

Environmental (e)DNA-Based Wildlife Monitoring & Human Effluent Quantification Water, Earth & Air

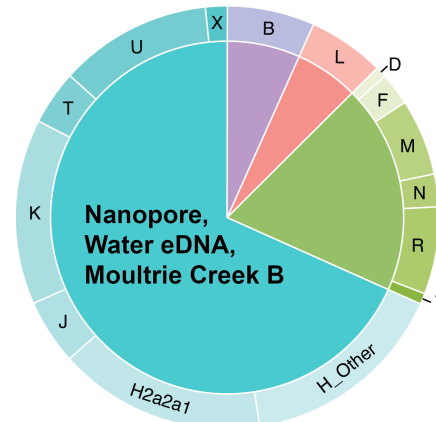


THE WHITNEY LABORATORY
for MARINE BIOSCIENCE



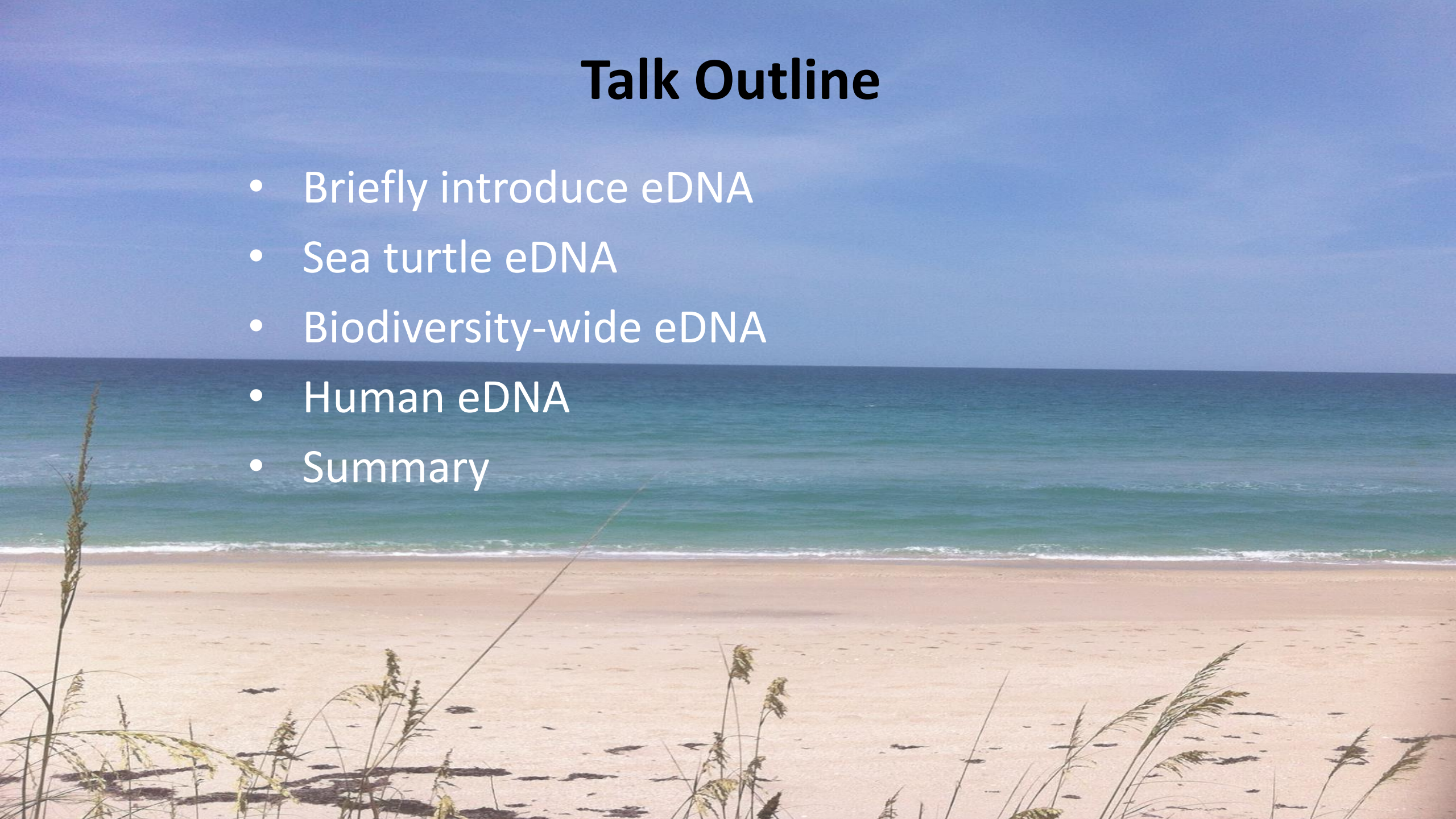
The Sea Turtle Hospital
at WHITNEY LABORATORY

Duffy Lab, University of Florida



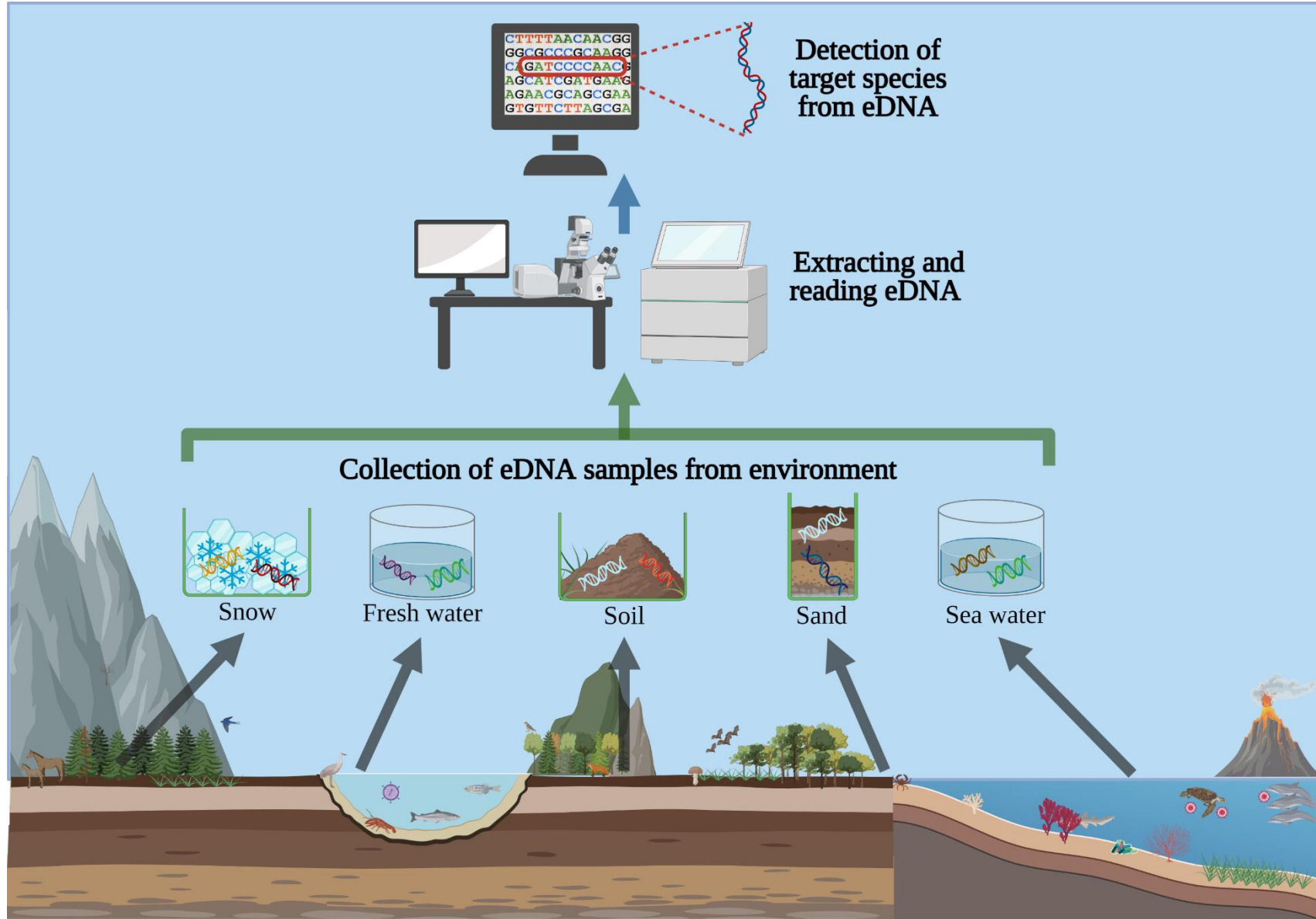
Talk Outline

- Briefly introduce eDNA
- Sea turtle eDNA
- Biodiversity-wide eDNA
- Human eDNA
- Summary



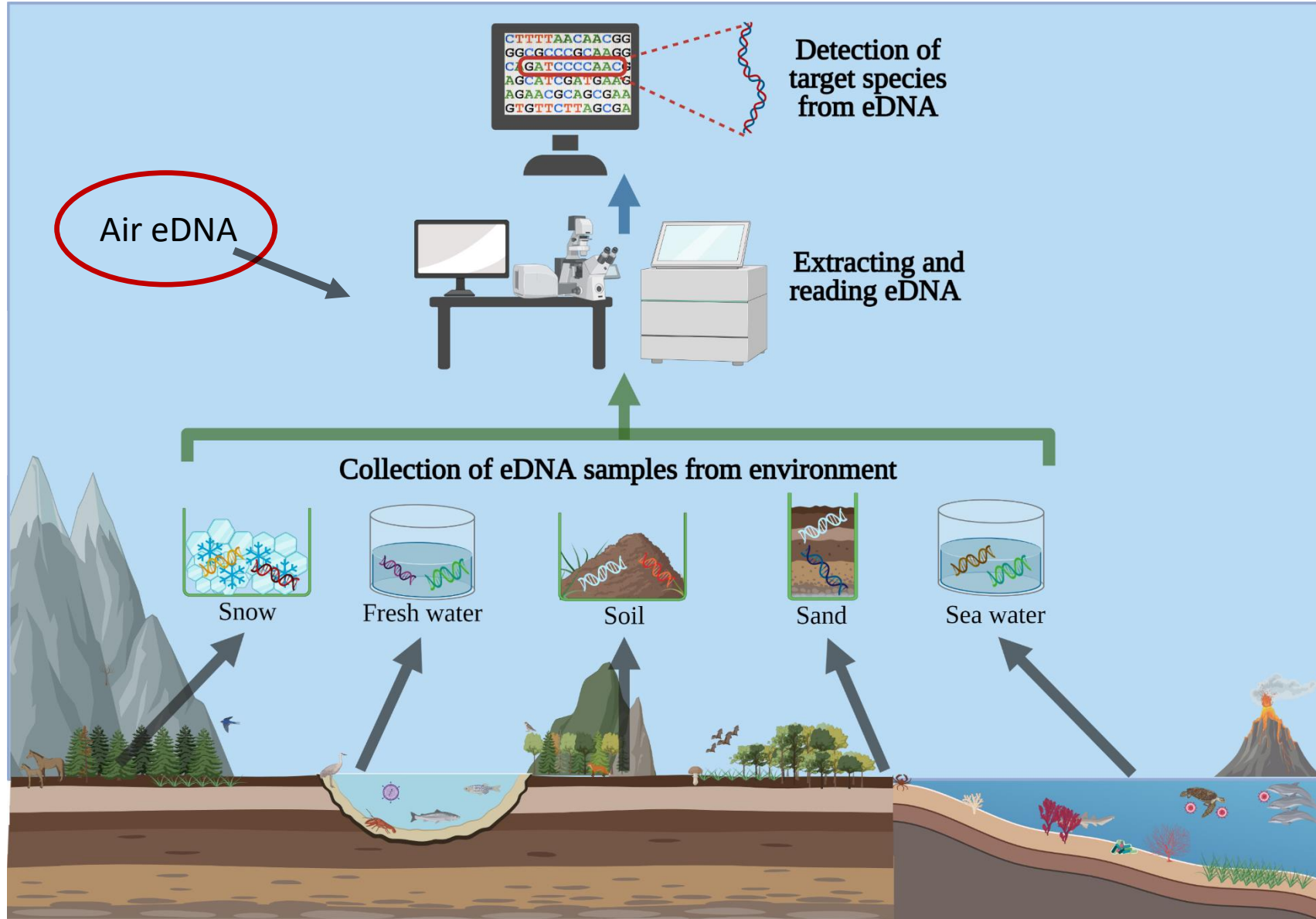
Environmental DNA (eDNA) – wildlife forensics

Organisms continually shed DNA into the environment (skin, hair, bodily fluids etc.)



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eDNA – a validated tool. Now being rolled out by federal & state environmental and health management agencies



- Our eDNA work:
- Specific wildlife sp.
 - Pathogens
 - Pan-biodiversity
 - Humans

ANIMALS NATIONAL GEOGRAPHIC

Hidden DNA is revealing secrets of animals' lives

New studies of genetic material, or eDNA, shed by plants and animals are providing astonishing biological insights.



How to protect yourself from fake payday loans
Scammers steal data and can easily imitate real lenders, Better Business Bureau says. In Money

Don't miss catching these 10 movies arriving in fall
From scary-movie fare to Oscar-season competitors, a list of must-see films. In Life

Stunning performance by Tiafoe in US Open
Frances Tiafoe, left, defeats No. 2 Rafael Nadal to advance to the quarterfinals of a major for just the second time in his career. In Sports

USA TODAY THE NATION'S NEWS | \$3 | TUESDAY, SEPTEMBER 6, 2022

EMPLOYMENT OUTLOOK
Hiring defies danger signs
Job market is solid even as economy continues to wobble

Paul Davidson USA TODAY
Economic data has been stable flatter this year, but that isn't stopping the Fed. Despite the usual marketing message from adding seven workers in coming months.
Last year, amid the labor shortage, the 26-employer firm struggled to attract job candidates as it battled larger competitors offering higher pay. "I'm preparing for the holidays," says CEO Terry Rana, citing "the hardship that we've faced in not finding enough people."
Some, with exceptions from raising off staffers, and Rana aims to scoop them up. "We're looking to take advantage of that," he says.
Millions of businesses are taking a similar approach, huffing the labor market's daily expectations of a sharp

THE FIGHT AGAINST CLIMATE CHANGE
Science's new weapon: Environmental DNA
Technology tracks biodiversity – like 'CSI' for the planet

Lucas Myers, part of the Mickler's Landing Turtle Patrol in Ponte Vedra Beach, Fla., collects sand samples from the track of a loggerhead sea turtle on June 16. The sample will be used to explore the DNA of tumors in sea turtles and help advance wildlife science and conservation – as well as monitor planetary health. In Environment

David Duffy and Catherine Eastman, of the University of Florida's Whitney Laboratory for Marine Biotechnology, sample water from Parker Creek.

ST. AUGUSTINE, Fla. – Even before sunrise, the tracks of a loggerhead sea turtle on the beach are hard to miss.
Searching for new nests one morning this summer, Mickler's Landing Turtle Patrol volunteer Lucas Myers looked from an all-terrain vehicle where he spotted a new scalloped trail in the sand.
"Puke crawl," Myers called out. For an unknown reason, the turtle returned to the sea without digging a nest for her eggs. That meant Myers didn't have to mark a protected area around the nest.

MAR-A-LAGO SEARCH
Special master to review records
A federal judge in Florida approved Donald Trump's request Monday for a special master to review documents seized from his Mar-a-Lago estate, paving the Justice Department's criminal probe of the records. 4A

USA TODAY SHOTS 0
With America, it's complicated
U.S. which says the U.S. is a good country with a proud history
Is a bad country with a painful history
62% Has good respect, considered history
23% Not sure
7% Not sure
SOURCE: Pew Research Center, Aug. 12-15, 2022. SEE USA TODAY

DAILY DISCOUNTS & SAVINGS...
Diving Deals USA
PAGE 24

Liz Truss era begins in Britain
Kim Hjeltnesgaard USA TODAY

LONDON – Liz Truss will become Britain's third female prime minister after Monday's announcement by the ruling Conservative Party that she would replace Boris Johnson.

Truss, 47, has portrayed herself as the political heir to Margaret Thatcher, Britain's formidable cheerleader for free markets and low taxes, a strong supporter of the Anglo-American alliance and a dynamo who played a key role in ending the Cold War. Johnson, an architect of Britain's exit from the European Union (known as Brexit), held the role for just over three years before resigning amid scandals over breaking his own coronavirus lockdown rules. Truss was foreign secretary in Johnson's government.

The new prime minister's first task may be to reassure the British public that she has a realistic plan to deal with spiraling energy costs that could reach a critical point this winter.

The latest
Truss is Britain's third prime minister in a little over three years and also its third female leader, after Thatcher (1979-1990) and Theresa May (2016-2019).
Truss beat Rishi Sunak, who served as Johnson's government's finance minister, after a weeks-long internal Conservative Party selection contest. In Britain, voters elect a party, not a specific leader, giving the government in power little to swap in a new prime minister. Truss won 57% of votes cast by about 17,000 eligible voters.
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See TRUSS, Page 6A

<https://www.usatoday.com/story/news/2022/08/26/staggering-dna-advances-could-improve-response-climate-change/10000538002/>
<https://www.nationalgeographic.com/animals/article/environmental-dna-sheds-light-on-animal-lives-edna>
<https://theconversation.com/environmental-dna-how-a-tool-used-to-detect-endangered-wildlife-ended-up-helping-fight-the-covid-19-pandemic-158286>

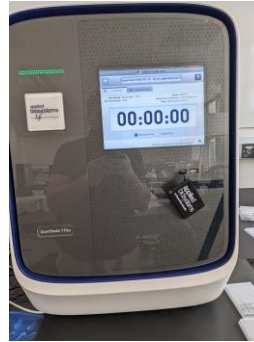
eDNA – coming of age



- Consistently outperforming conventional monitoring approaches for:
 - Native species detection
 - Invasive species monitoring
 - Pathogen monitoring
 - Biodiversity assessments
- Large federally backed initiatives for the adoption of eDNA by managers & conservationists
 - Including eDNA-specific funding to USGS & other agencies in the Bi-partisan Infrastructure Bill (2022)
 - **Autonomous samplers & remote sensing**
- Health assessments and other metrics still require more traditional survey approaches

Targeted versus untargeted (shotgun) eDNA

- Targeted (qPCR, ddPCR or metabarcoding sequencing)
 - Cost-effective
 - Limited information, more upfront effort required (assay/metabarcoding design etc.)
- Shotgun (sequencing)
 - More costly, but vastly more information recovered per sample
 - Simultaneous detection and population genetics/viral variant analysis
 - Can study pan-biodiversity in single sample – flora, fauna, microbes & pathogens (e.g. algal blooms, waste/pollution-associated microbes, charismatic megafauna and prey species)
- Combining Targeted & Shotgun can be highly complementary
 - Targeted for large sample numbers & shotgun for deep info. on most crucial samples



eDNA & the GTM NERR



- GTM or GTM-adjacent sites usually make up the majority of our initial test locations
 - Waterways
 - Beaches
 - Air

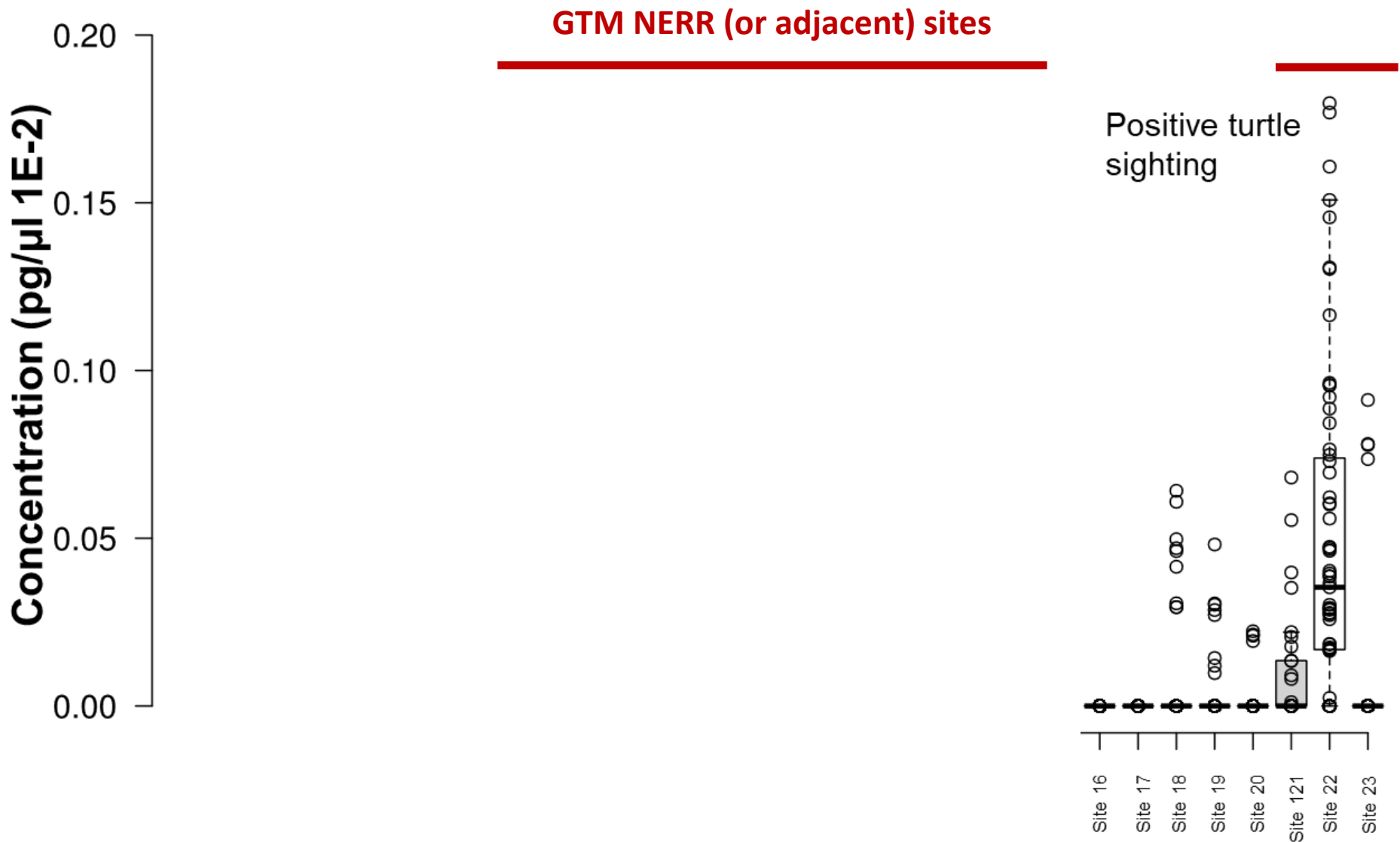
i.e. in and around the UF Whitney Lab




<https://gtmnerr.org/about/>



Green sea turtle eDNA for in-water monitoring (oceanic & lagoon), targeted qPCR



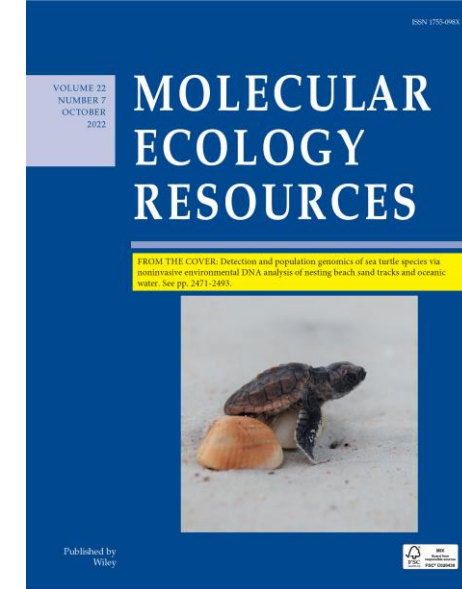
Sand eDNA - Not possible without support of nesting beach patrol volunteers!!

Detection and population genomics of sea turtle species via noninvasive environmental DNA analysis of nesting beach sand tracks and oceanic water 

Jessica A. Farrell^{1,2} | Liam Whitmore^{1,3} | Narges Mashkour¹  |
Devon R. Rollinson Ramia¹ | Rachel S. Thomas¹ | Catherine B. Eastman¹ |
Brooke Burkhalter^{1,4} | Kelsey Yetsko^{1,5} | Cody Mott⁶ | Larry Wood⁷ |
Bette Zirkelbach⁴ | Lucas Meers⁸ | Pat Kleinsasser⁹ | Sharon Stock¹⁰ |
Elizabeth Libert¹⁰ | Richard Herren¹¹ | Scott Eastman¹² | Whitney Crowder¹³ |
Caitlin Boverly¹³ | David Anderson¹³ | David Godfrey¹¹ | Nancy Condron^{1,8} |
David J. Duffy^{1,2} 

Beach permit holders & volunteers

<https://onlinelibrary.wiley.com/doi/10.1111/1755-0998.13617>

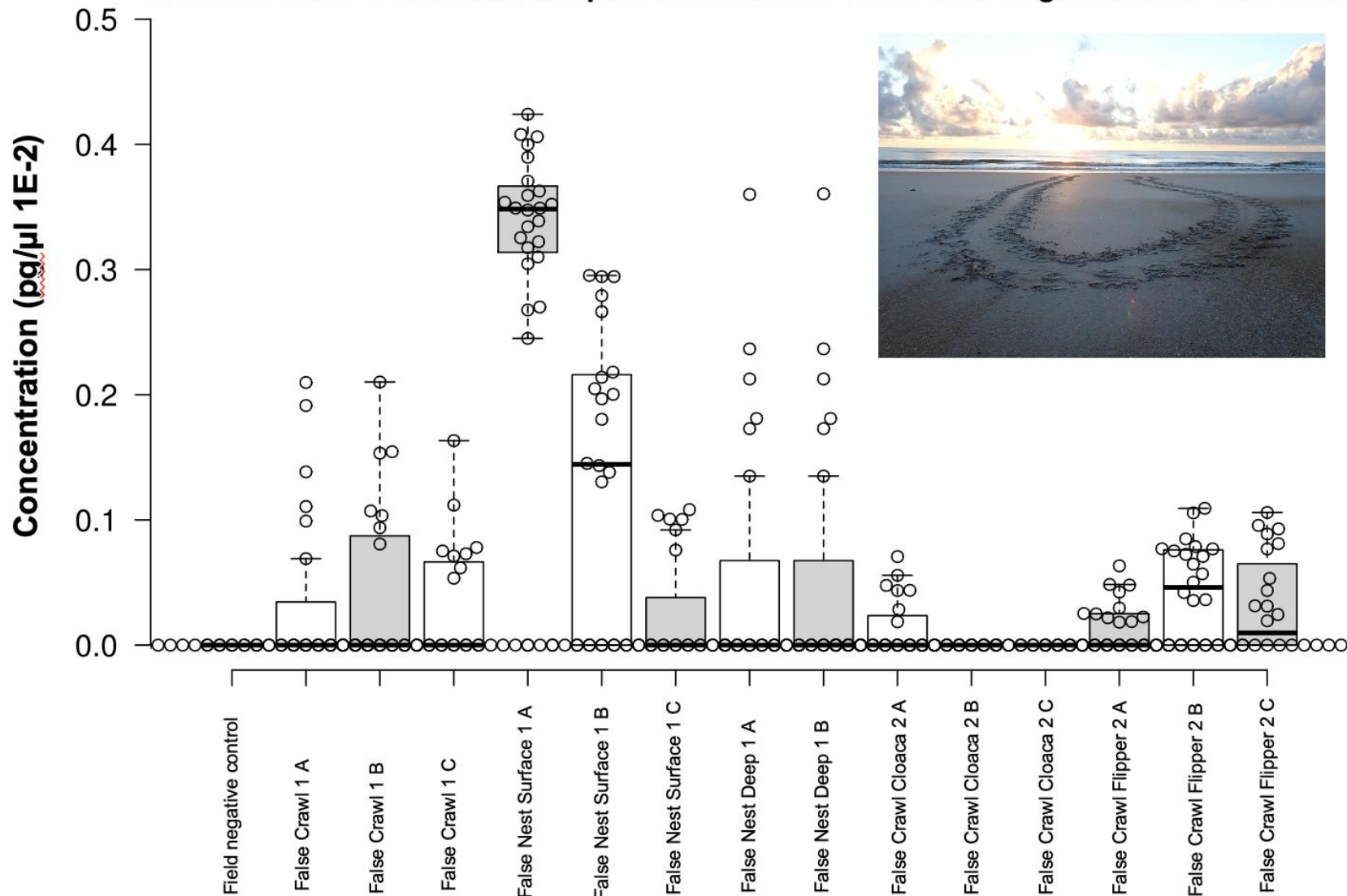


Patrols:

GTM Research Reserve
Mickler's Landing
Crescent Beach
Vilano Beach
Marineland
Summer Haven
Boca Raton Beaches

Sea turtle eDNA detectable in nesting beach sand tracks

C. caretta eDNA in wild sand samples collected over 24hrs after single maternal false crawls



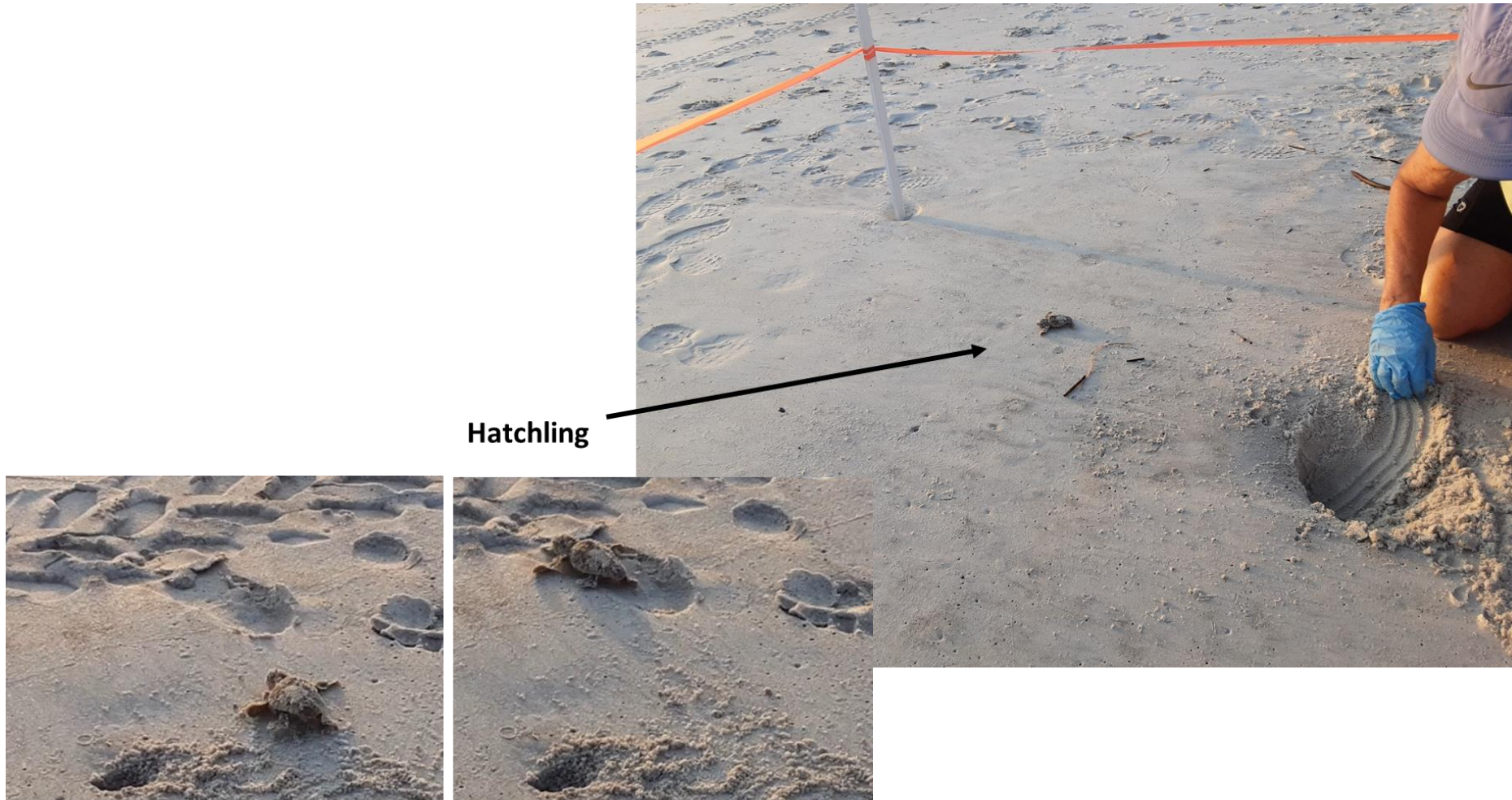
Turtle eDNA present for at least 24hr after crawl occurred!

Some of our earliest trials were on GTM NERR beaches Scott & Cat Eastman & team

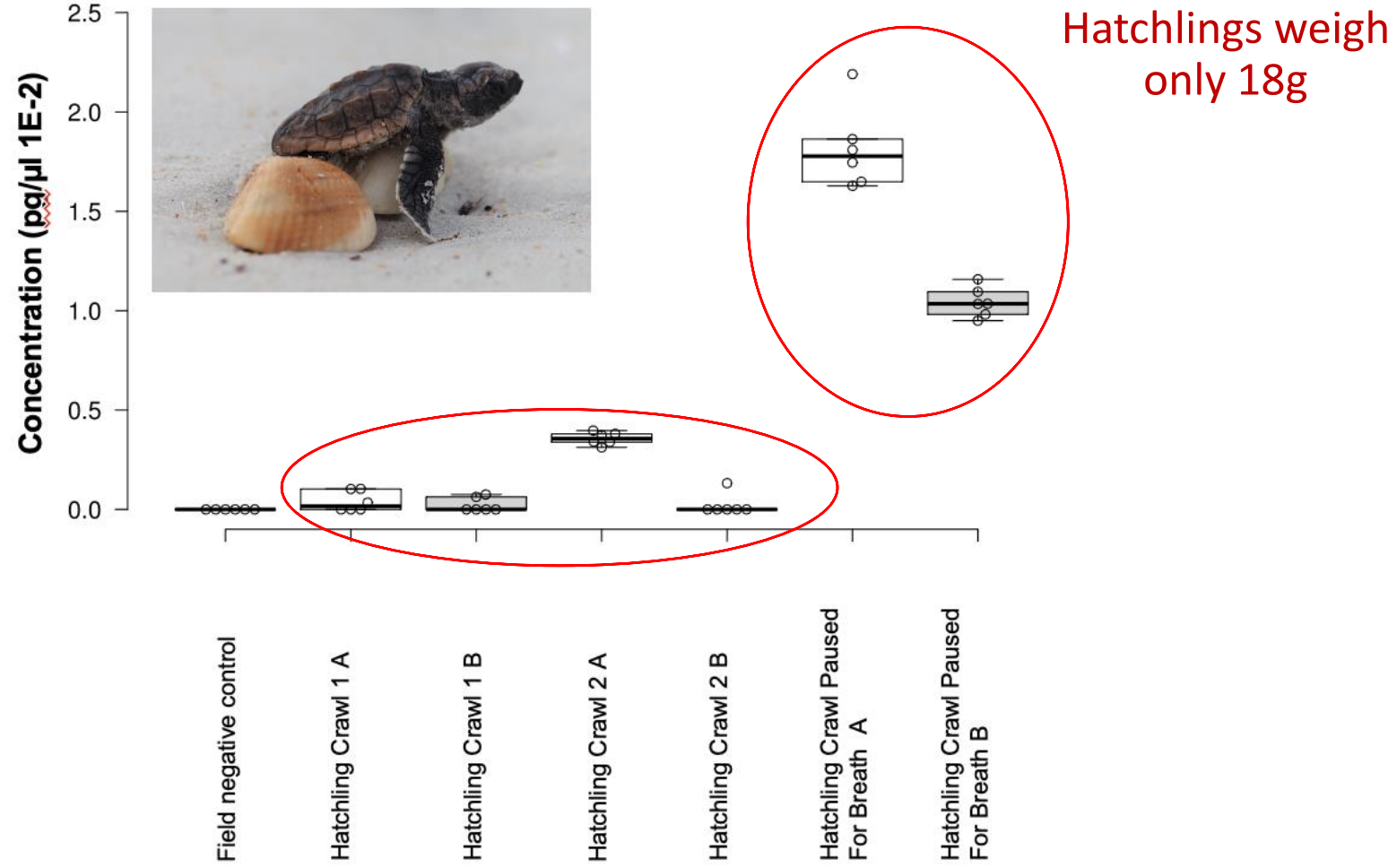
Samples now range from FL, GA, TX, PR, SM

Sand eDNA

- Can we detect eDNA left by a single hatchling (lightly) crawling over sand on its way to the ocean?



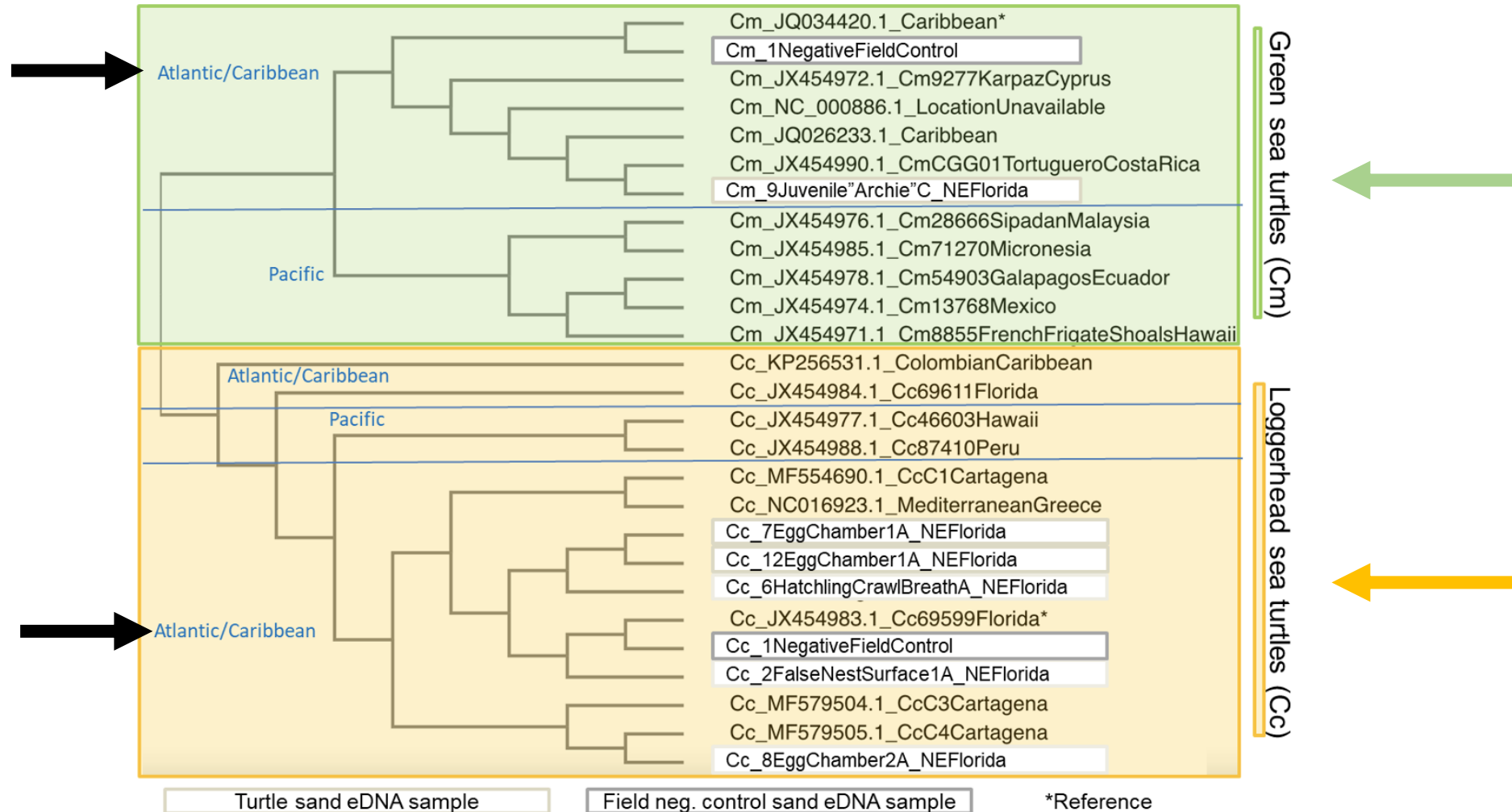
Sea turtle eDNA detectable even from a single hatchling crawl



eDNA not just for quantification!

Sea turtle sand eDNA can non-invasively determine geographic origin

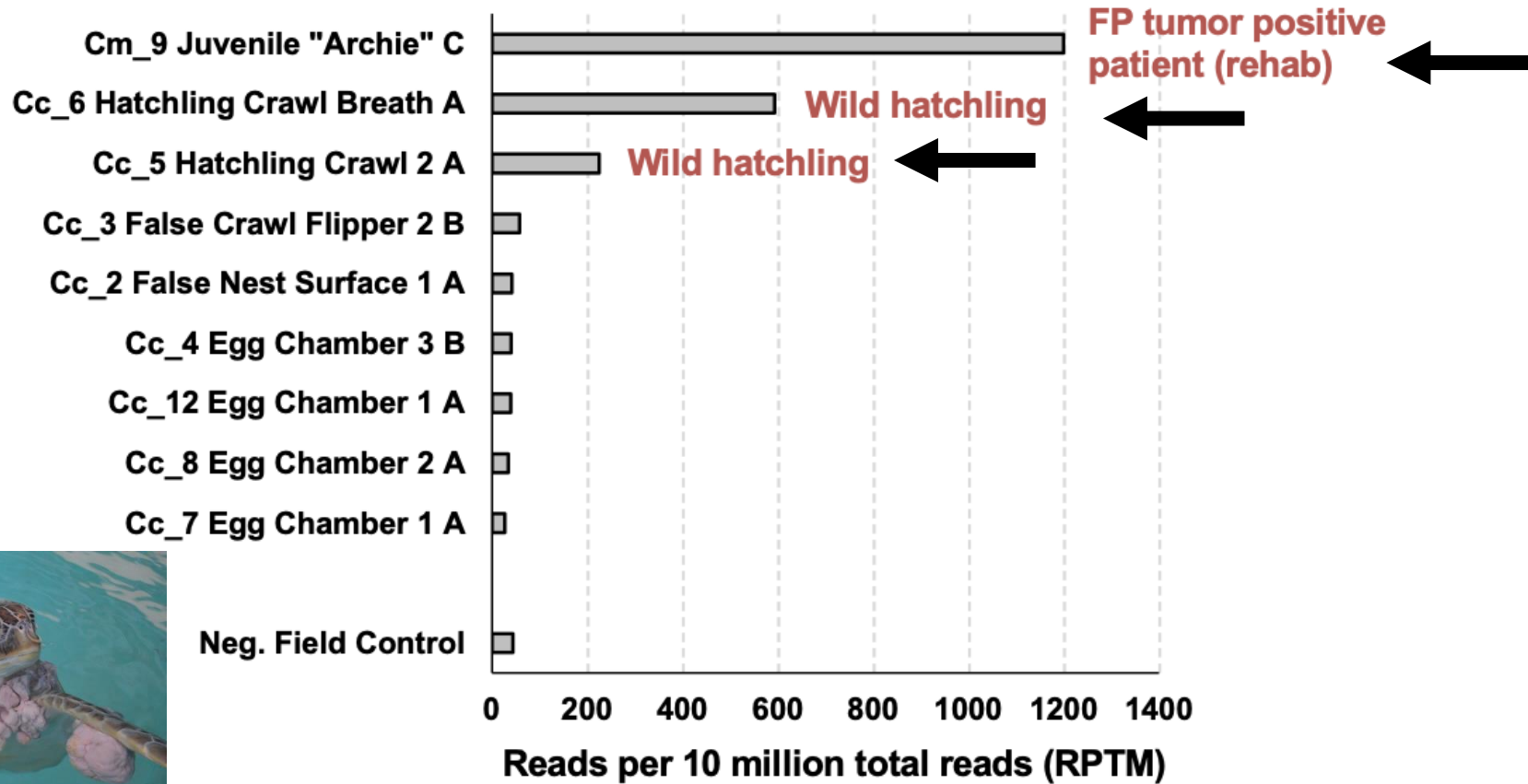
Phylogenetic analysis of *C. mydas* and *C. caretta* mitochondrial genomes from sea turtle sand samples and NCBI deposited sequences (whole genome shotgun sequencing)



eDNA for simultaneous pathogen detection

Sand eDNA-based detection of wild tumor-associated pathogen ChHV5

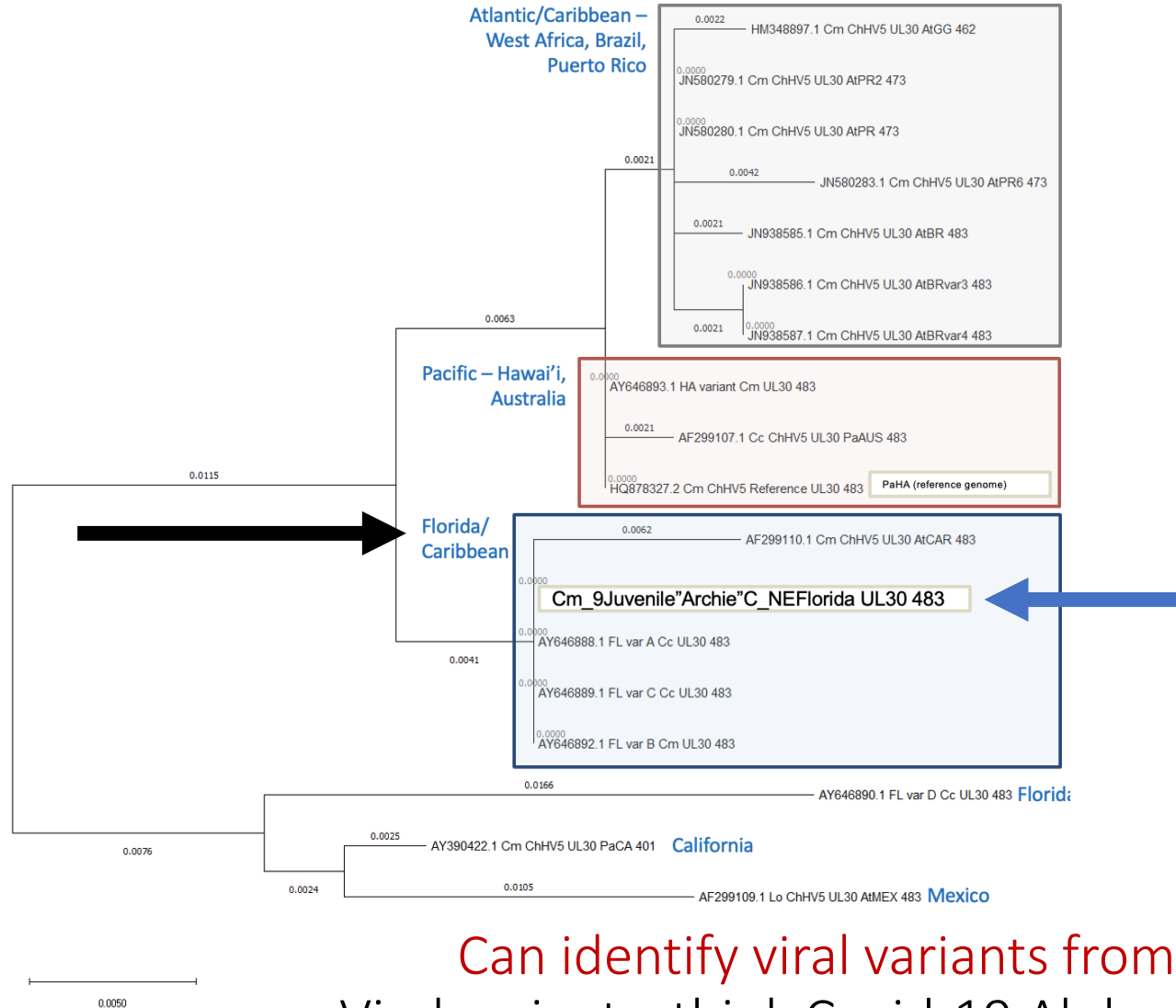
ChHV5 viral genome aligning reads from non-targeted shotgun sequencing of sand eDNA samples (whole genome shotgun sequencing)



ChHV5 viral variant analysis from turtle sand

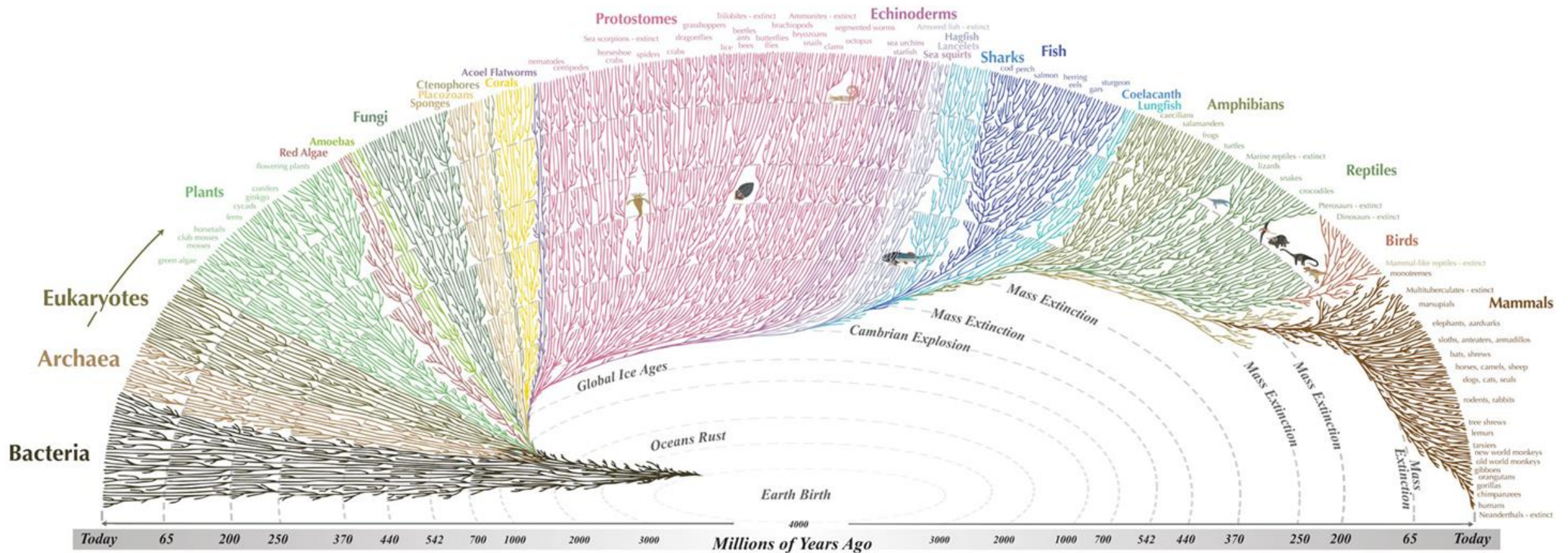
ChHV5 variant calling from sand eDNA of patient "Archie" (Illumina shotgun sequencing)

Farrell J.A, Whitmore L, Mashkour N et al. (Submitted for review) Detection, quantification and population genomics of sea turtle species via non-invasive environmental DNA (eDNA) analysis of nesting beach sand tracks and oceanic water samples.



Can identify viral variants from sand alone!
 Viral variants: think Covid-19 Alpha, Omicron & Delta

eDNA Metagenomics possible from shotgun sequencing eDNA (pending)

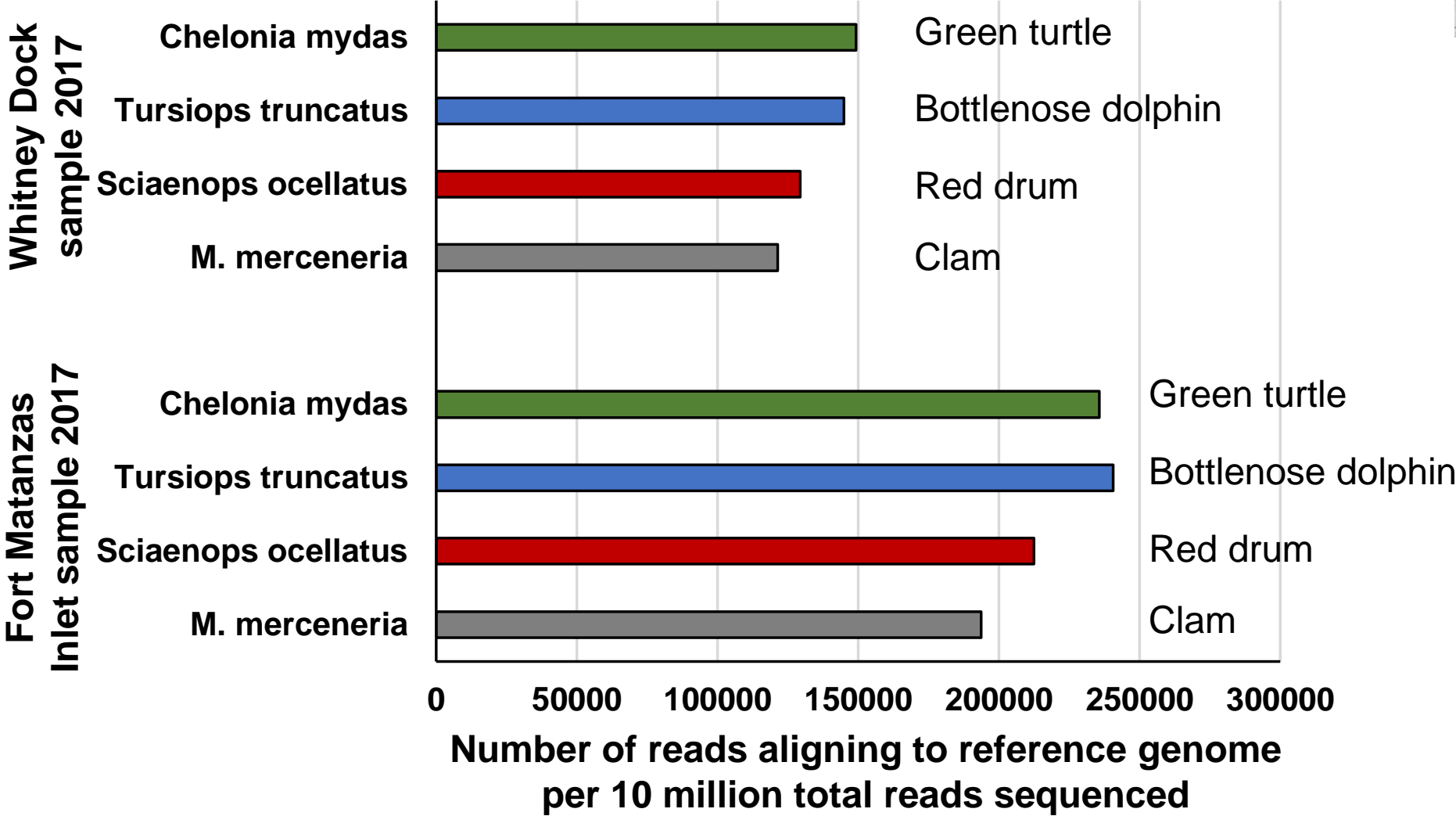


All the major and many of the minor living branches of life are shown on this diagram, but only a few of those that have gone extinct are shown. Example: Dinosaurs - extinct

Pan-species Environmental Metagenomics (NGS-based)

http://evogeneao.s3.amazonaws.com/assets/tree_of_life/tree-of-life_2000.jpg

eDNA – shotgun sequencing interrogated for specific species reptile, mammal, fish & invertebrate



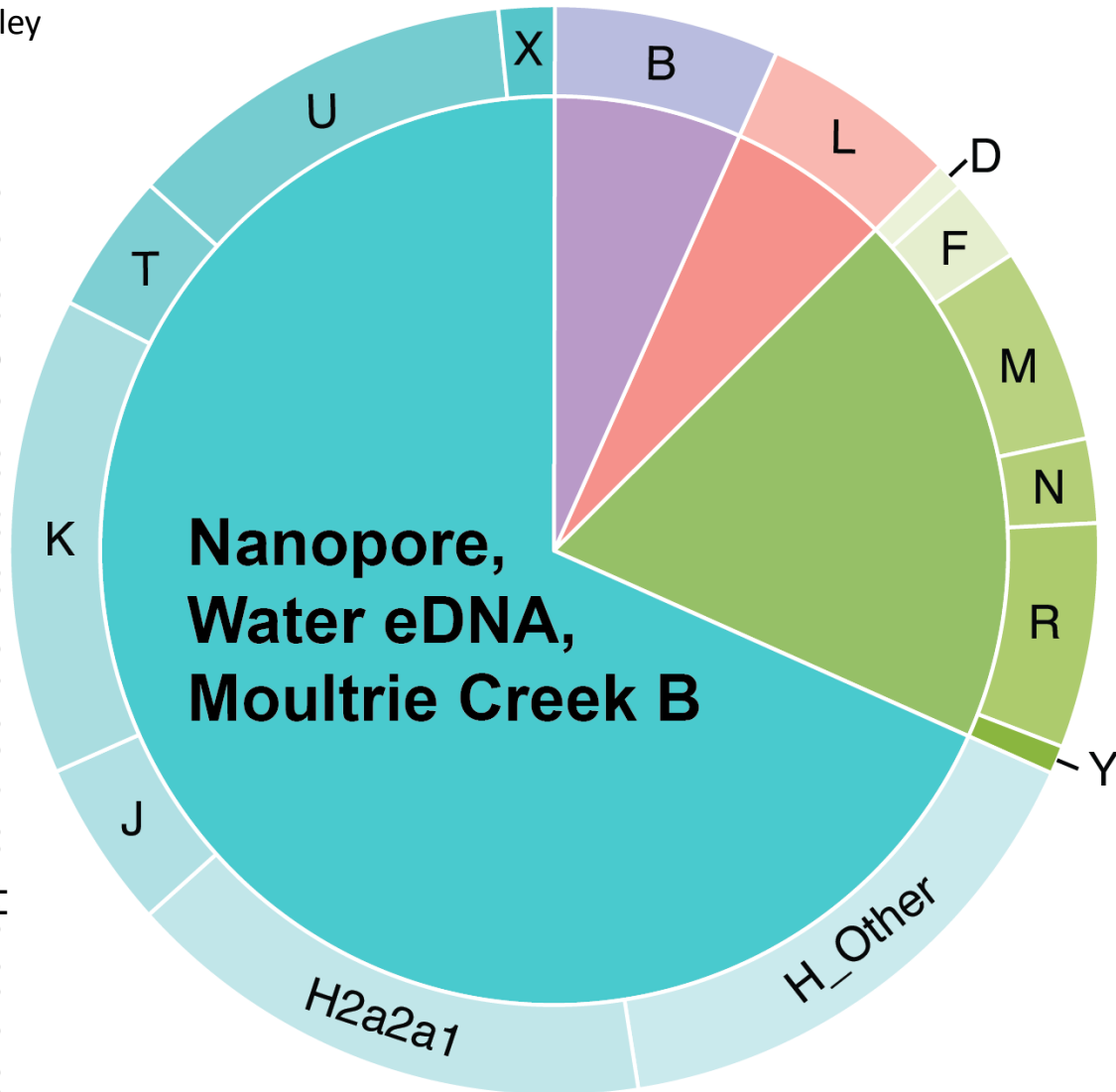
Where is eDNA going next?

- Wildlife population genetics from water?
 - Heterogenous mix of species per sample
- Not just what species are present, but where do they come from?

Wildlife population genetics from sea water? (500ml) Human eDNA as proof-of-principle

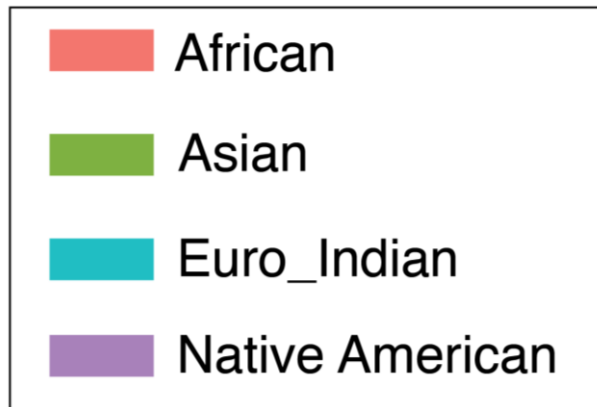
Mark McCauley

Whitmore et al. (2023) Human environmental DNA: inadvertent human genomic bycatch and intentional capture raises novel beneficial applications and ethical concerns. Under review.



Also juvenile sea turtle habitat from where we have detected sea turtle eDNA

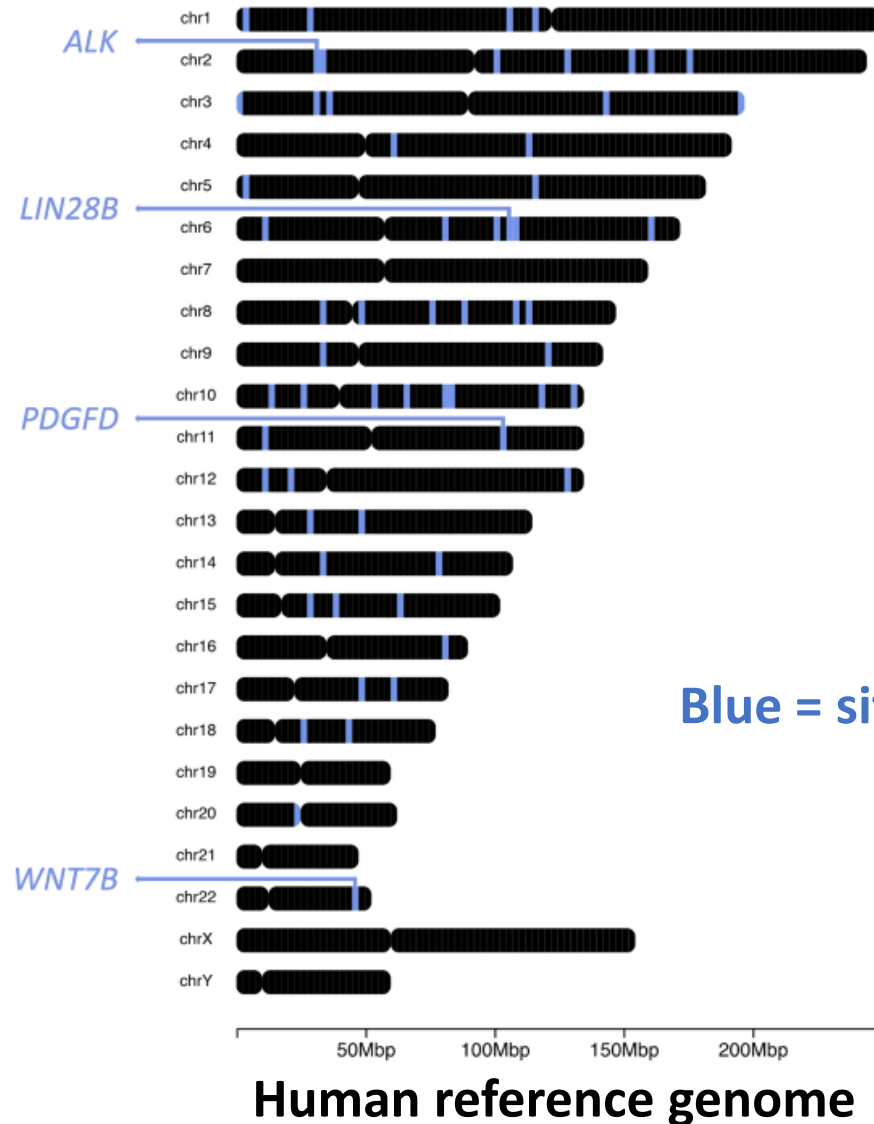
Human haplogrouping & haplotyping



eDNA can also reveal disease-associated mutations / any alleles of interest in the population

Max
Stammitz

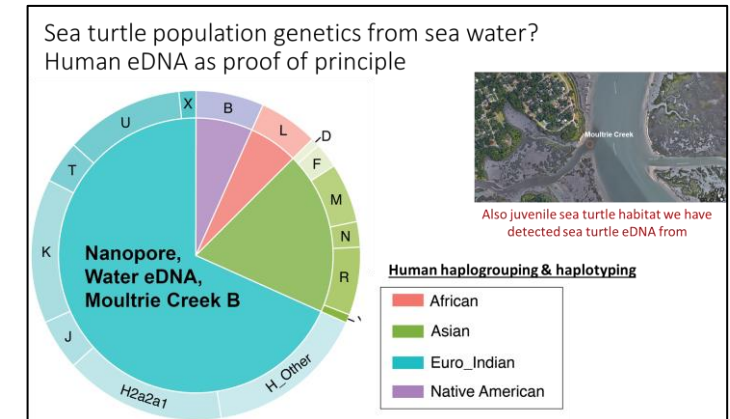
Whitmore et al. (2023) Human environmental DNA: inadvertent human genic bycatch and intentional capture raises novel beneficial applications and ethical concerns. Under review.



Prominent human cancer-associated genes with or adjacent to deletions detected by gnomAD from Moultrie Creek B water eDNA sample (Oxford Nanopore shotgun long read sequencing)

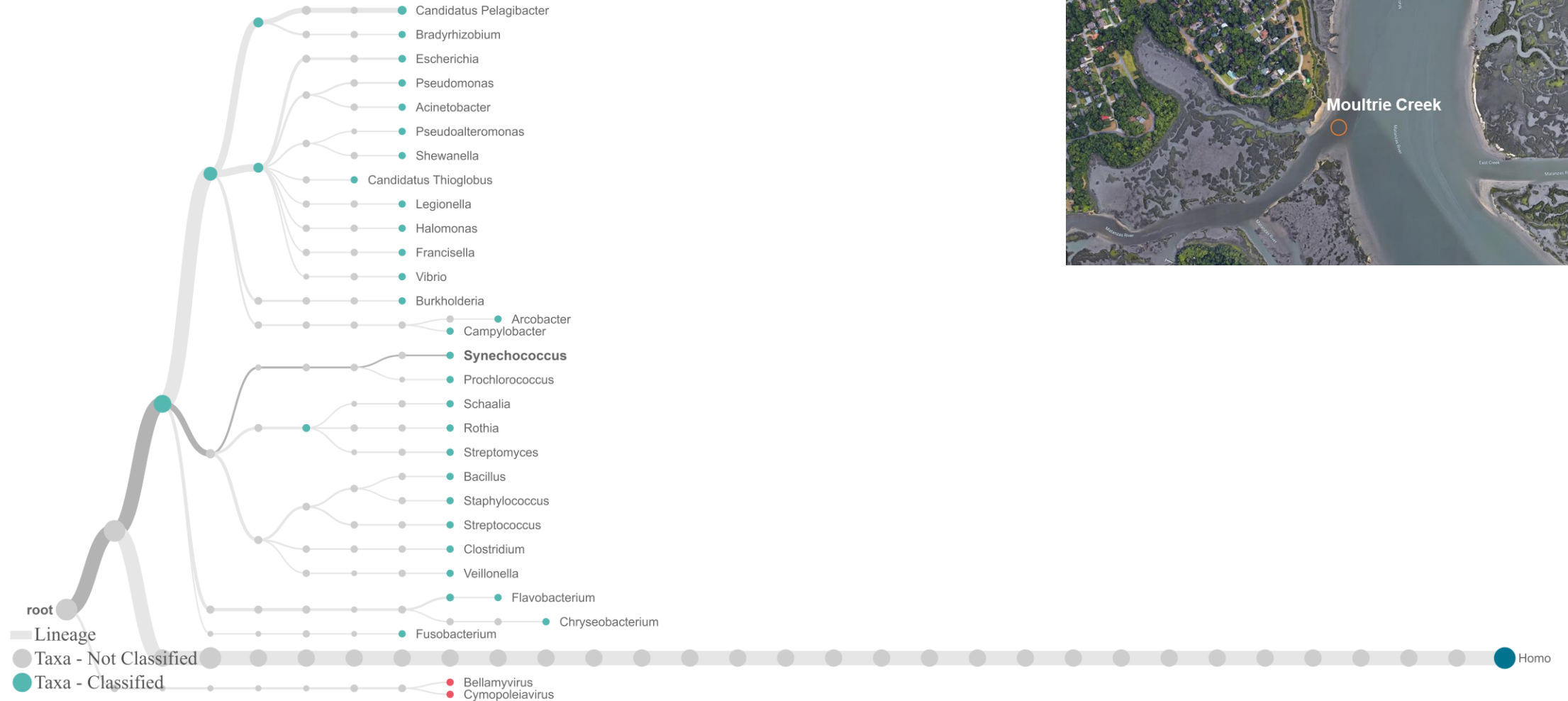
Blue = site of deletion

Same water eDNA Nanopore sample used for haplotyping



Same shotgun eDNA also recovers microbial reads & all other species

Metagenomics taxonomy (human & microbe) high human water
(no enrichment Oxford Nanopore shotgun long read sequencing, **Moultrie Creek B**)



Human eDNA research arose from identifying human eDNA as bycatch in our wildlife studies

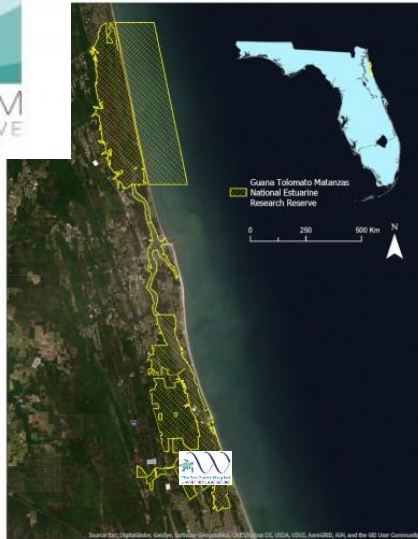
Is human eDNA collection possible? **Yes**

Is human eDNA useful?

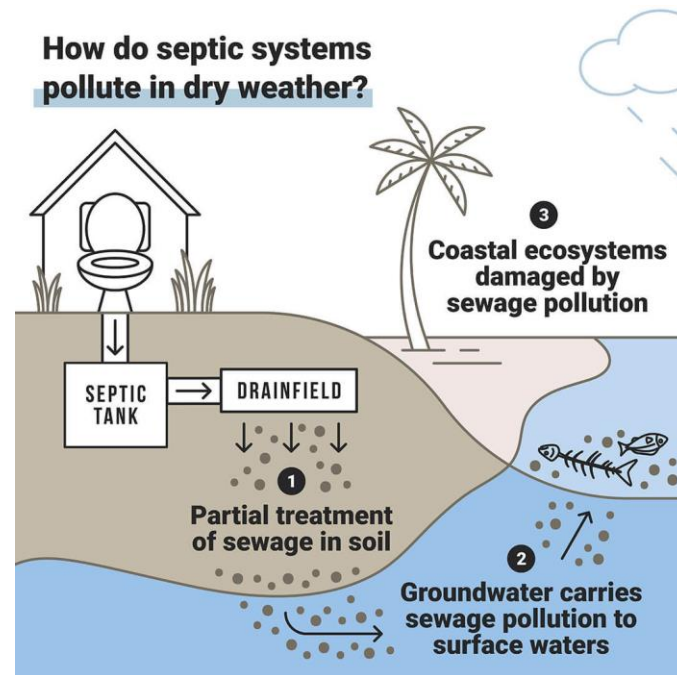


Human-specific eDNA assays for rapid and cost-effective wastewater release/septic tank leeching monitoring

- Pollution of aquifers & waterways
- A problem internationally, including NERRs



<https://gtmnerr.org/about/>



<https://www.surfrider.org/coastal-blog/entry/how-do-septic-systems-pollute-coastal-watersheds>



<https://www.studyread.com/wp-content/uploads/2012/01/Water-release-of-industrial-waste-1.jpg>

Effluent Quantification

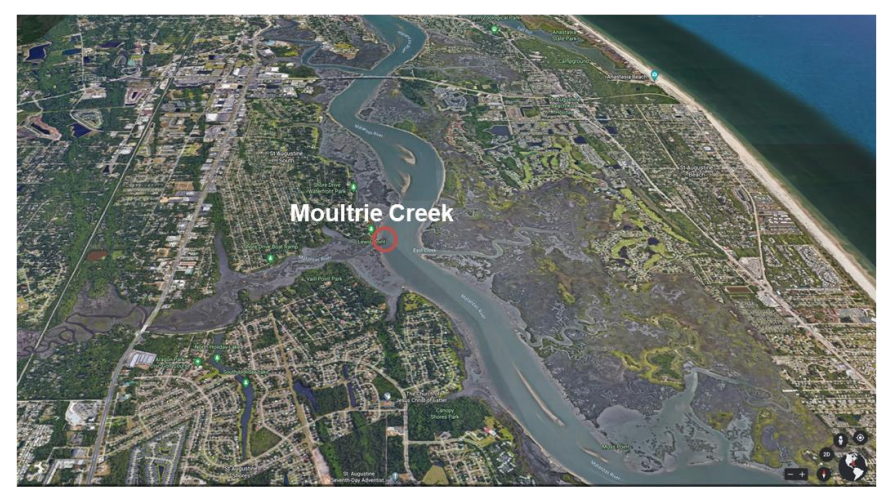
Presumptive high and low human eDNA sites, FL samples

Todd Osborne



<https://wusfnews.wusf.usf.edu/environment/2022-09-04/efforts-restore-nature-best-pollution-filters>

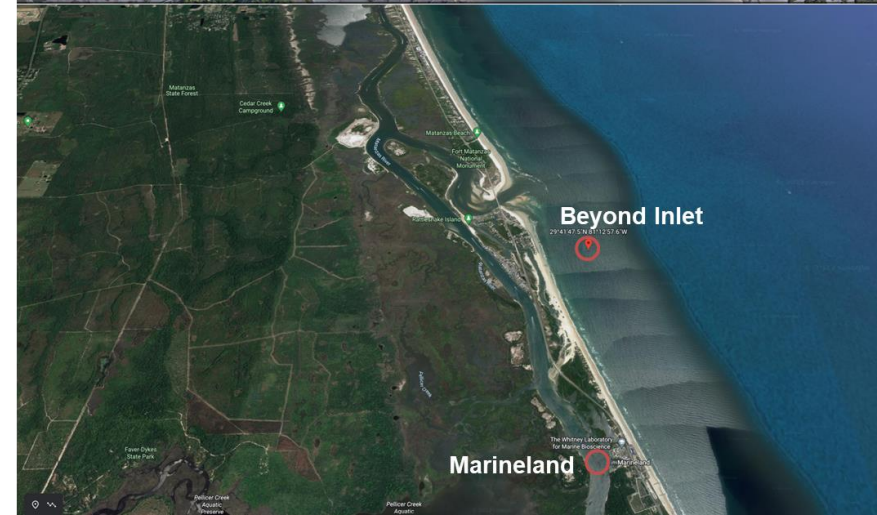
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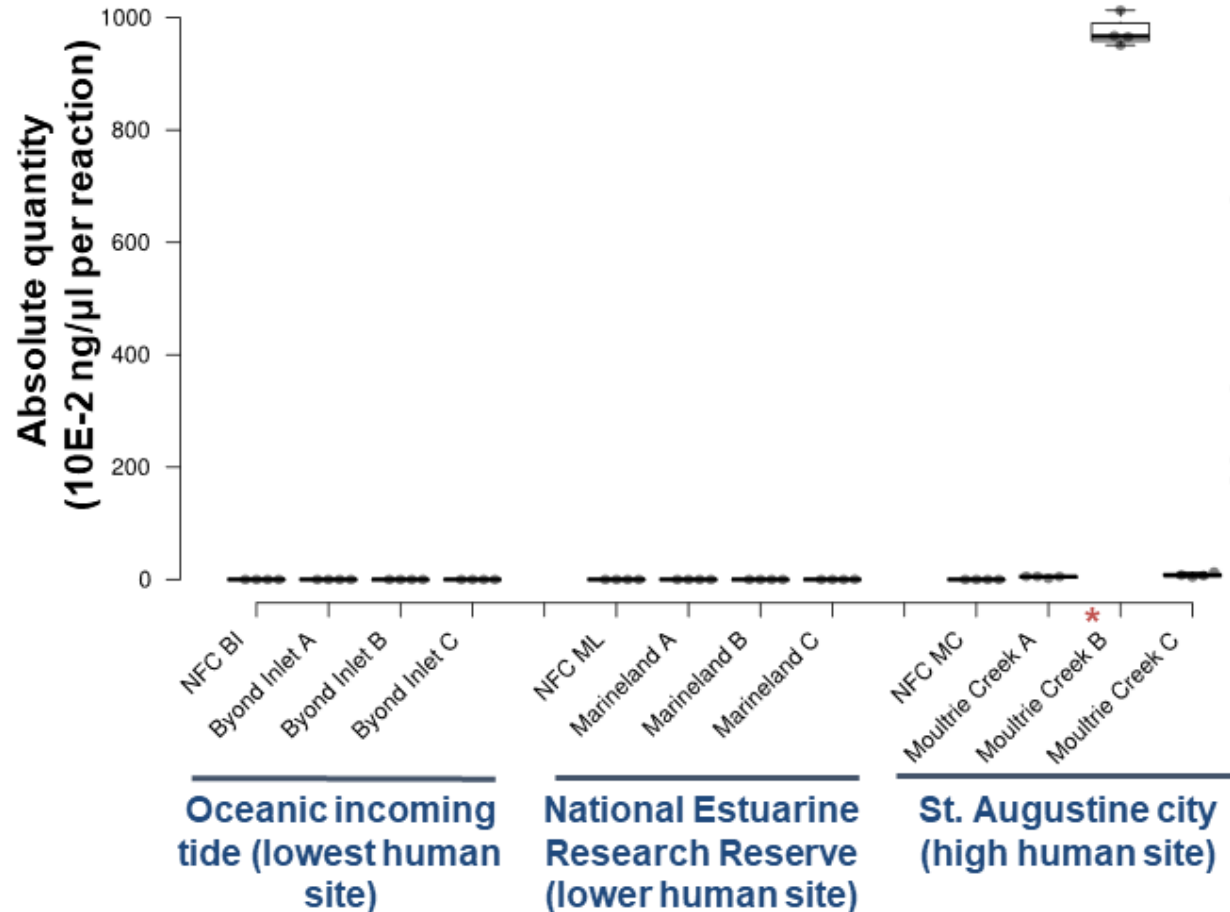


c



Human eDNA from intentional water sampling, Florida, human species-specific qPCR assays

ZNF285

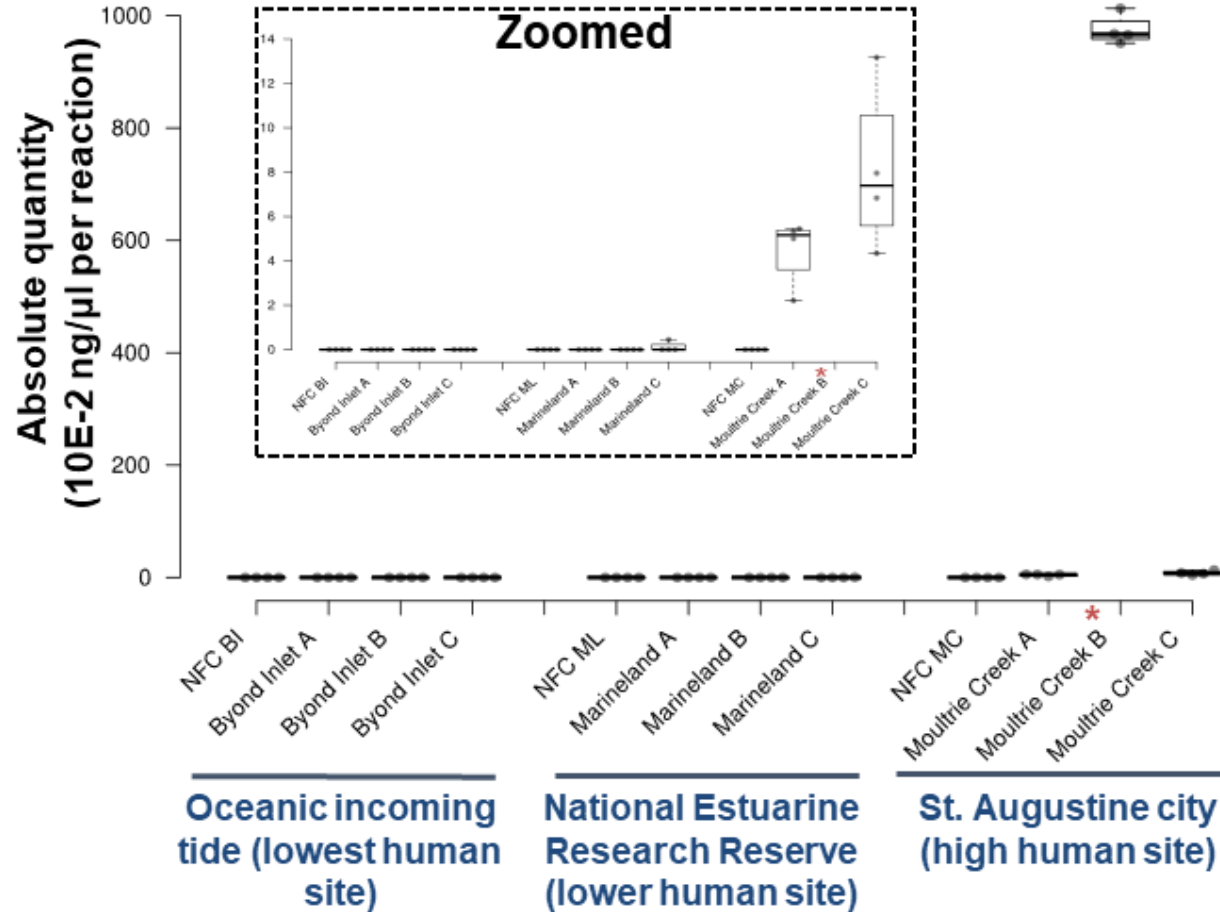


* Off the scale in zoomed inset view

Human-specific eDNA assays

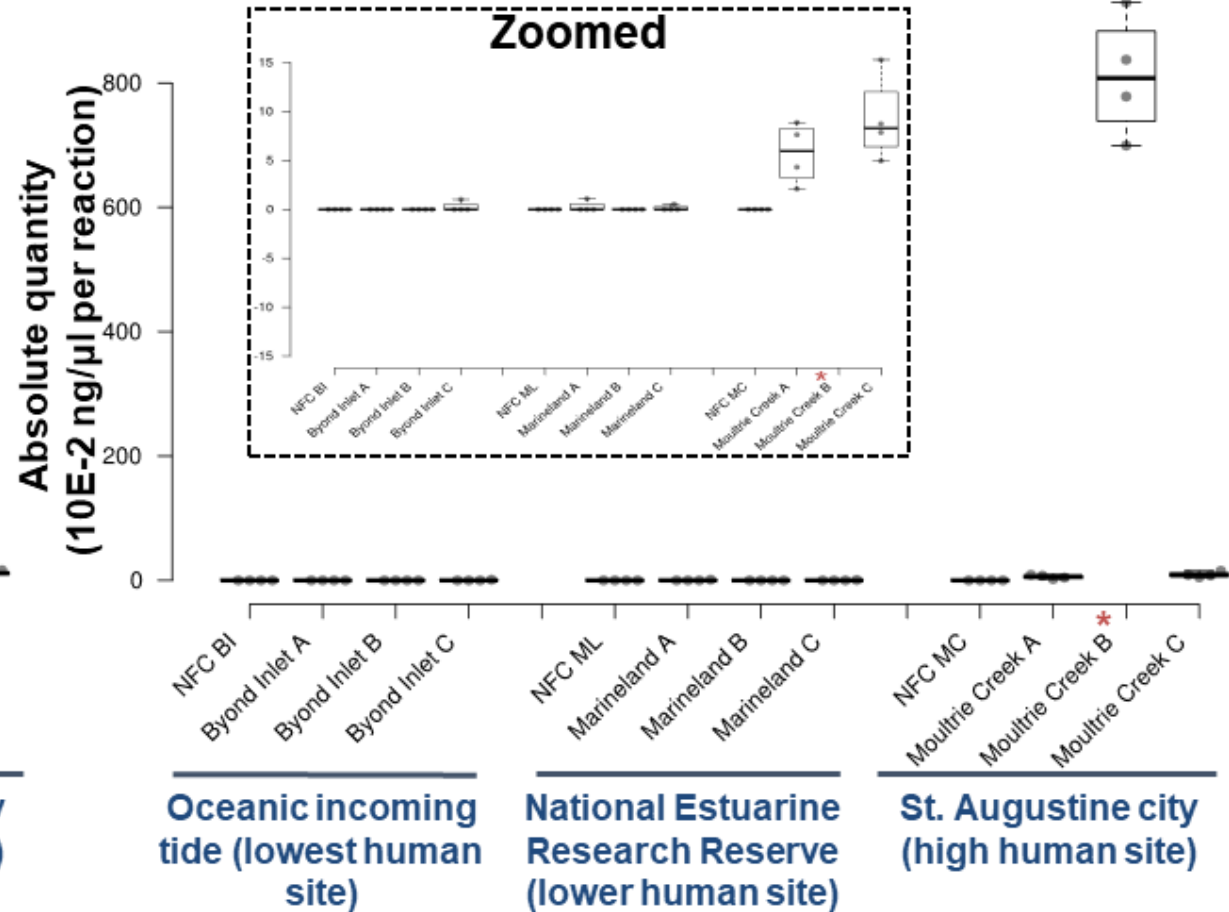
Human eDNA from intentional water sampling, Florida, human species-specific qPCR assays

ZNF285



* Off the scale in zoomed inset view

LILRB2



* Off the scale in zoomed inset view

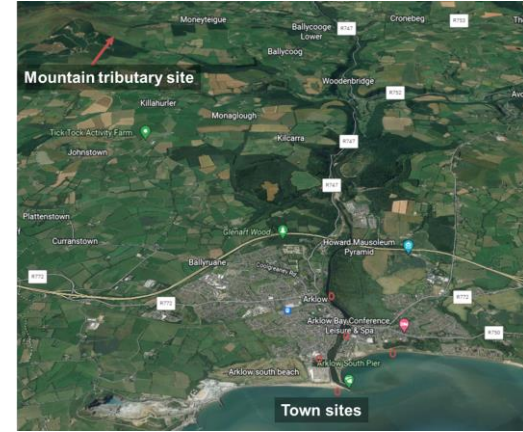
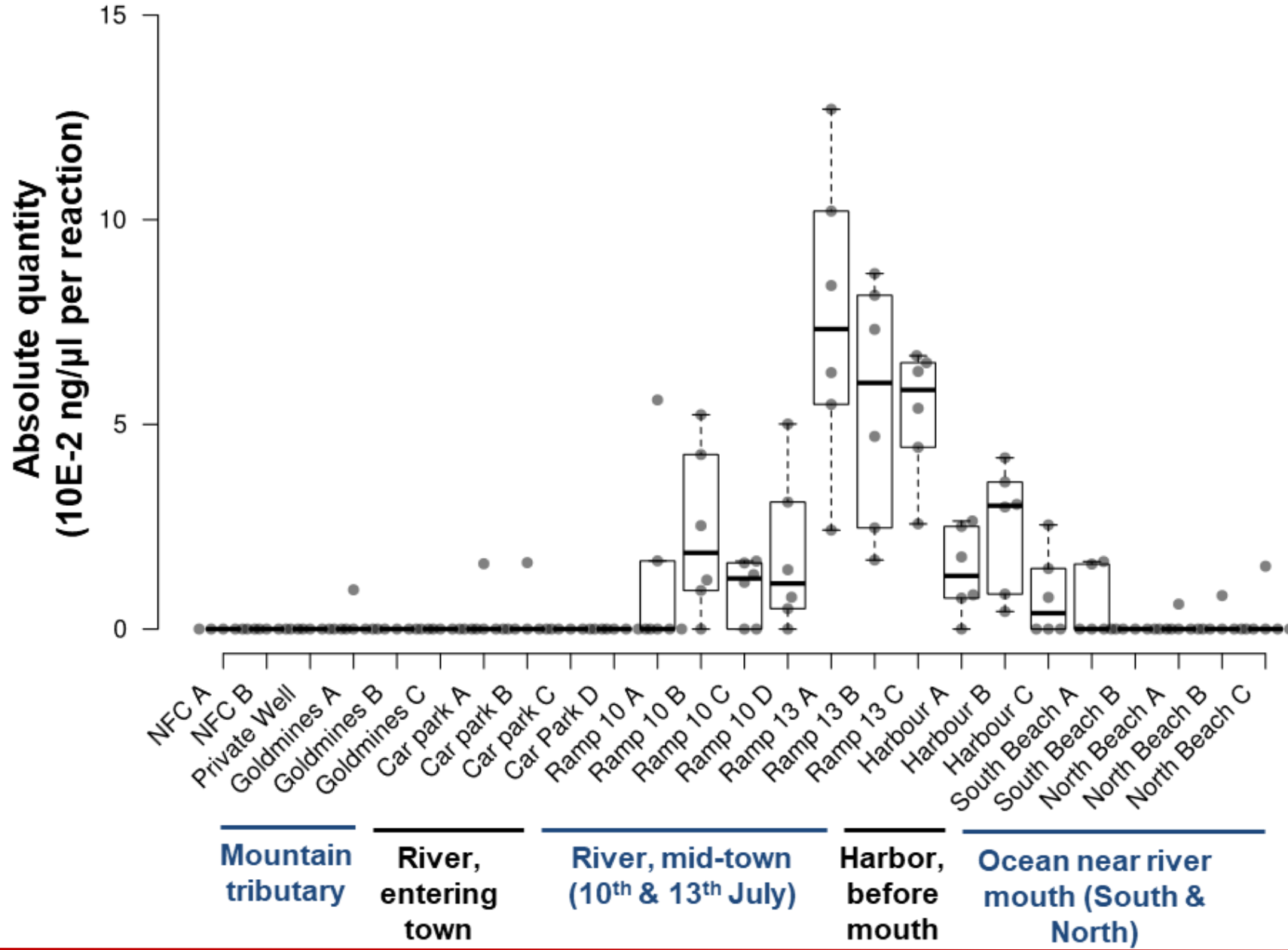
Human-specific eDNA assays

Irish river course sampling

- Avoca River, Co. Wicklow



Human eDNA from intentional water sampling, Avoca River, Ireland, LILRB2 species-specific (human) qPCR assay



All collected on an outgoing tide

Identifies source of human effluent pollution

eDNA-based Human Effluent Quantification

GTM NERR & Irish validation

- Human-DNA-specific wastewater, septic tank leeching, human fertilizer run-off monitoring
- Those responsible can't claim the source to be wildlife or animal agriculture (as is often the case currently)
- Rapid and cost effective (approx. \$20 per sample), could be coupled with remote monitoring
- Absolute Quantification
 - Can be compared across global sites and large time courses
 - qPCR-based, cost-effective

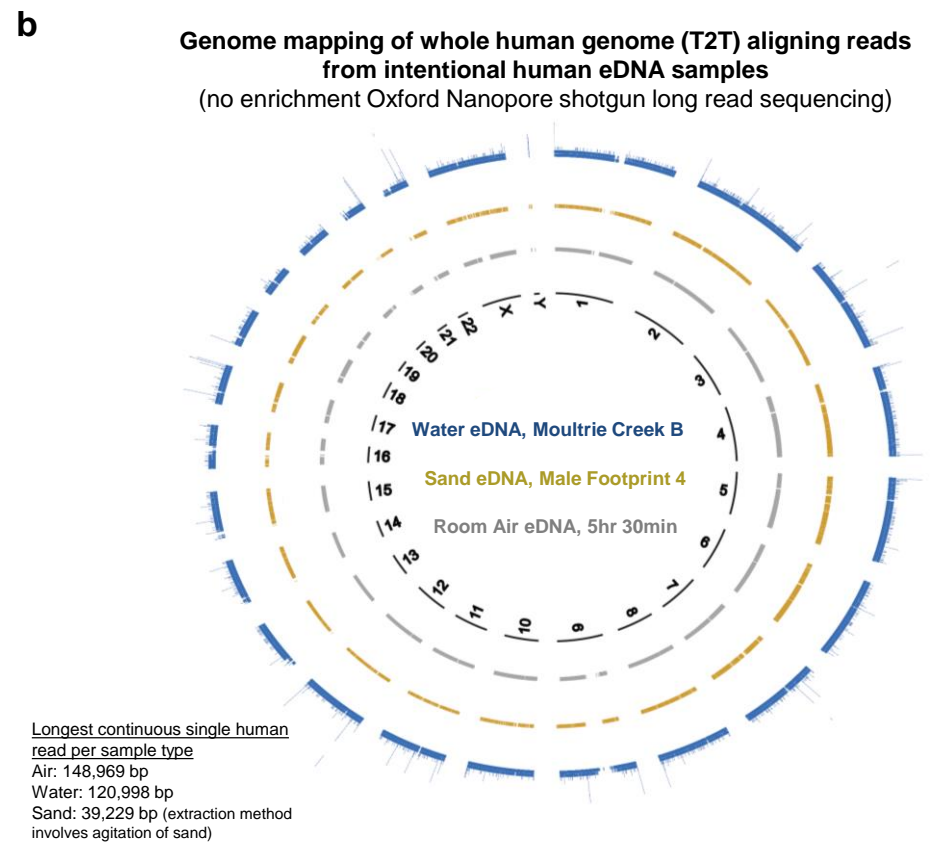
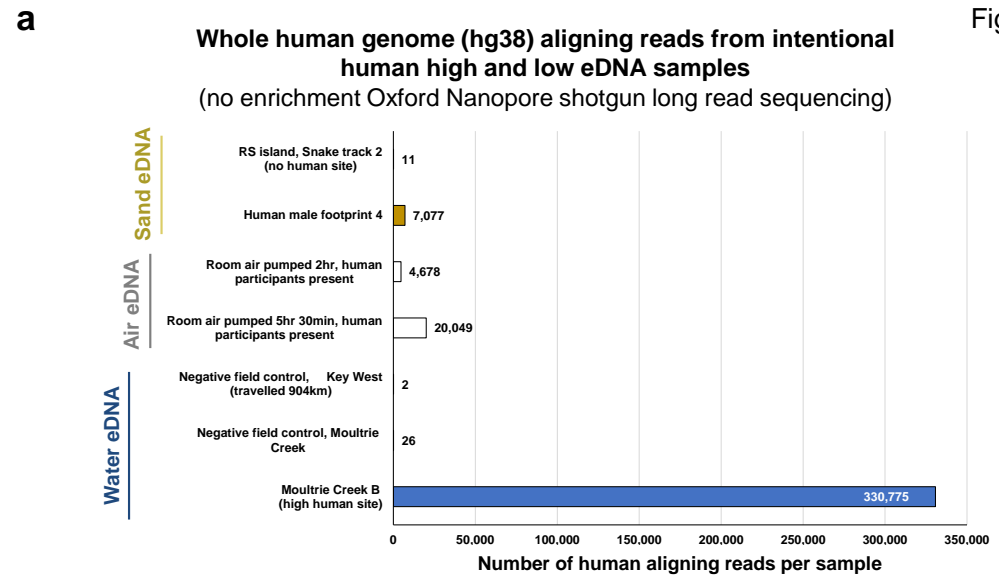
Human eDNA

- Not just quantification
- Whole genome recovery possible. Utility for:
 - Medical (population-level disease susceptibility)
 - Recover of ancient/contemporary remains & fallen heroes
 - Informing future directions in wildlife/biodiversity eDNA
 - Ethical implications

Human environmental DNA: inadvertent human genomic bycatch and intentional capture raises novel beneficial applications and ethical concerns.

Short title: Promises and dilemmas of human eDNA

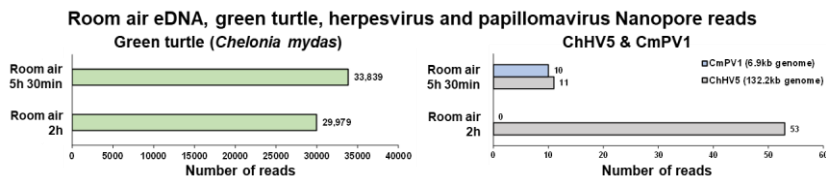
Liam Whitmore^{1,2*}, Mark McCauley^{1,3*}, Jessica A. Farrell^{1,4*}, Maximilian R. Stammnitz⁵, Samantha A. Koda¹, Narges Mashkour¹, Victoria Summers¹, Todd Osborne¹, Jenny Whilde¹ and David J. Duffy^{1,4}



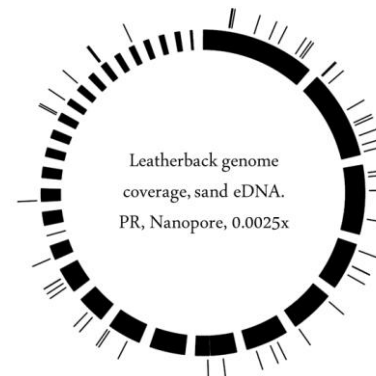
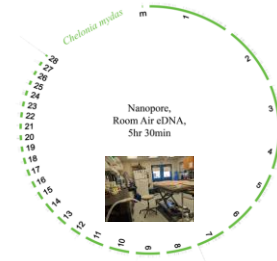
eDNA Summary



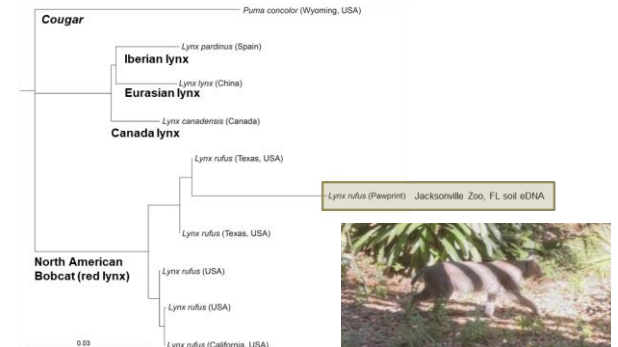
- **What** species are present?
- **Where** do the present individuals come from? (population genetics of fauna)
- Conservation-relevant genetic loci study (adaptability, disease susceptibility etc.)
- Pathogens (human, livestock and wildlife) & invasive species
- Air eDNA: we have applied to human, sea turtle & pathogens, now working on pan-biodiversity air assessments
- Federal & regulatory support for eDNA to be widely utilized by management agencies



Genome mapping of whole green turtle genome aligning reads from room air eDNA samples (no enrichment Oxford Nanopore shotgun long read sequencing)



Phylogenetic placement of Zoo housed *L. rufus* "Abby" based on mitochondrial reads recovered from soil eDNA (no enrichment Oxford Nanopore MinION shotgun sequencing)



Take-home message eDNA:

- Has HUGE POTENTIAL
- Will be a ROUTINE CONSERVATION & RESILIENCE MANAGEMENT TOOL
- A lot of information in 10g of sand, 500ml water or thin air!



Sea Turtle Funding



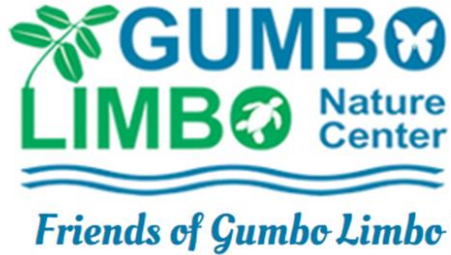
PRIVATE CITIZEN
DONATION



Llywodraeth Cymru
Welsh Government



Human eDNA:
My UF lab
start up



NATIONAL
SAVE THE SEA TURTLE
FOUNDATION



save our seas
foundation



Marie Skłodowska-Curie
Actions COFUND



IRISH
RESEARCH
COUNCIL
An Chomhairle um
Thaighde in Éirinn





Acknowledgements

- Jessica Farrell (UF)
- Liam Whitmore (UL/UF)
- Samantha Koda (UF)
- Mark McCauley (UF)
- **Scott Eastman** (DEP)
- **Catherine Eastman** (UF)
- **NE FL nesting beach patrols**
- Nancy Condron (UF)
- Mark Q. Martindale (UF)
- Sea Turtle Hospital (UF)
- Jenny Wilde (UF)
- Larry Wood, IRG & STC
- Kelsey Yetsko (UF)
- Narges Mashkour (UF)
- Maximilian Stammnitz (CU)
- Todd Osborne (UF)
- Kurt Foote (NPS)
- Andrew Rich (NPS)
- NPS staff, Fort Matanzas National Monument
- Voluntary participants (human study)
- P. Murphy & R. Rolfe (TCD)
- A. Krstic & W. Kolch (UCD)
- State Permitting Agencies



Questions?



duffy@whitney.ufl.edu

<https://www.bbc.com/sport/av/63125522>

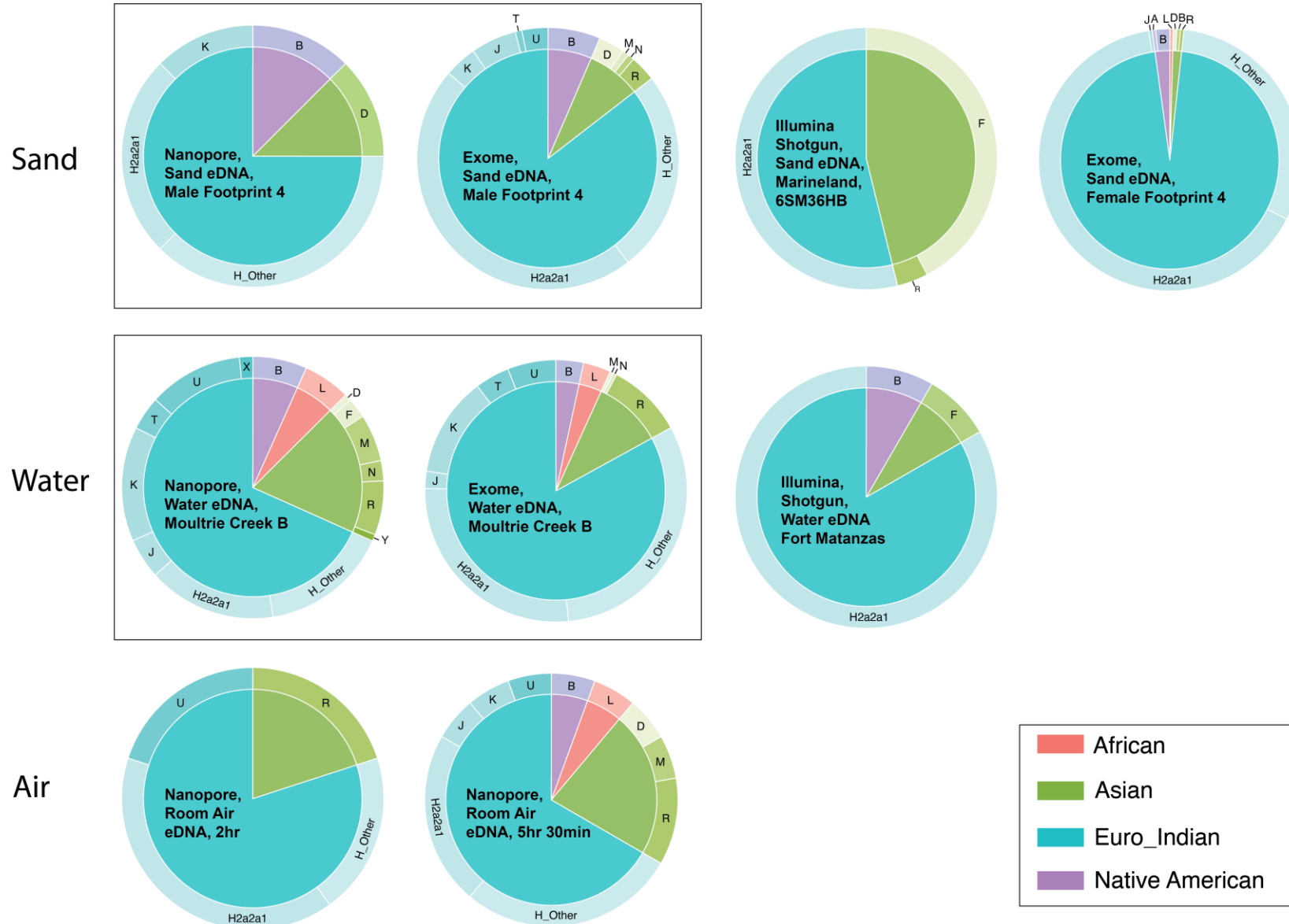
<https://news.ufl.edu/2022/05/sea-turtle-edna/>

www.facebook.com/SeaTurtleHospitalatWhitneyLab

www.whitney.ufl.edu/people/current-research-faculty/david-duffy-phd/

Population genetics from water, sand & air?

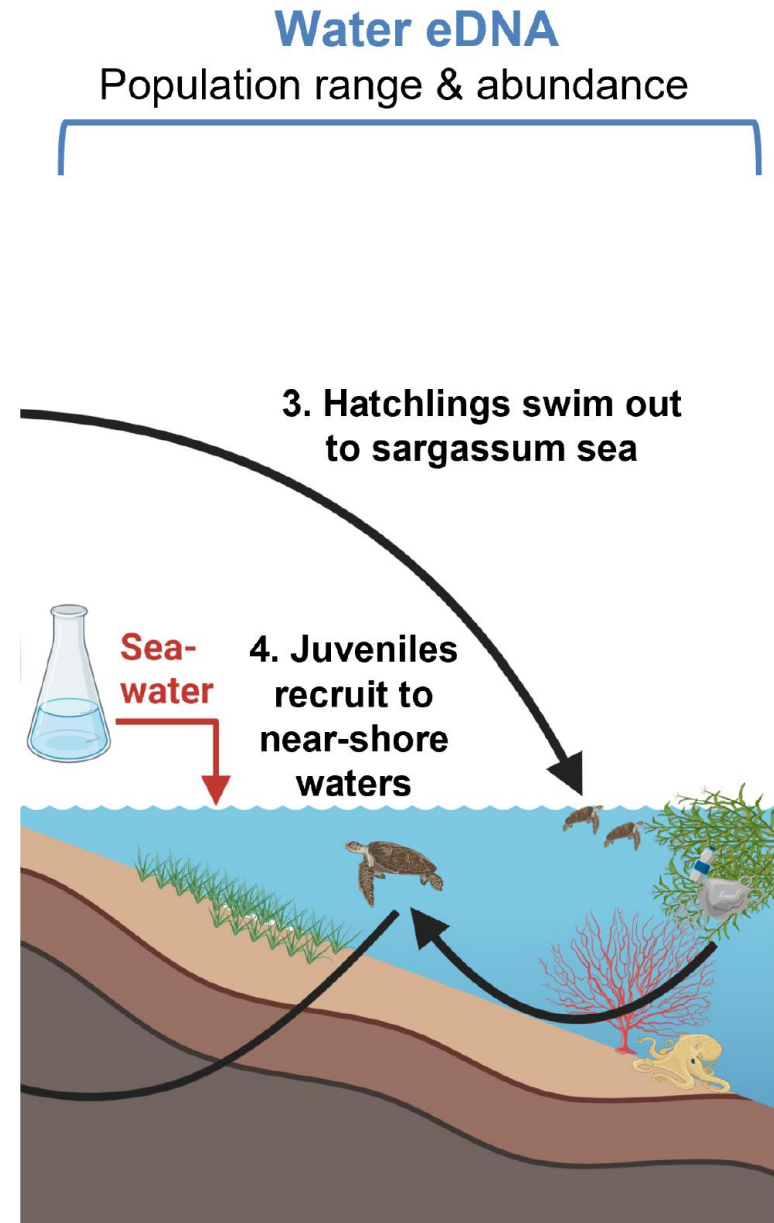
Human eDNA as proof of principle



Voluntary participants – approved by UF ethics board

Environmental DNA (eDNA) – useful for whole sea turtle lifecycle

<https://onlinelibrary.wiley.com/doi/10.1111/1755-0998.13617>



Human footprint sand Vs 'No human' restricted island sand eDNA



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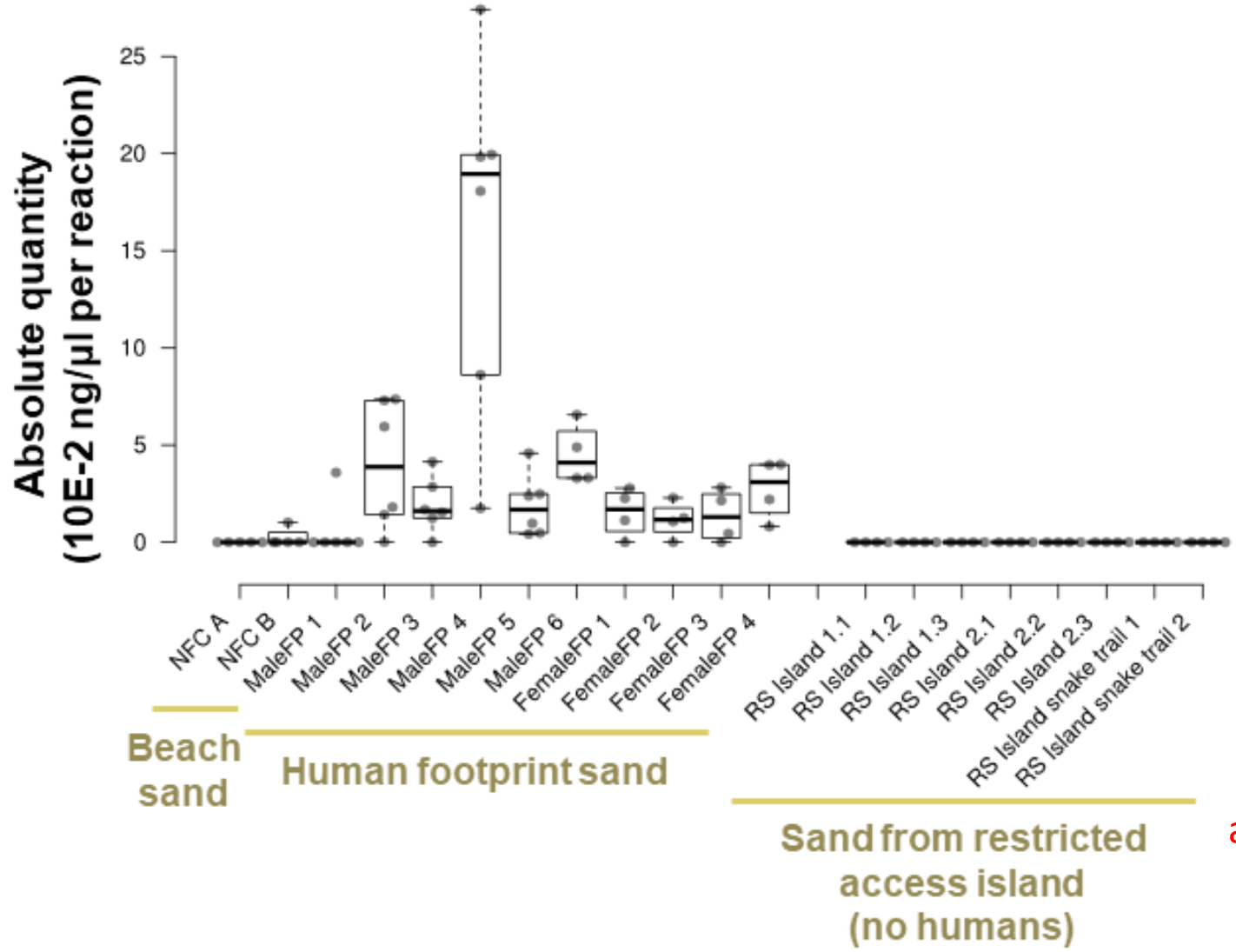
b



Kurt Foote, Andrew Rich and the NPS staff of the Fort Matanzas National Monument

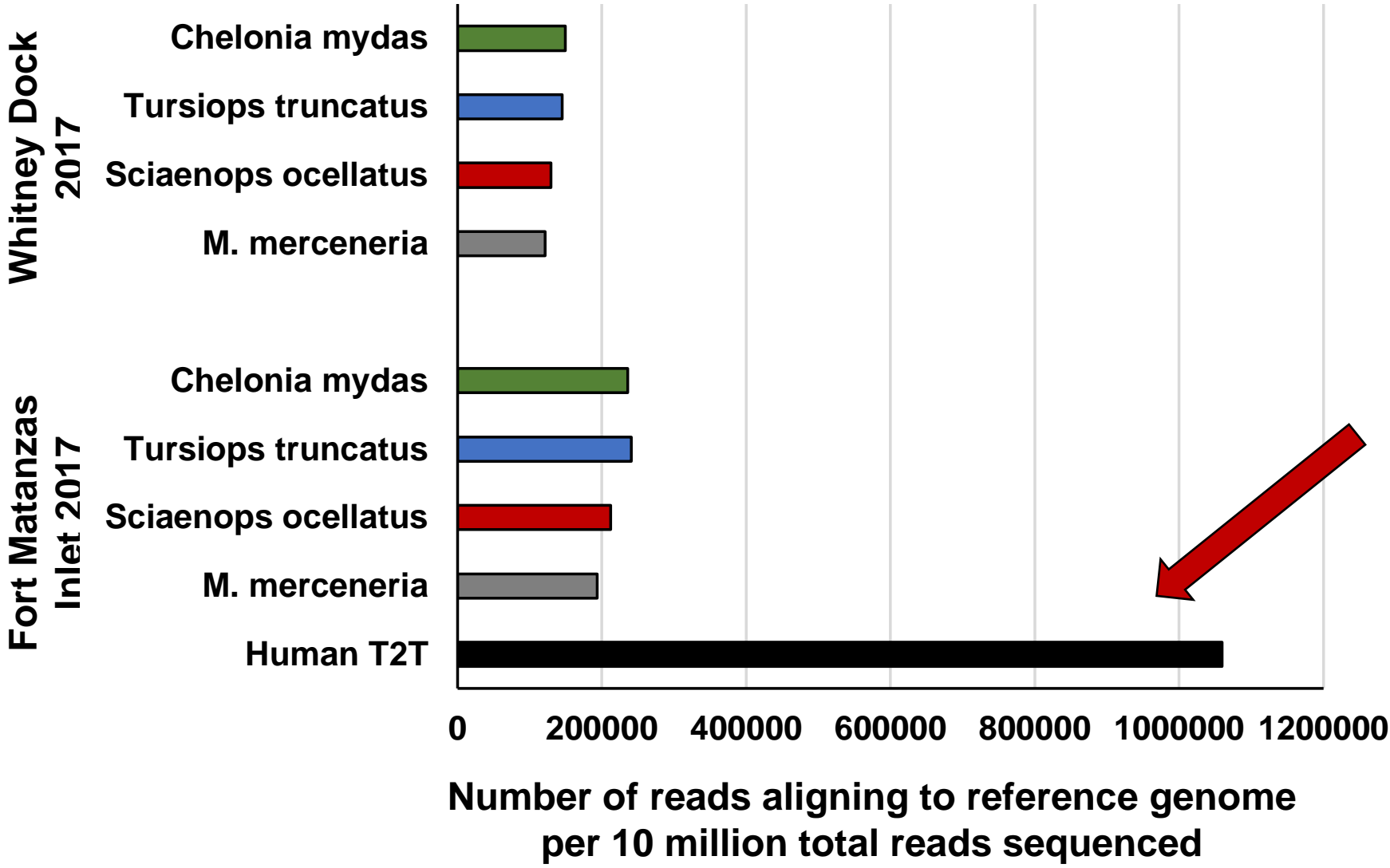
Human eDNA from intentional sand sampling, Florida, human species-specific qPCR assays

ZNF285



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eDNA – shotgun sequencing interrogated for specific species



Irish river, town samples

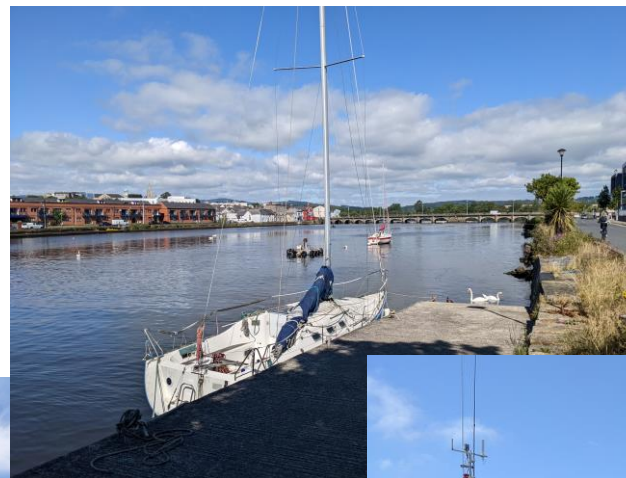
- Avoca River,
Co. Wicklow



Mountain tributary



Entering town



Mid-town



Harbour



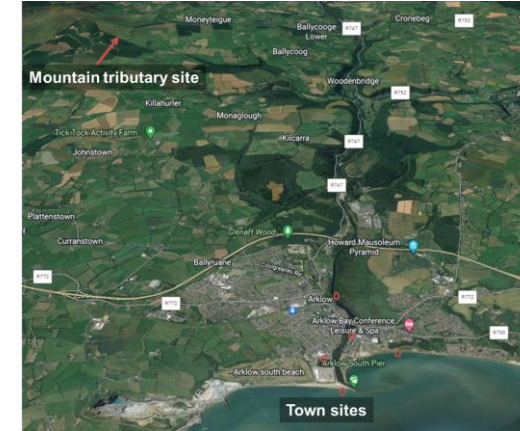
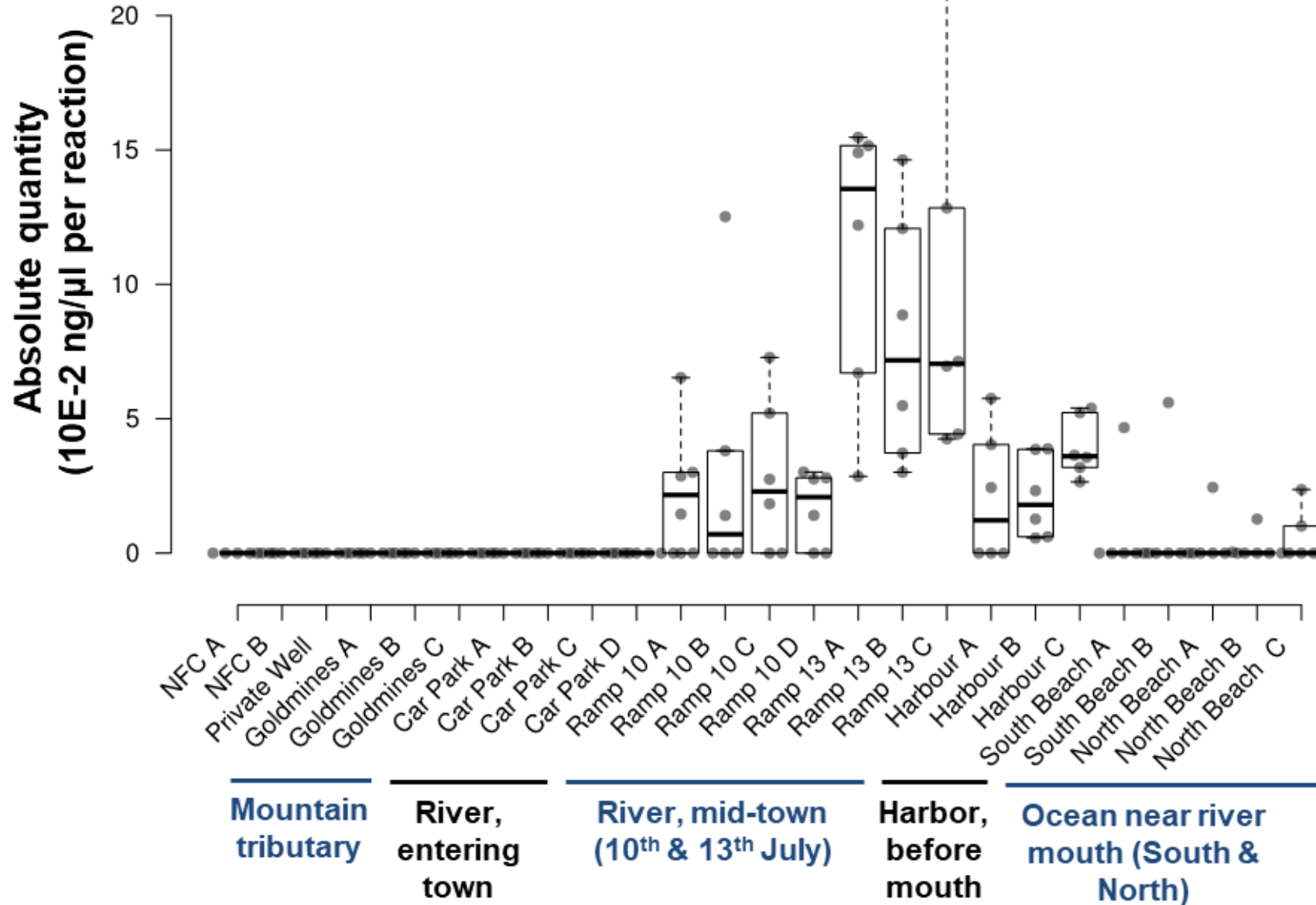
Beach/mouth

Species-specific qPCR/ddPCR eDNA assays can benefit from reference genomes

- Species-specific assays for (mitochondrial-based):
 - **Green (Cm)** – published (Yetsko et al. 2020, Harper et al. 2020, Yetsko et al. 2021, Farrell et al. 2022)
 - **Loggerhead (Cc)** – published (Farrell et al. 2022)
 - **Leatherback (Dc)** – MS in preparation (Farrell et al.)
 - **Kemps (Lk)** – Active development (Komoroske group)
- Probably wait/need until full references available. Mito. sequences not great for assay design for these species
 - **Hawksbill (Ei)** – need full reference?
 - **Olive (Lo)** – need full reference?
 - **Flatback (Nd)** – need full reference?

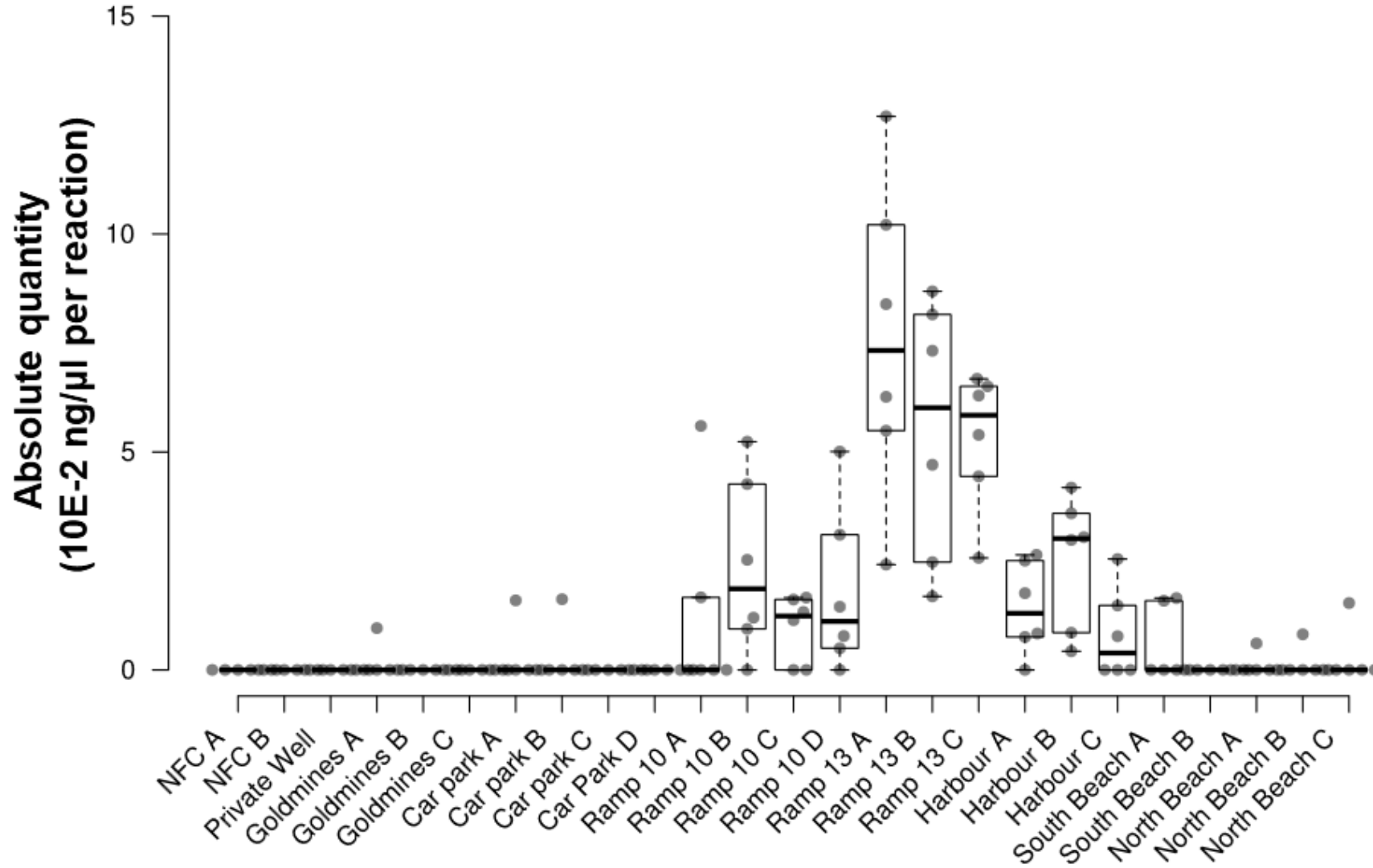


Human eDNA from intentional water sampling, Avoca River, Ireland, ZNF285 species-specific (human) qPCR assay



All collected on an outgoing tide

Human eDNA from intentional water sampling, Avoca River, Ireland, LILRB2 species-specific (human) qPCR assay



Same samples, different human assay

Source

Mountain tributary

River, entering town

River, mid-town (10th & 13th July)

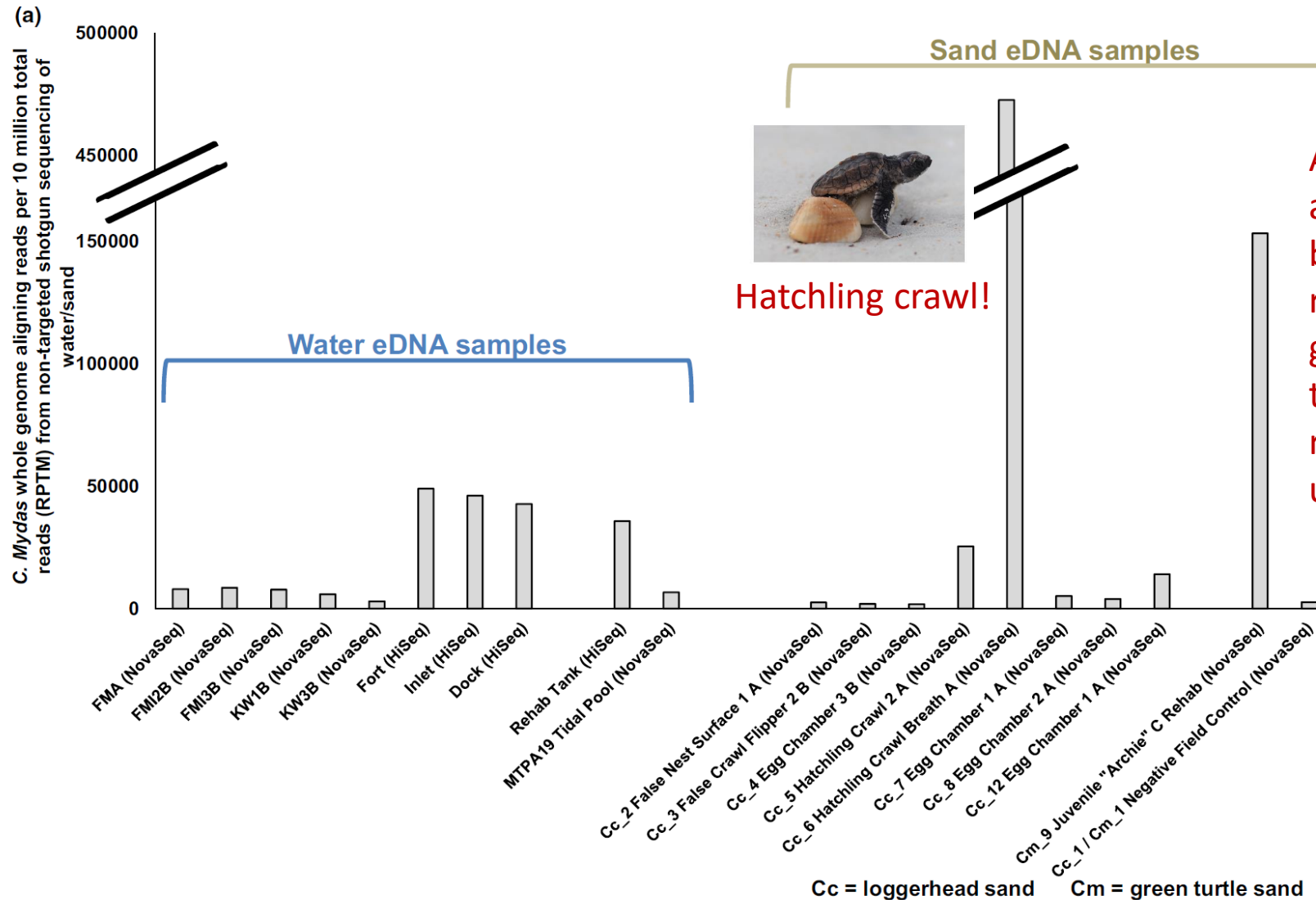
Harbor, before mouth

Ocean near river mouth (South & North)

Sea



Shotgun sequencing (Illumina) of water and sand eDNA

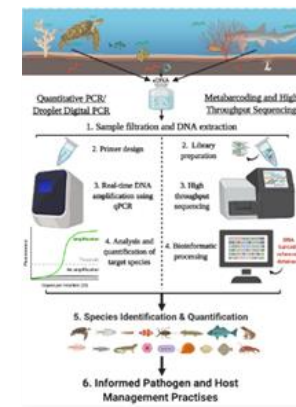


All but 1 sand samples are loggerhead crawls, but had to align to green reference as no Cc genome available at the time, likely under-reporting & should now use new Cc genomes



Problems eDNA can overcome

- Sea turtles are difficult to detect and survey in the wild
 - eDNA no need for visual confirmation or capture of turtles
 - More cost-effective, so can survey more areas
- Population genetic data for conservation and research requires invasive blood/tissue sampling, and egg sacrifice is used
 - eDNA means no disturbance of individuals, especially females during nesting
 - Samples can be taken from sand hours after the nesting event
 - So don't need to see nesting, can get samples the next morning

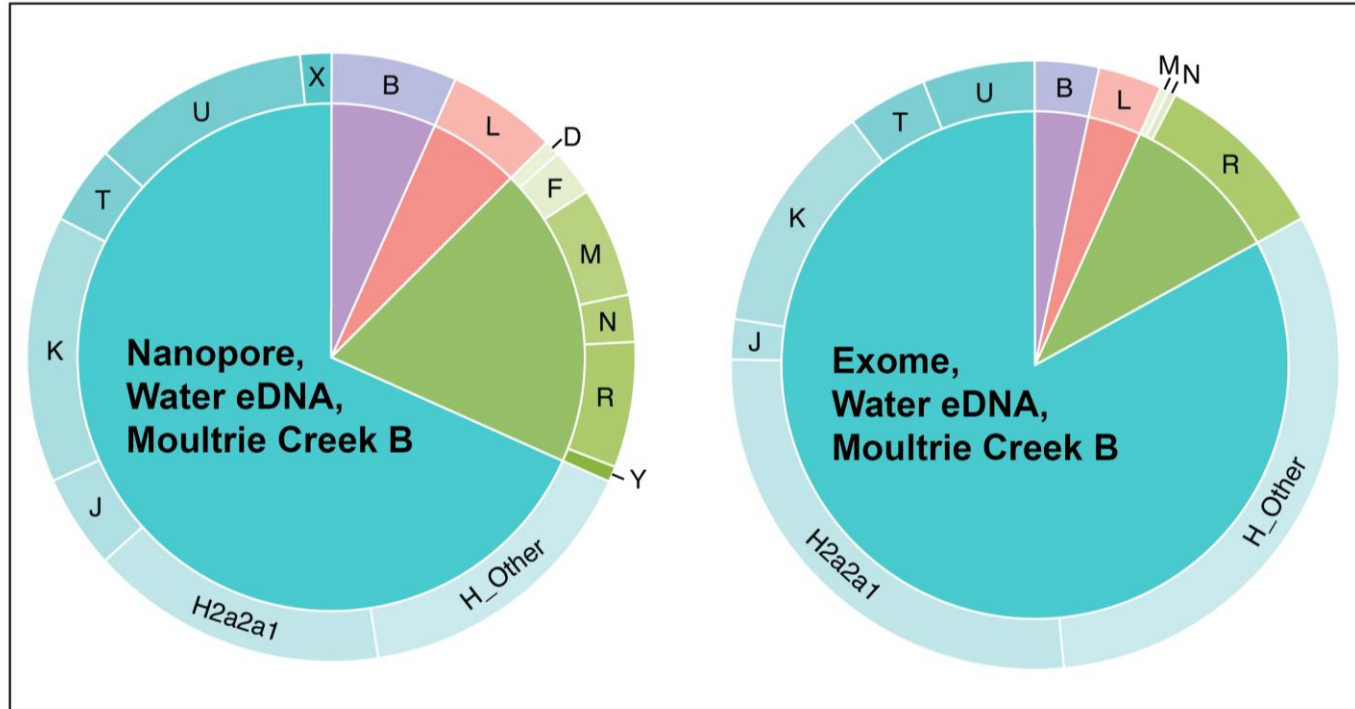


Sea turtle eDNA detectable in nesting beach sand tracks



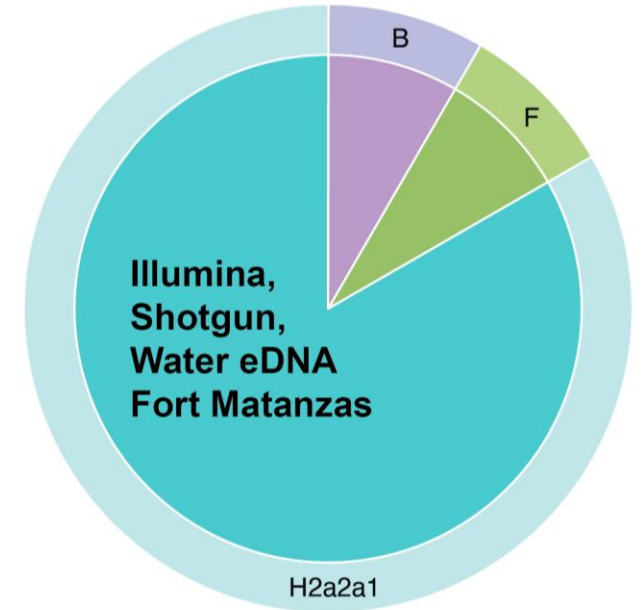
Sea turtle population genetics from sea water? Human eDNA as proof of principle

Water



Sample from previous slide

Same sample with human exome enrichment and Illumina sequencing



- African
- Asian
- Euro_Indian
- Native American

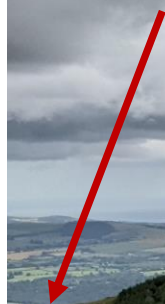
Goldmines Tributary



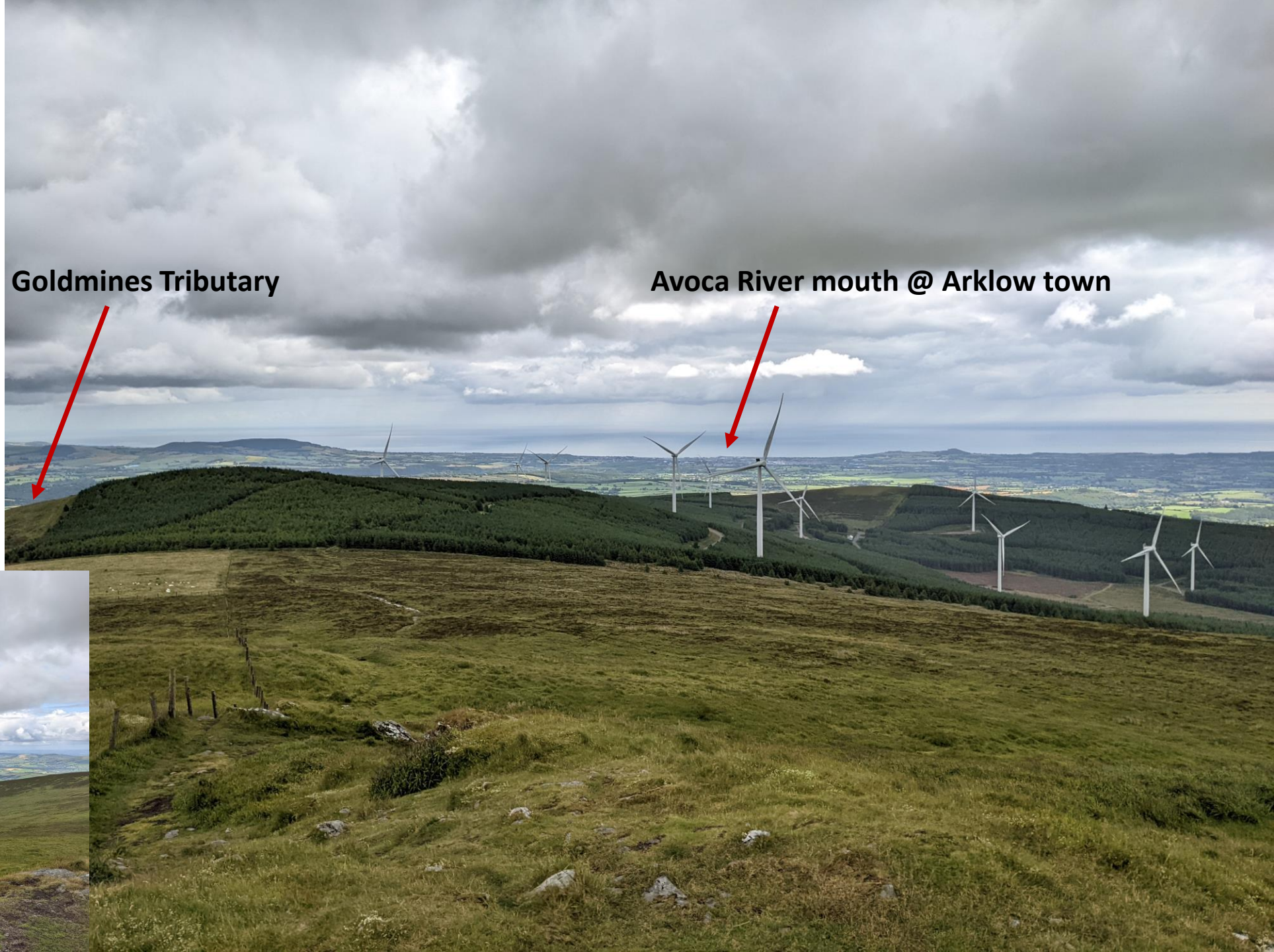
Avoca River



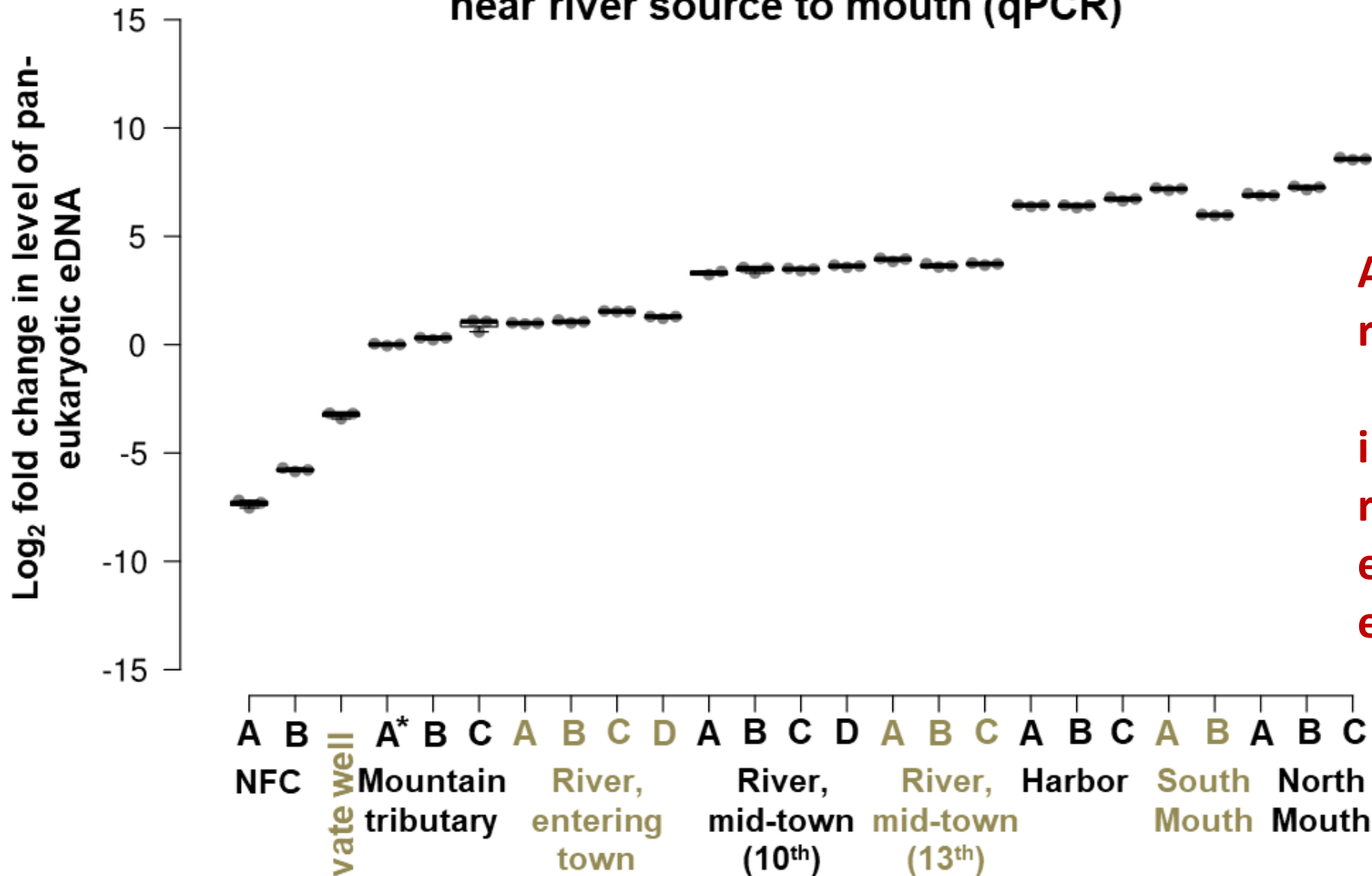
Goldmines Tributary



Avoca River mouth @ Arklow town



18s rRNA pan-eukaryotic eDNA level within each Irish sample, from near river source to mouth (qPCR)



All eukaryotic eDNA recovered per sample

i.e. eDNA successfully recovered from all samples, even those with no human eDNA

Source

* Set as reference sample, i.e. eDNA level set as 1.

Sea