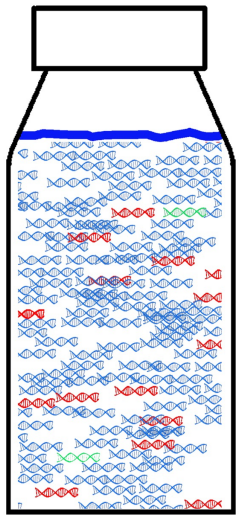
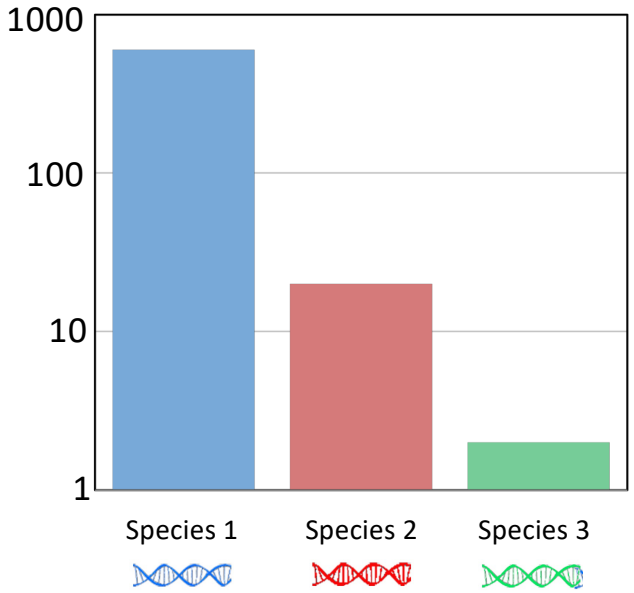
Riaz 12s Metabarcoding with DNA Standard Quantifies Marine Fish eDNA

also identifies threshold for reproducible amplification, and overcomes distortion due to non-fish vertebrate eDNA

Water sample



eDNA copies/species



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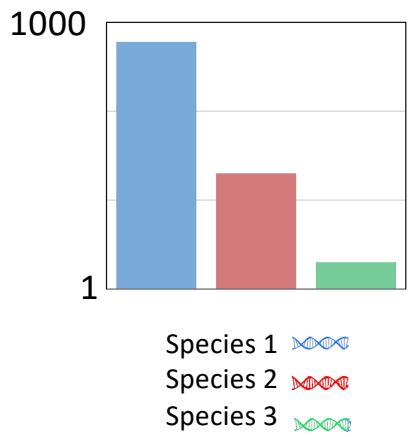
BACKGROUND

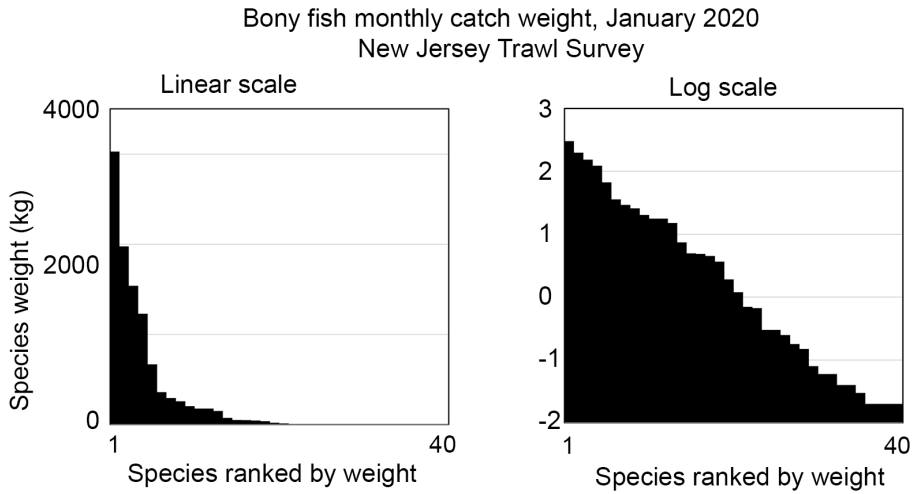
Why quantify marine fish eDNA?



- Quantitative censusing needed to
  - manage fishing impacts (set commercial, recreational quotas)
  - assess ocean protected areas, restoration efforts
- Potential advantages eDNA vs traditional methods
  - Low cost
  - Harmless to fish, environment
  - Applicable to difficult environments, elusive species
- Potential disadvantages, challenges
  - Relating eDNA to traditional survey techniques (capture, acoustic, visual)
  - No gold standard—all methods have biases

BACKGROUND

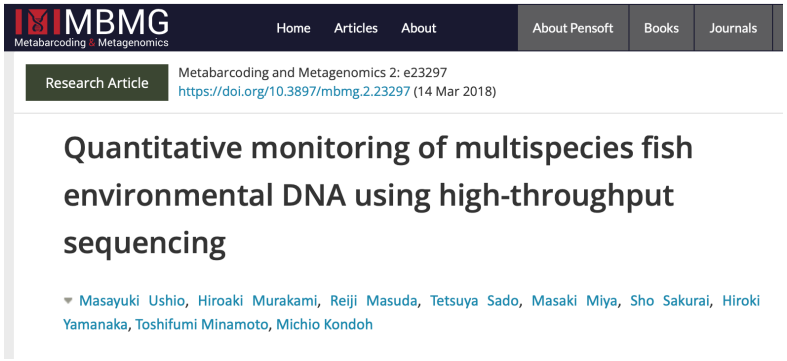
- Metabarcoding generally considered qualitative tool for relative eDNA abundance
  - *Commensurate experimental goal*
    - Develop metabarcoding as quantitative tool for absolute eDNA abundance



- Marine fish species differ in abundance over multiple orders of magnitude
  - *Commensurate experimental goal:*
    - Measure eDNA concentration with error less than 1 order of magnitude

BACKGROUND: Related studies

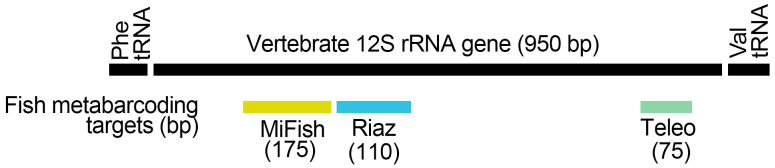
Ushio et al., 2018 ("qMiSeq" metabarcoding)



qMiSeq PROTOCOL

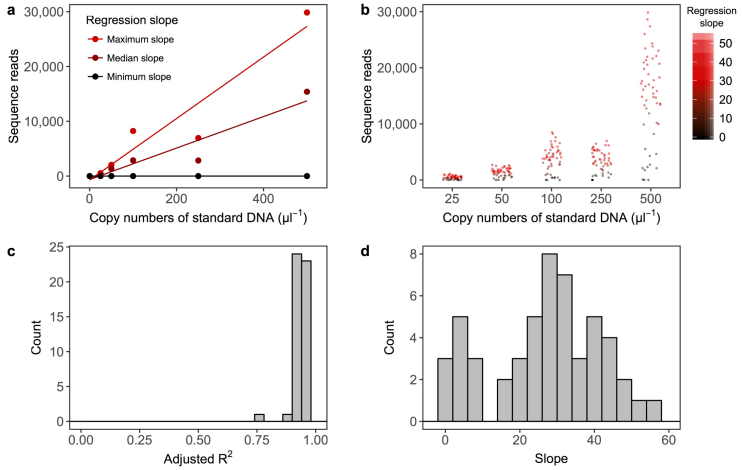
- Spike PCRs with mix 5 standard DNAs (25, 50, 100, 250, 500 copies)
- Calculate reads per copy standards, use to convert fish reads to fish copies
- qPCR to assess accuracy

Vertebrate metabarcoding targets



qMiSeq results (1)

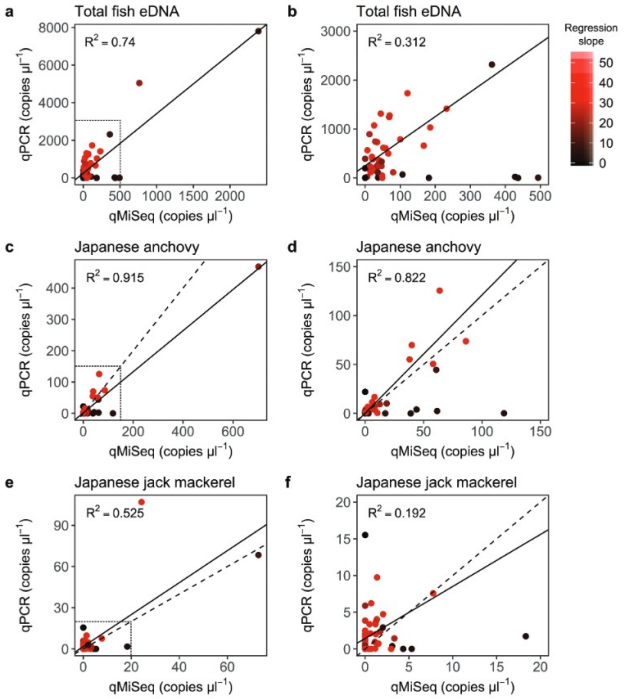
- DNA standards



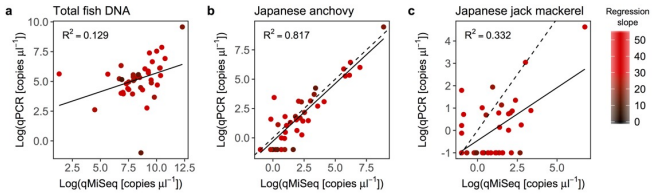
- Multiple samples little or or no amplification
- Standard 4 weak amplification

BACKGROUND: Related studies (Ushio et al. 2018, cont)

Linear scale



Log scale (base 2)

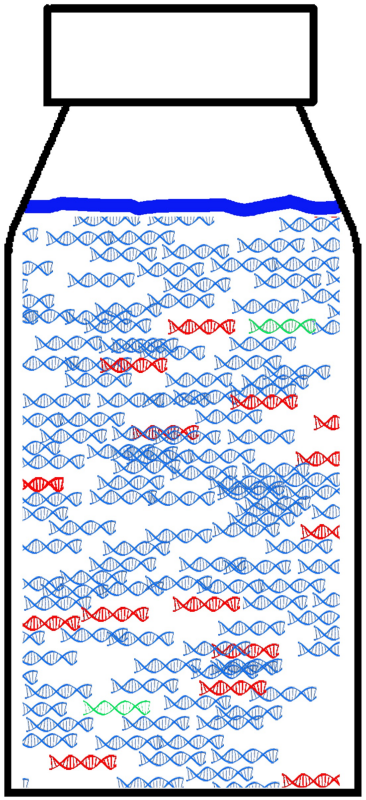


- Some samples qMiSeq>>qPCR
- Some samples qPCR>>qMiSeq
- Total fish eDNA weak correlation qMiSeq, qPCR
  - Total fish qMiSeq assay amplifies non-fish eDNA
- Overall mostly positive correlation reads vs copies but a lot of variation among samples, species

"calculated copy numbers showed significant positive correlation with those determined by quantitative PCR, suggesting that eDNA metabarcoding with standard DNA enabled useful quantification of eDNA"

- Major limitation may be that fish eDNA concentration was too low for reproducible amplification
- For example, median total fish eDNA 40 copies/ul; most (80%) species detections had <10 copies/ul sample

QUESTIONS

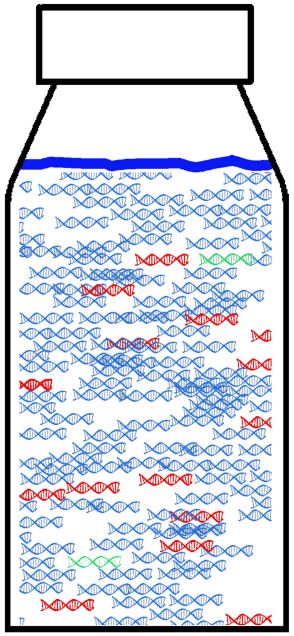


For marine bony fish

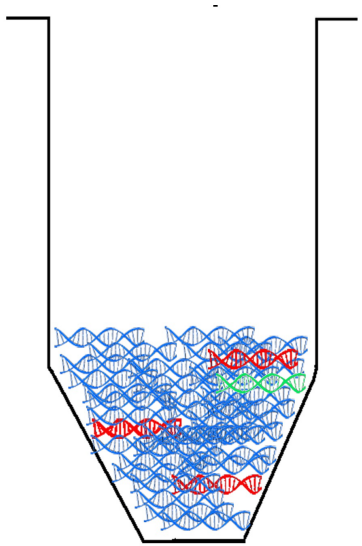
- Can 12S metabarcoding quantify relative, absolute concentration eDNA?
  - Does non-fish vertebrate DNA distort metabarcoding results?
  - How important are primer, PCR bias?
  - What are lower limits to quantification, detection?

METHODS

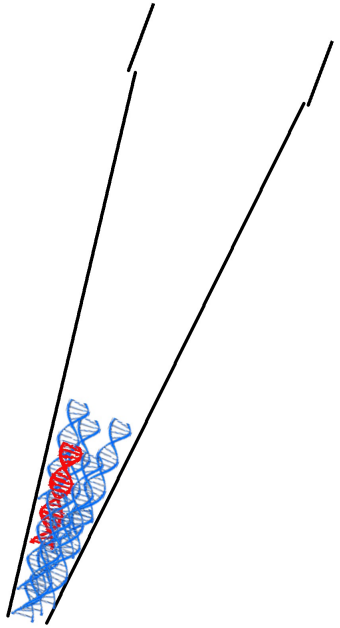
USUAL EXPERIMENTAL VOLUMES



1 L
WATER COLLECTION



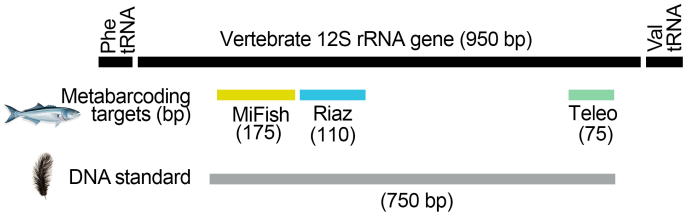
100 µl
DNA EXTRACTION



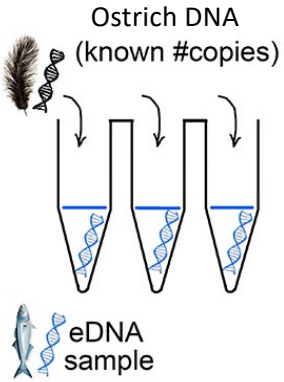
5 µl
PCR INPUT

METHODS-OVERVIEW

1. PREPARE DNA STANDARD



2. SPIKE PCRs WITH DNA STANDARD



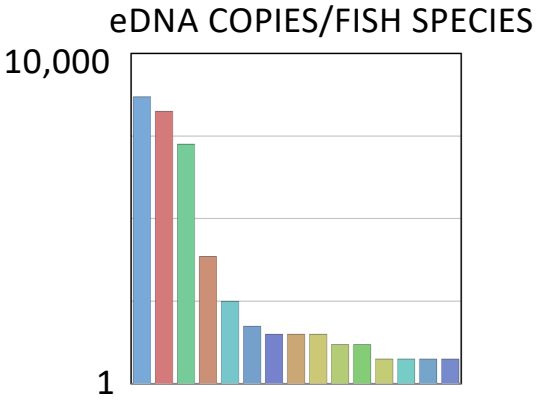
3. SEQUENCE, ANALYZE READS

Illumina MiSeq

SEQUENCE	LIBRARY	655-05AFAR-2022mar	655-600AFAR-2022mar	655-60AFAR-2022mar	655-6AFAR-2022mar	655AFAR-2022mar	657-06AFAR-2022mar	657-600AFAR-2022mar
Seq_1		670	839	206	655	827	17629	17926
Seq_2		0	5368	101	17	0	102	54761
Seq_3		29	50	19	14	12	106	211
Seq_4		69006	86614			3	8676	8264
Seq_5		5973	7907				68022	70963
Seq_6		0	0	0	0	0	0	0
Seq_7		0	0	0	0	0	0	0
Seq_8		11327	15439	4473	13228	11439	26942	30010
Seq_9		10127	13765	3951	11614	9909	16430	15049
Seq_10		7	38	0	15	13	0	142

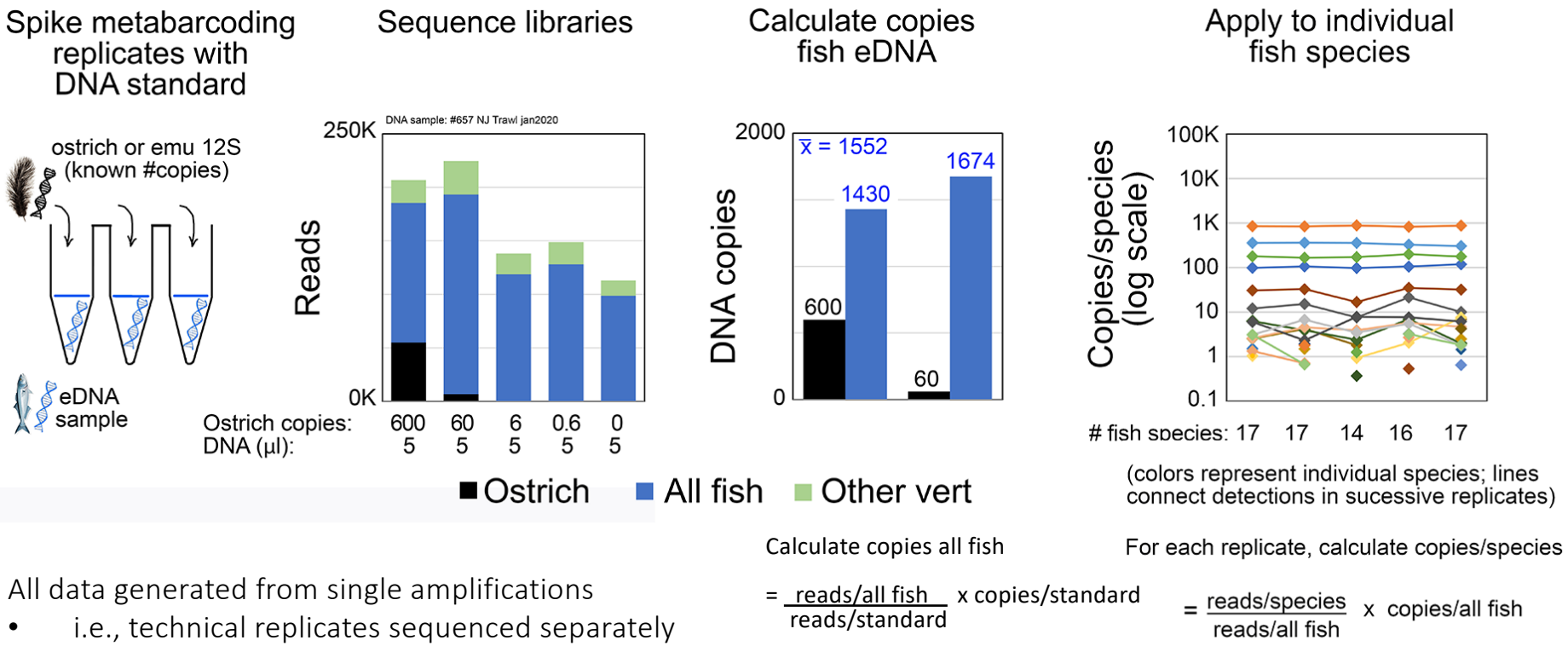
READS

4. CALCULATE COPIES FISH eDNA USING DNA STANDARD



METHODS

Quantifying Marine Fish eDNA with Metabarcoding



- All data generated from single amplifications
  - i.e., technical replicates sequenced separately

Calculate copies all fish

$$= \frac{\text{reads/all fish}}{\text{reads/standard}} \times \text{copies/standard}$$

For each replicate, calculate copies/species

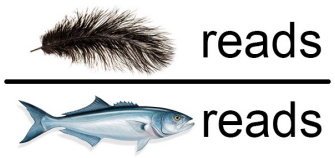
$$= \frac{\text{reads/species}}{\text{reads/all fish}} \times \text{copies/all fish}$$

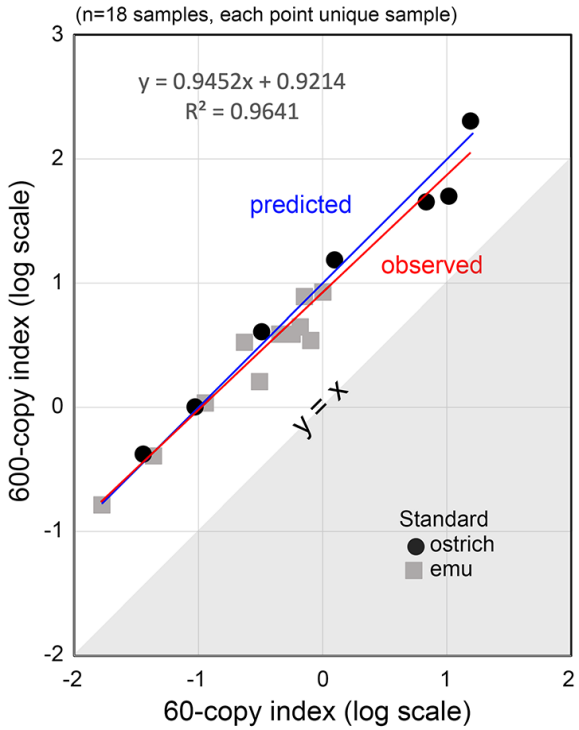
RESULTS

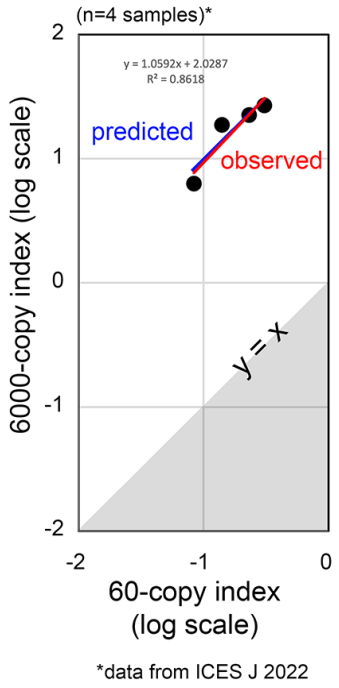
RELATIVE READS DIRECTLY PROPORTIONAL TO RELATIVE COPIES

- Linear over at least 1000-fold range (10 to 10,000 copies eDNA)
- Average deviation 1.2-fold; range, 1- to 2-fold (arithmetic scale)
  - Results within goal of <1 order of magnitude

INDEX FORMULA =





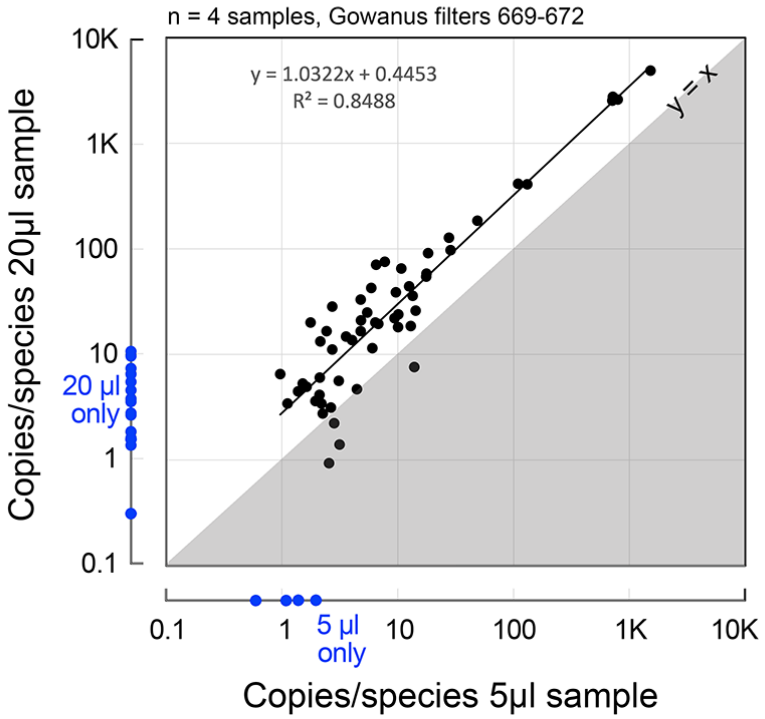


*data from ICES J 2022

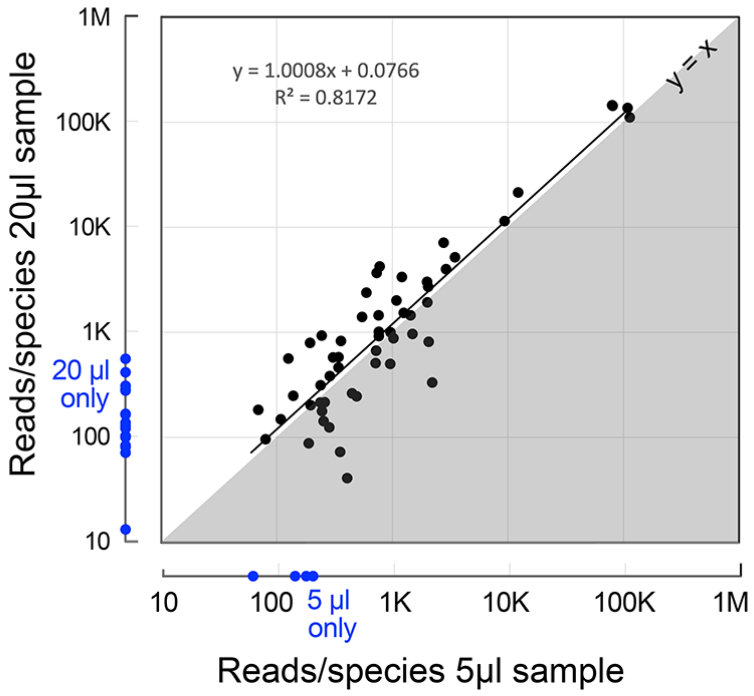
RESULTS

- Copies reflect amount DNA analyzed, reads don't
- eDNA rarity accounts for drop-outs, pick-ups (threshold ~10 copies/species)

COPIES

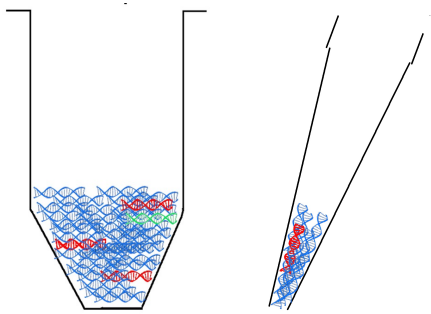


READS

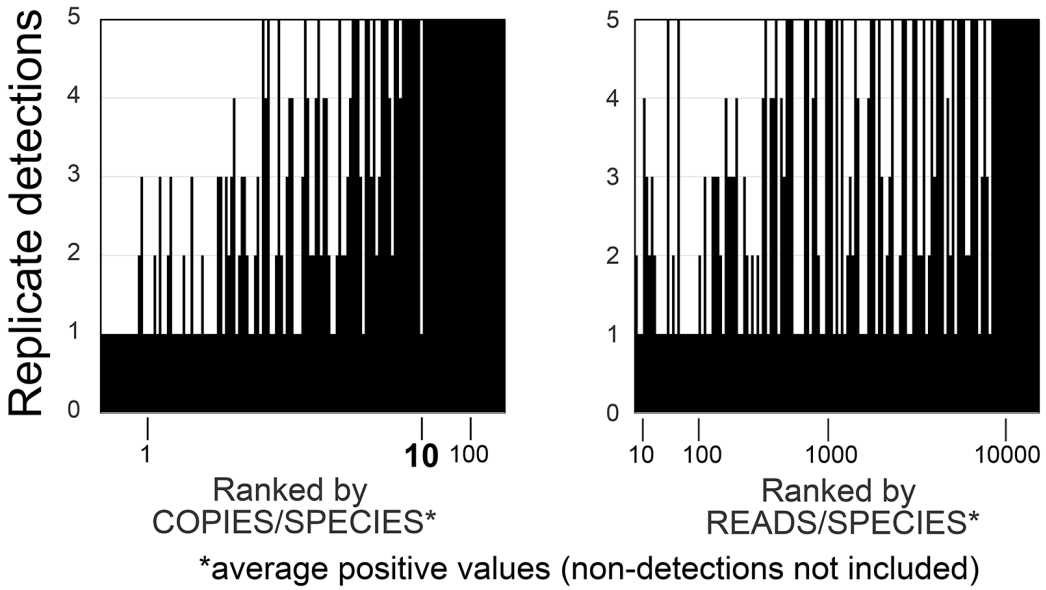


RESULTS

- Increased drop-outs below 10 copies/species
- Consistent with Poisson distribution rare eDNA among PCR aliquots

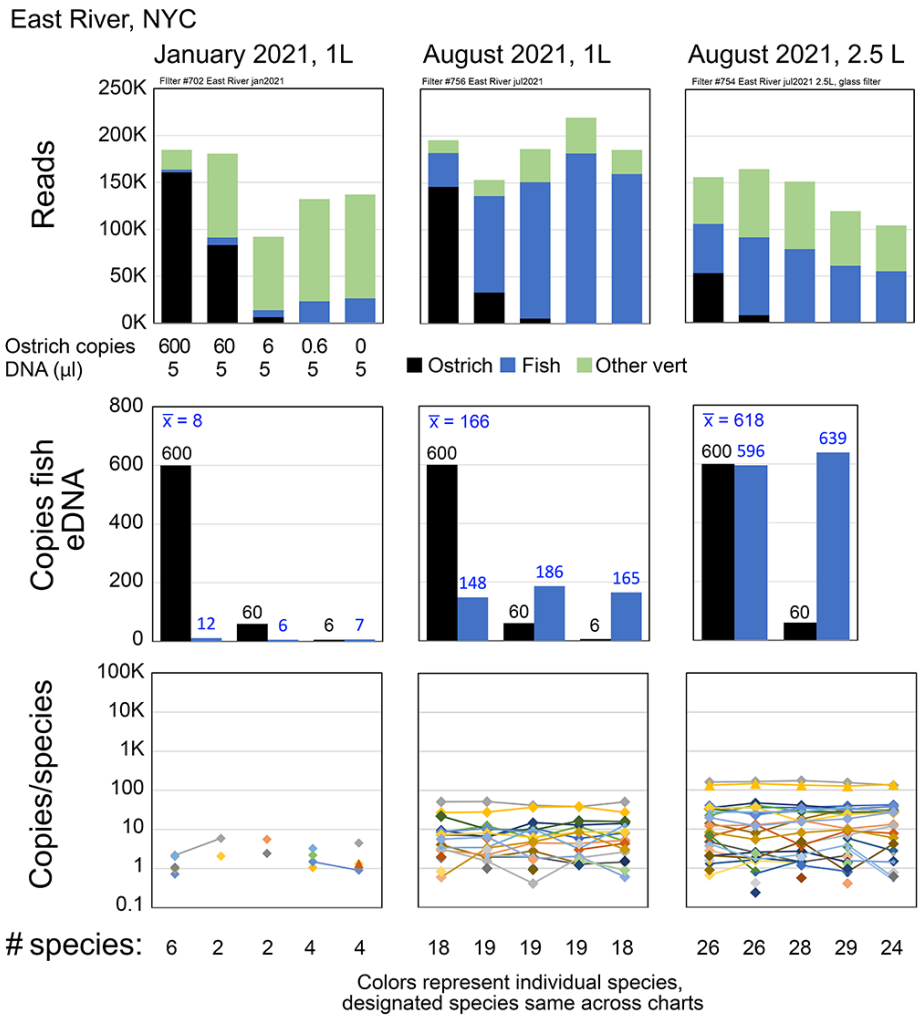


- Copies better than reads as predictor of drop-outs



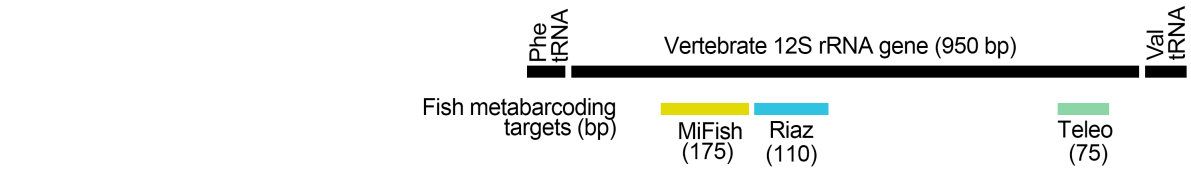
RESULTS

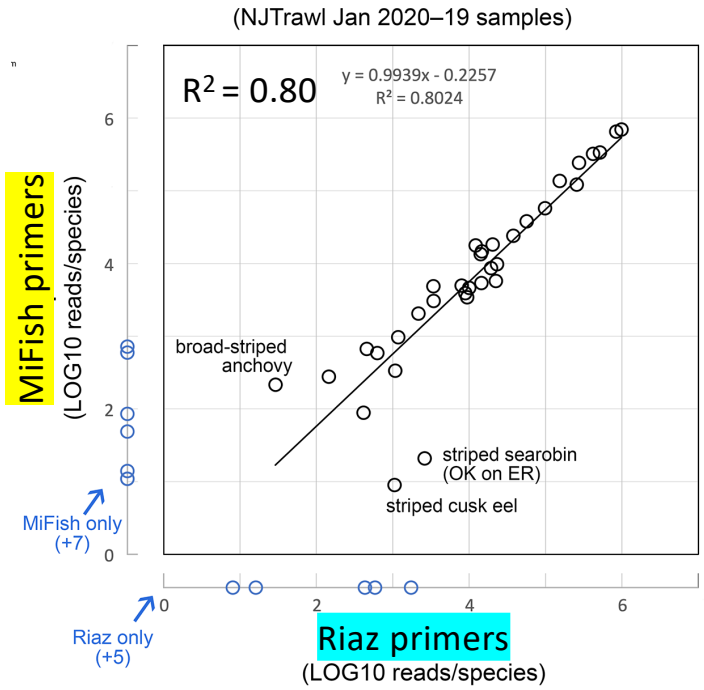
- Species detection robust to non-fish DNA
- Copies robust to non-fish DNA, reads not
- Copies consistent with season, water volume, reads aren't

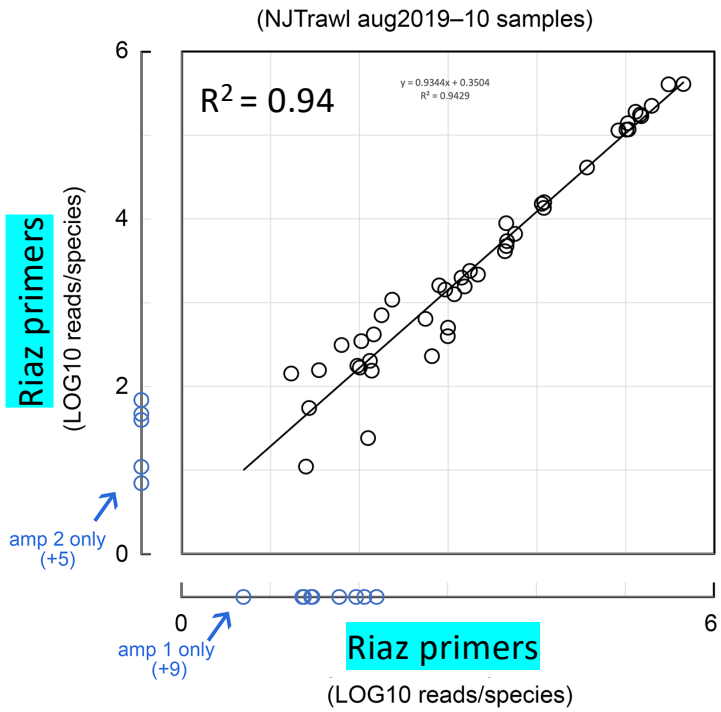


RESULTS

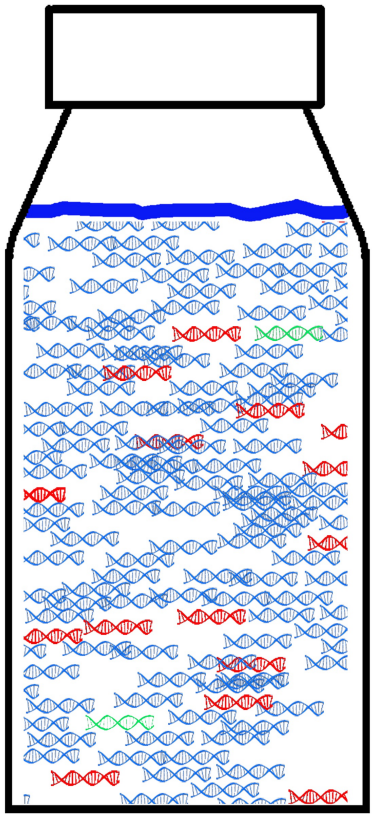
- Different primers, similar species detection, relative reads
- Consistent with modest primer, PCR bias







ANSWERS

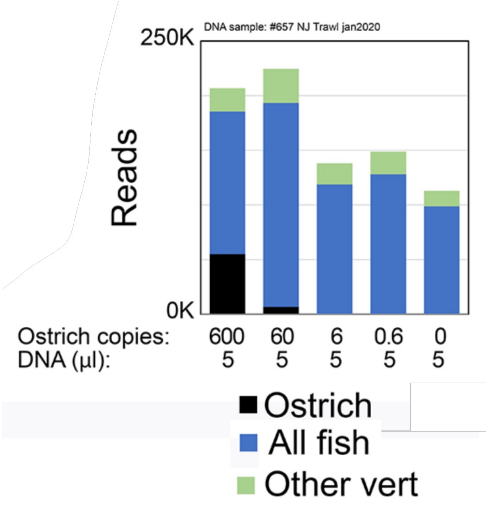


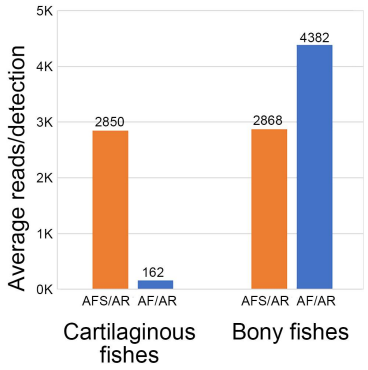
For marine bony fish,

Can 12S metabarcoding quantify relative, absolute concentration of species eDNA?	YES, WITH DNA STANDARD
Does non-fish vertebrate DNA distort metabarcoding results?	FOR COPIES, NO
How important is primer, PCR bias?	NOT CRITICAL
What are lower limits of quantification, reproducible detection?	10 COPIES

LIMITATIONS

- Bioinformatic pipeline filters out low level detections (<1/1000 reads per taxon); may eliminate true positives including DNA standard; could address with unique dual index primers
- Very abundant eDNA (>100,000 copies) suppresses amplification rare eDNA; could address with deeper sequencing
- Primer mismatch, PCR bias significant with some species, primer sets (e.g, Riaz primers not suitable for sharks, rays)

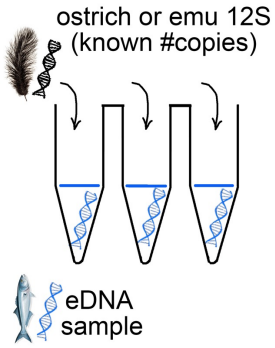




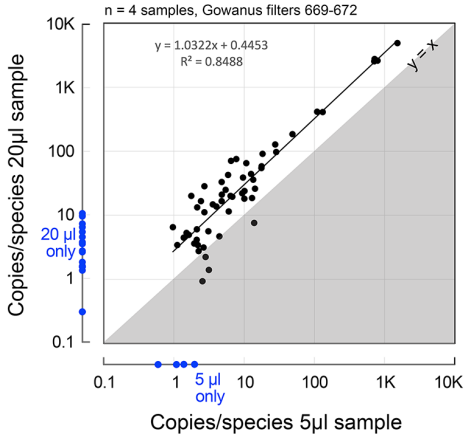
Frontiers Mar Sci 2020

PRACTICAL INFERENCES

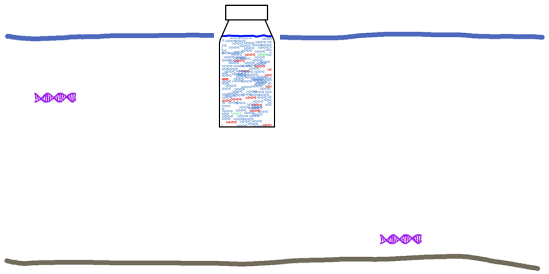
- DNA standard quantifies eDNA

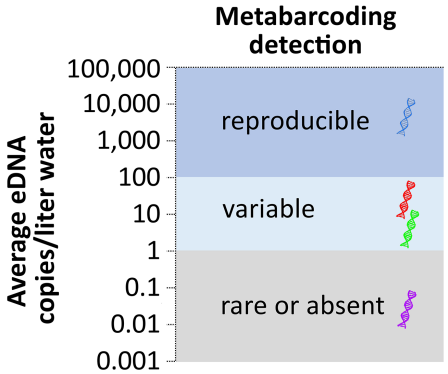


- To find less abundant eDNAs, analyze larger proportion DNA sample



- Larger or multiple water samples needed for rare eDNA

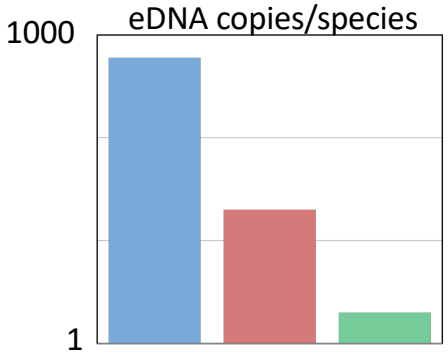


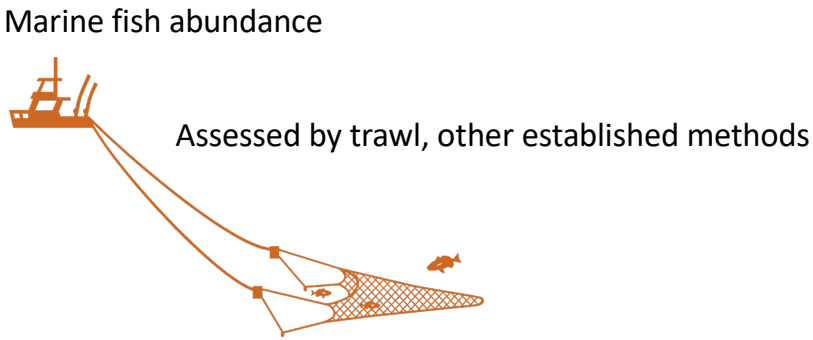


- Gloves during water collection, human blocking primers not routinely needed? (DNA standard corrects for contaminants)

LOOKING AHEAD

COMPARE quantitative eDNA metabarcoding to traditional survey methods





- Test quantified eDNA vs traditional measures fish abundance
  - Improve eDNA performance as index of absolute fish abundance
  - Monitor ecological restoration