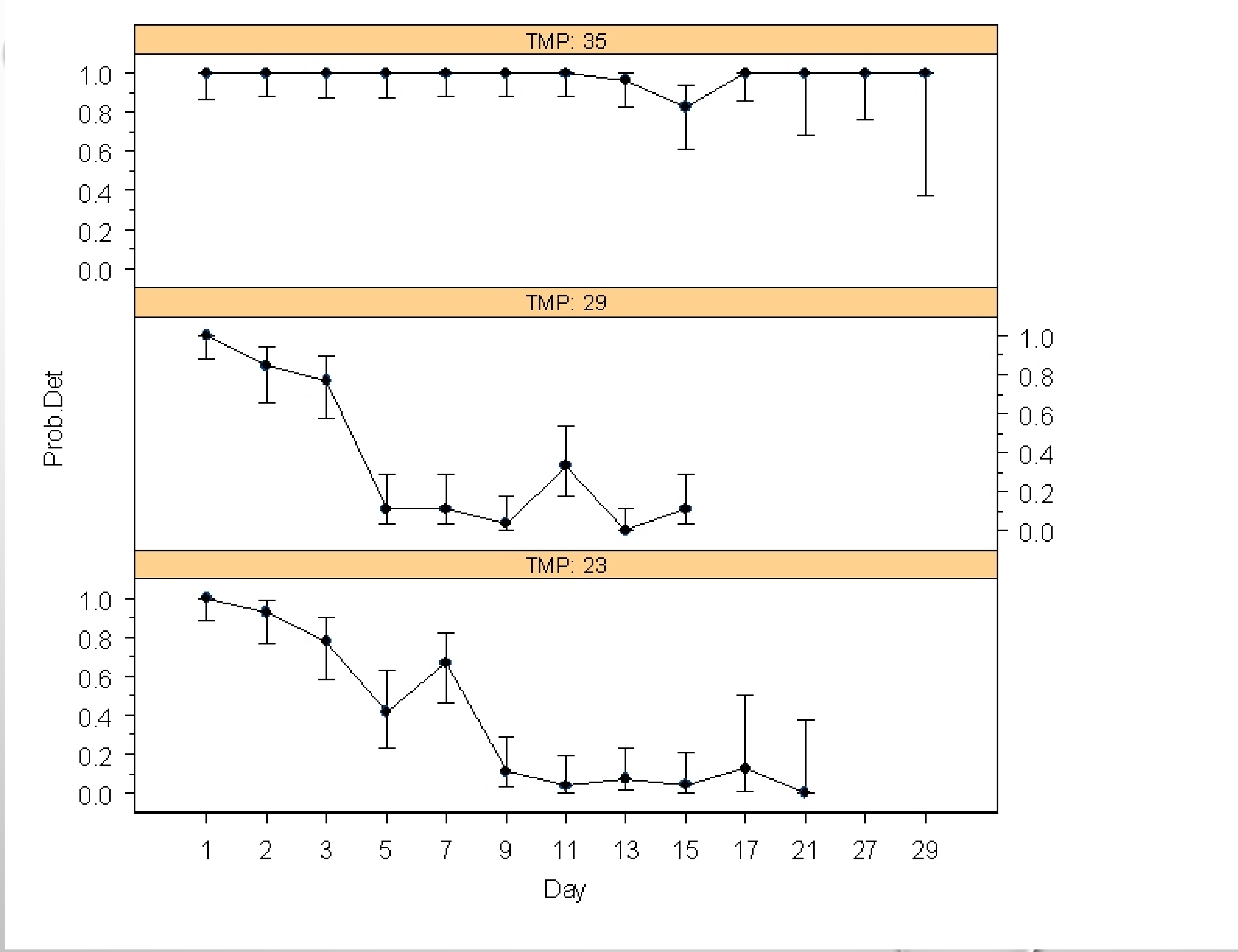

Figure 2. Probability of eDNA detection (+/- 95% confidence limits) at three temperatures (23, 29 and 35° C) showing the degradation of eDNA following the removal of tilapia.