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Applied molecular bio-surveillance in the northeast Pacific Part II:

Improving targeted detection and quantification

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Implement eDNA tools into invasive species management:

(1) Develop new eDNA tools to meet current management requirements.

(2) Understand how eDNA behaves with respect to traditional survey metrics.

OUTLINE

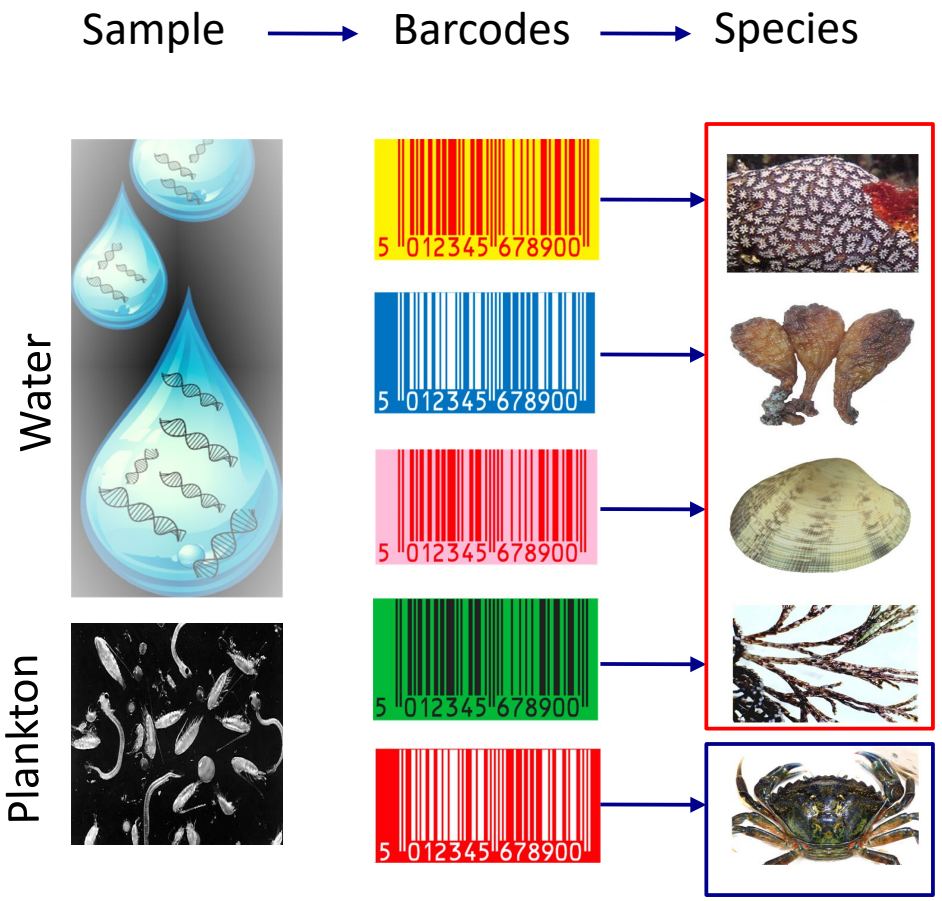
Background:

- Non-indigenous species (NIS) and Environmental DNA: targeted detection.

Targeted NIS detection, case study of the European Green Crab:

- Invasion of the green crab in the northeast Pacific.
- A new quantitative eDNA-based assay “targeted next generation sequencing”.
- Quantifying the relationship between traditional survey CPUE and eDNA availability.
- Compare detection probability and power between quantitative techniques.

Environmental DNA



Metabarcoding: broad detection

- multiple species
- biodiversity
- not quantitative*
- multiple markers (loci)
- high throughput sequencing

Barcoding: targeted detection

- single species
- can be quantitative
- qPCR & high throughput sequencing

Invasion of the European Green Crab

Carcinus maenas

- Came to North America in the 1980's and moved northward to the west coast of Vancouver Island in the late 90's.
- They exert considerable pressure on native biota with detrimental impacts.
- Major efforts in Canada and the USA to monitor the spread and eradicate new populations as they arise.
- Considerable potential for eDNA to monitor spread of populations, quantify abundance and assess eradication efforts.

Photo: https://wsg.washington.edu/crabteam/greencrab/

Targeted detection green crab

Improving detection and quantification of invasive green crab from eDNA[1].

Targeted Next Generation Sequencing (tNGS):

- eDNA amplified from qPCR primers in a single step reaction and sequenced directly on a next generation sequencer at equal volumes.
- Circumvents limitations of fluorescence based methods for DNA at low concentrations.
- Standard curves generated for each sequencing run used to extrapolate eDNA concentration of an unknown sample.

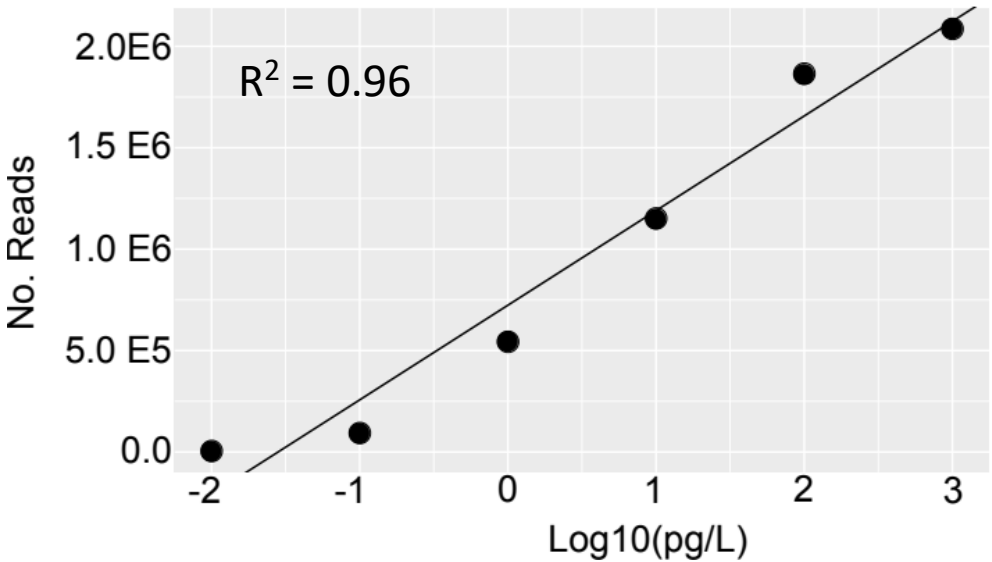


Figure 1: Example standard curve tNGS.

[1]Westfall, K.M., Therriault, T.W. & C.L. Abbott (prepared) Targeted Next Generation Sequencing of environmental DNA improves detection and quantification of invasive European green crab (*Carcinus maenas*).

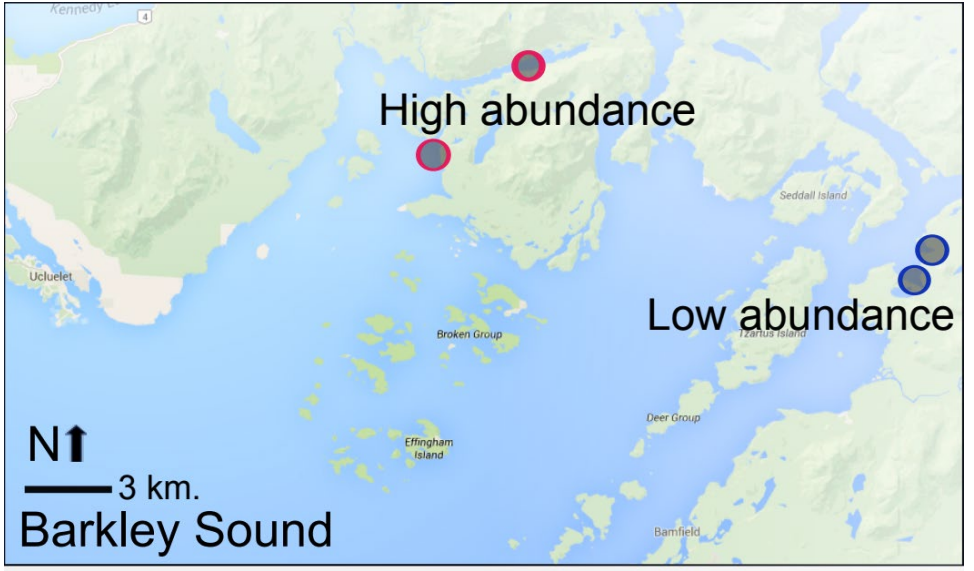
Targeted detection green crab

Improving detection and quantification of invasive green crab from eDNA[1].

i. Define relationship between traditional trapping CPUE and eDNA concentration.

ii. Estimate detection probability of green crab.

iii. Which method is more powerful at detecting changes in green crab eDNA concentration.



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Targeted detection green crab

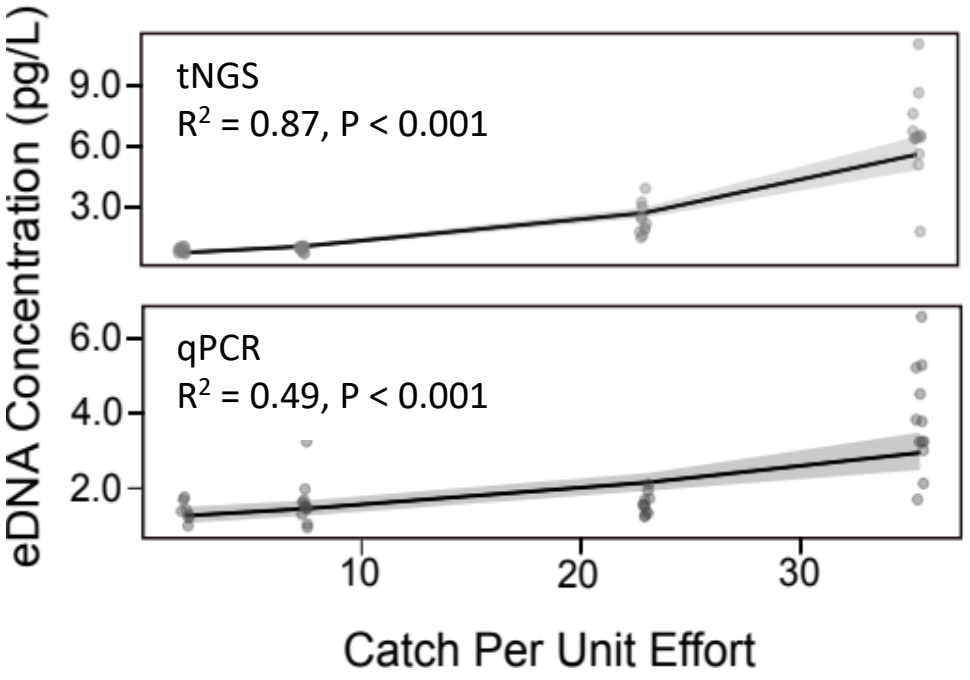

Figure 1: Predicted eDNA concentration from CPUE based on linear models. Shaded area is 95% confidence interval.

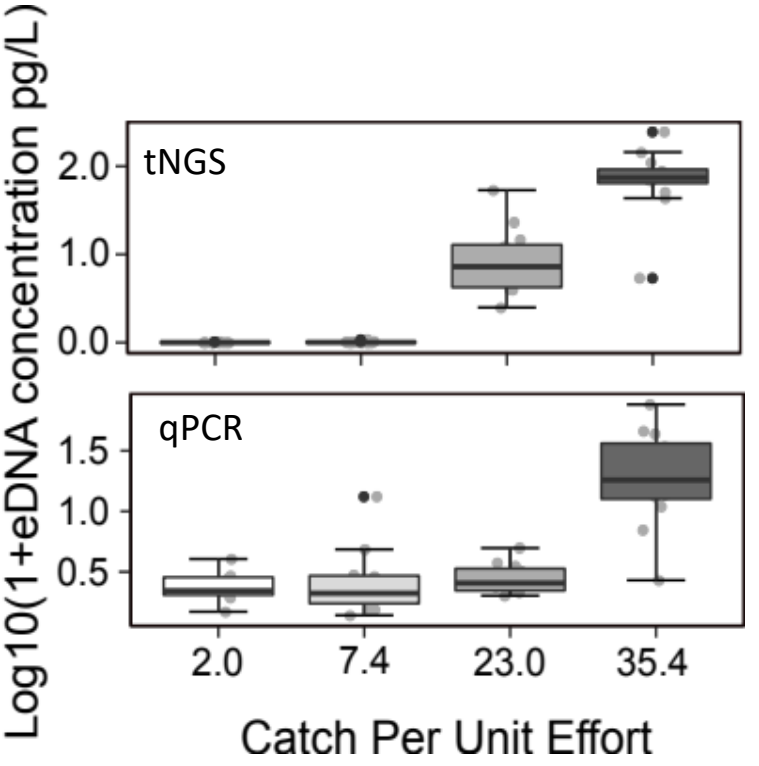

Figure 2: Box/dotplots of eDNA concentration by site marked by CPUE.

Targeted detection green crab

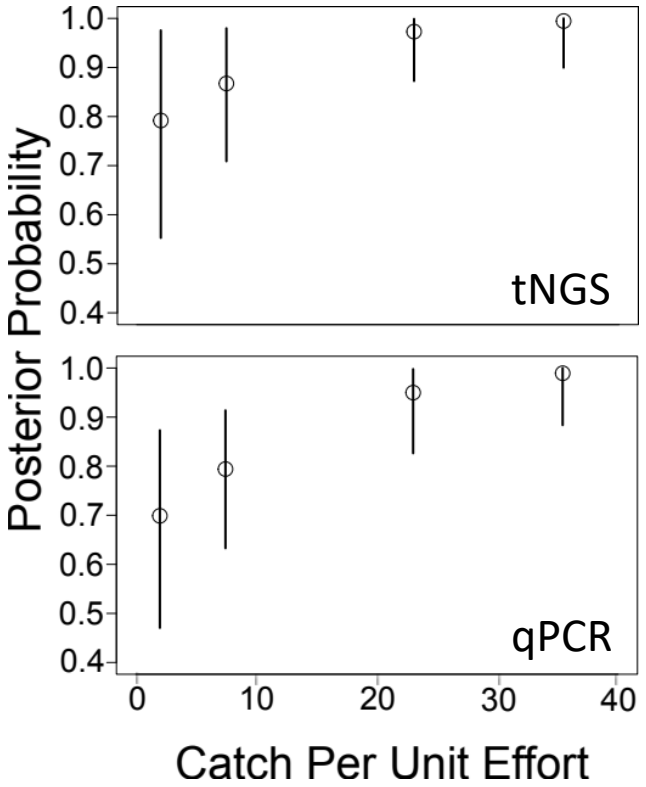

Figure 1: Estimated probabilities of occurrence of green crab eDNA related to CPUE.

Hierarchical multi-scale eDNA Occupancy Modelling:

- Estimates conditional probability of green crab occurrence in a sample given it is present at a site.
- Positive correlation between CPUE and probability of eDNA occurrence.
- High abundance sites: 95% to 99%, similar.
- Low abundance sites: 71% to 81%, different.

⟹ 7% - 10% increase in detection probability at low abundance.

Targeted detection green crab

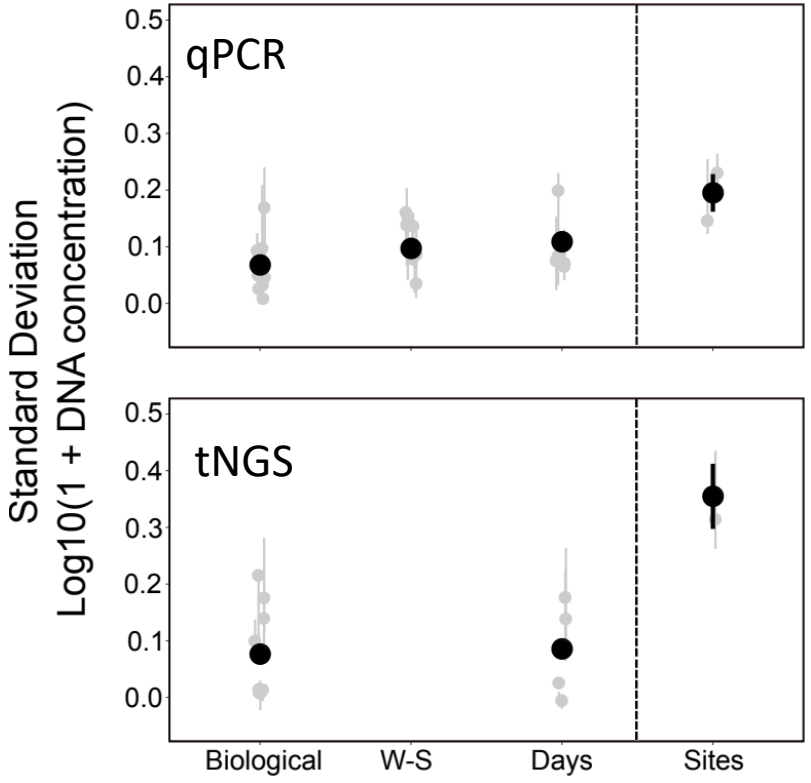

Figure 2: Partitioning sources of variation for qPCR and tNGS.

Sources of variation:

- Biological = variation among 6 replicates within a site within a day.
- W-S = added variation from qPCR technical replicates.
- Days = variation within sites between 2 sampling days.
- Sites = variation among sites within days (variation across Barkley Sound).

⇒ Greater variation between sites = more power to detect changes in eDNA concentration (and abundance).

Concluding remarks

New quantitative tool developed to meet specific management needs.

Quantitative eDNA surveys are a useful tool for monitoring green crab:

- eDNA concentration correlated with CPUE.
- new tNGS method increases power and sensitivity of eDNA detection.

Where do we go from here?

- standardized methods (sampling, laboratory, bioinformatics, reference libraries).
- implementation into monitoring programs.

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