Combating Seafood Fraud: Development of two Seafood Assays

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Background

- Seafood mislabeling is a rampant issue in the U.S.
- Up to 96% of shrimp served in restaurants in West Florida are fraudulently labeled as locally caught but, upon testing, were found to be imported shrimp [1].

Seafood species substitution is associated with a slew of risks [2], and they are as follows:

- *Allergens* - the consumer is making allergen decisions based on what is labeled and not what is received
- *Pathogens* – The level of food safety enforcement in different parts of the world varies, which makes the seafood imported from areas with less strict enforcement prone to foodborne pathogens.
- *Contamination* – species substitution can expose the consumers to a species that is associated with potentially worse contamination (e.g., mercury)

- The most direct impact of seafood fraud is the economic suffering of domestic fisheries, as seafood substitution reduces the demand for their products. This hurts the US domestic shrimpers, as they have no chance to compete with the cheap imports. [1]

Introduction

- Seafood mislabeled is common in the US, and they are frequently replaced with frozen imported alternatives.
- According to the NOAA, up to 40% of seafood may be mislabeled in the US. [3]
- The current method for identification of a seafood sample is DNA barcoding, which takes 2-3 days, and is very costly, as samples must be shipped offsite and processed with sophisticated equipment and highly trained staff.

Fig.1: Royal Red shrimp from Coast of Alabama

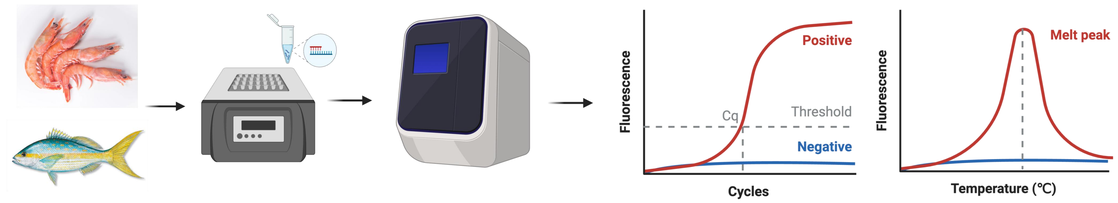
Fig.2: Yellowtail snapper from Jacksonville

Aim of the Research

- The goal of this project is to develop two rhPCR-lateral flow assays for the identification of Royal Red Shrimp (*Pleoticus robustus*) and Yellowtail Snapper (*Ocyurus chrysurus*).
- The study aimed to standardize and validate an assay for the identification of the target species within 120 minutes using a low-cost PCR instrument

Methods

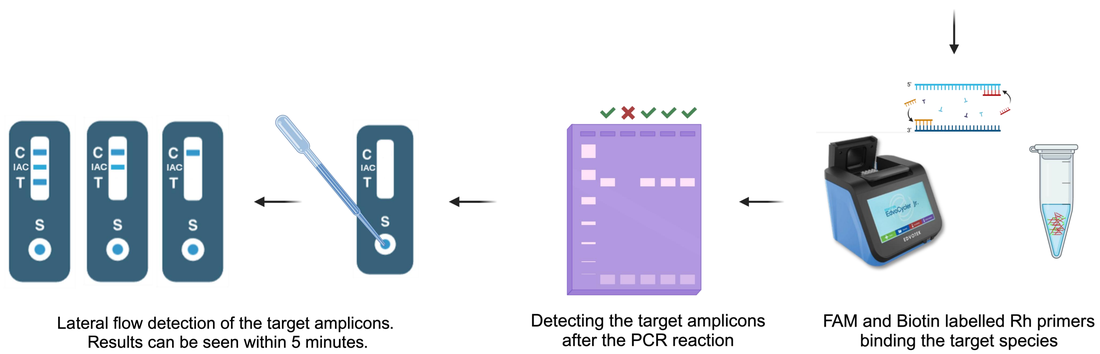
- The cytochrome oxidase 1 (COI) gene of the target species was used to design rhPCR primers for the identification of Royal Red Shrimp and Yellowtail Snapper species.



Rapid heat lysis-based extraction of DNA from tissue sample

Rh primers binding to target species with SybR Green mastermix

Data analysis using the mean Cq values and the melting peaks of the target amplicons



Lateral flow detection of the target amplicons. Results can be seen within 5 minutes.

Detecting the target amplicons after the PCR reaction

FAM and Biotin labelled Rh primers binding the target species

Fig. 3: Overview of the methods used for optimization and standardization of the assay

Results

- Both assays were optimized using 8 Royal Red samples and 11 Yellowtail Snapper, respectively. The assays were further tested using 33 negative samples that included other closely related snapper and shrimp species.

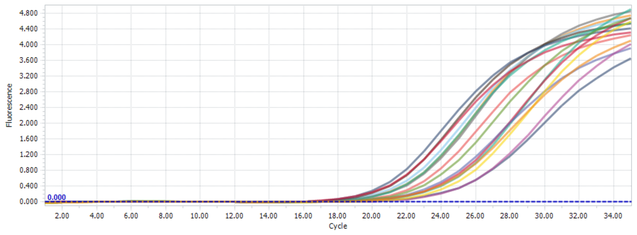


Fig.4 Amplification curve showing positive amplification of Royal Red samples in qPCR:

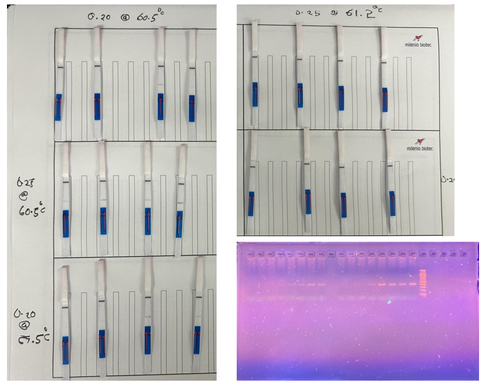


Fig. 5 Lateral flow and agarose gel Results showing bands for different optimization conditions for Yellowtail Snapper

References:

[1] SeaD consulting, “Exposing the Dark Underbelly of the Seafood Scene in Tampa-St. Pete.” Accessed: Feb. 28, 2025. [Online]. Available: https://www.seadconsulting.com/news-and-media/media-kits/exposing-the-dark-underbelly-of-the-seafood-scene-in-tampa-st-pete

[2] Oceana, 2013, “Oceana Study Reveals Seafood Fraud Nationwide,” Oceana. Accessed: May 30, 2024. [Online]. Available: https://oceana.org/reports/oceana-study-reveals-seafood-fraud-nationwide/

[3] N. Fisheries, “Seafood Fraud | NOAA Fisheries,” NOAA. Accessed: Feb. 28, 2025. [Online]. Available: https://www.fisheries.noaa.gov/national/sustainable-seafood/seafood-fraud