Development of a Rapid Assay and a Portable Device to Detect Tuna Species

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Background

Illegal, **u**nreported, and **u**nregulated (**IUU**) fishing **violates the rights of Indigenous Peoples** to traditional fishing grounds, **compromises the food security** of legitimate fishers and coastal populations, **and facilitates human labor trafficking**.

Why Yellowfin Tuna?

- Bluefin and yellowfin tuna, because of their highly valued meat, are among the 13 species **most impacted by IUU**.

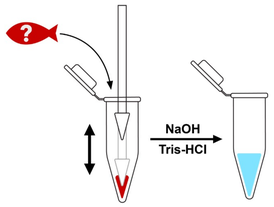
Problem

- Identification by **phenotype is nearly impossible**.
- **Current methods** require expensive polymerase chain reaction (PCR) instruments to detect species-specific DNA biomarkers. This process can take up to a week or more to receive results, **preventing** authorities from **promptly acting on the information**.
- To address this issue, we are **developing a novel biological assay and a portable heater/reader device** to detect different tuna species.

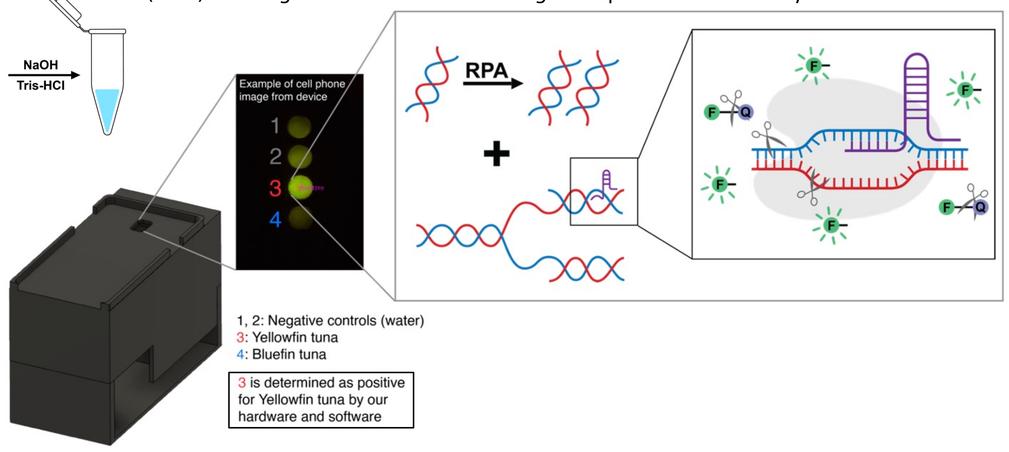
Materials and Method

Our biological assay consists of two simple steps:

(1) 10-min sample preparation by alkaline lysis (to **release DNA without purification**)

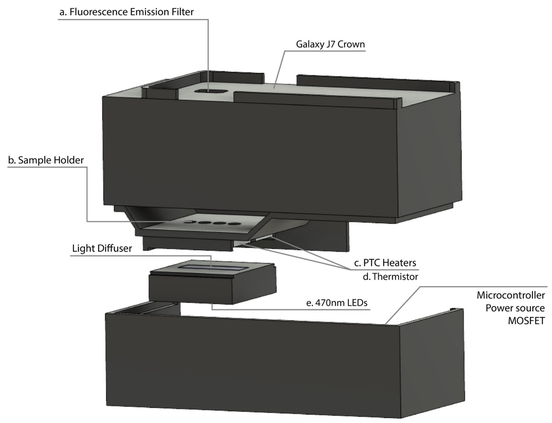


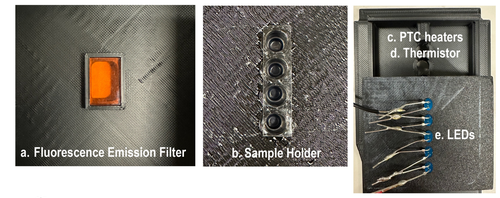
(2) 20-min single-pot isothermal detection by recombinase polymerase amplification (**RPA**) and target-activated trans-cleavage of reporter substrates by **CRISPR Cas12a**



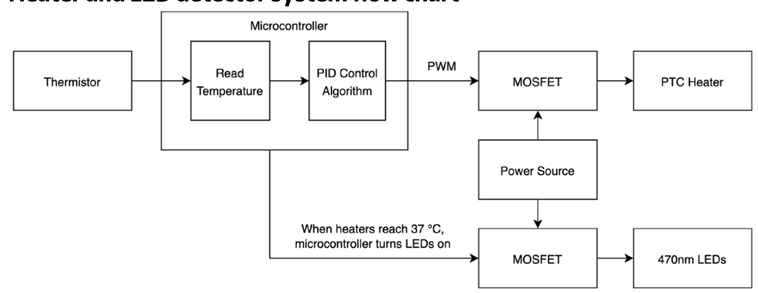
1, 2: Negative controls (water)
3: Yellowfin tuna
4: Bluefin tuna

3 is determined as positive for Yellowfin tuna by our hardware and software



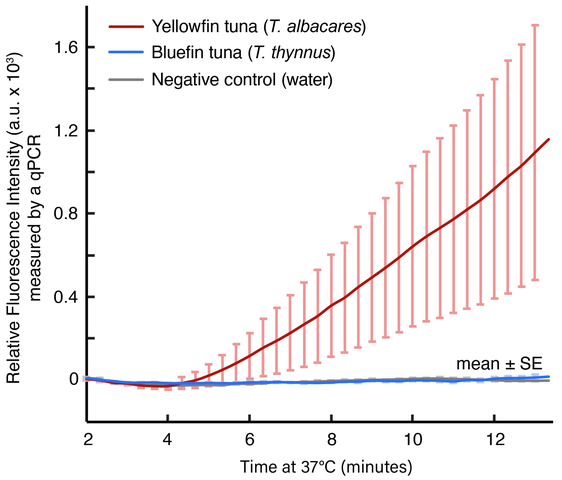


Heater and LED detector system flow chart



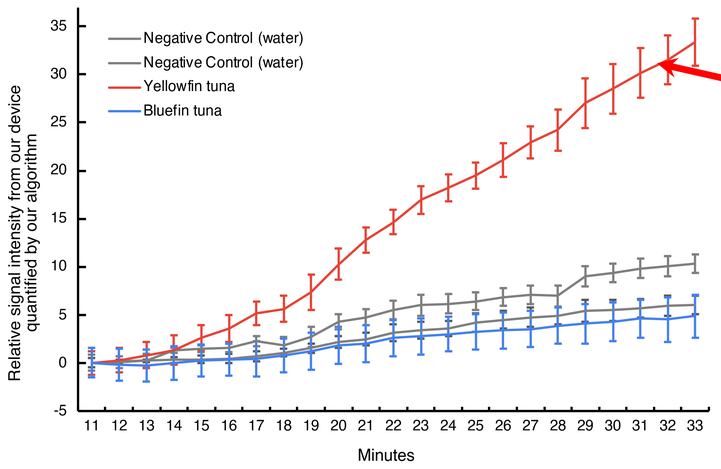
Results and Discussion

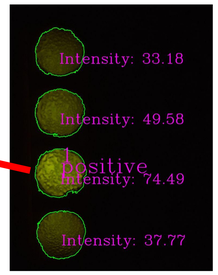
Our assay performed on a commercial qPCR machine



Analysis of our final optimized assay on lysates derived from yellowfin, bluefin, and water control (n=3 for each group) using a commercial qPCR machine with detection as early as 5 minutes. **After 13-14 min** signal crossed 1000 relative fluorescence units, a level **detectable by our heater/detector device.**

Our assay performed on our heater/detector device





Water, bluefin tuna lysate, and yellowfin tuna lysate were **tested by our heater/detector device**. Cell phone images **after 15 minutes** of incubation were classified by our in-house software, correctly indicating positive results for yellowfin tuna lysates and negative results for bluefin lysates and water controls.

Our algorithm analyzes the images every minute during the reaction. A threshold was set to classify negative samples and/or background (lower intensity) from the positive sample (intensity above the threshold).

Conclusions

- We demonstrated a workflow that can sensitively and specifically detect yellowfin tuna samples within 30 minutes from sample to result.
- Our novel assay and portable device can offer a rapid method for identifying yellowfin tuna on-site, providing actionable info to aid in combating IUU fishing.

Acknowledgments

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