Rapid Smelt Species Identification in the San Francisco Estuary using CRISPR-based SHERLOCK

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Objective

Design a diagnostic assay for rapid species identification in the field

Introduction

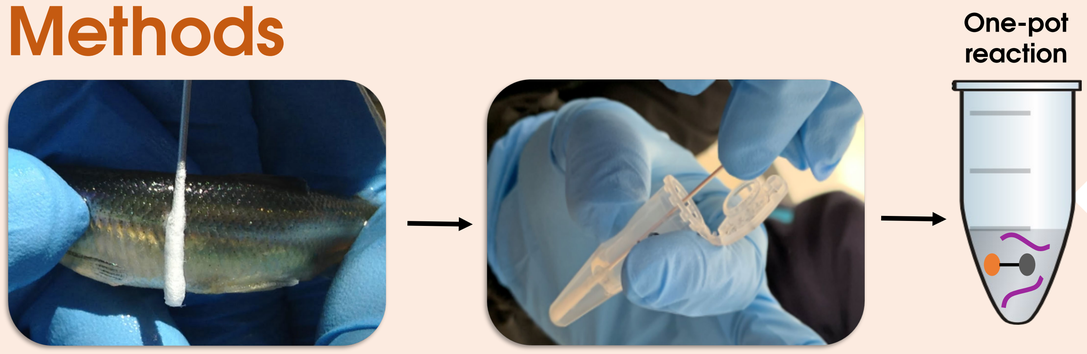
CRISPR-Cas13a-based SHERLOCK (Specific High-sensitivity Enzymatic Reporter unlocking)

- SHERLOCK is a powerful molecular tool that enables real-time species or subspecies identification from nearly any location
- This technology has been pioneered by the healthcare field for viral diagnostics, but has yet to be implemented by molecular ecologists

Photos by René Reyes, USBR

- We focused on three morphologically similar Osmerid species co-occurring in California's San Francisco Estuary, the U.S. threatened and California endangered Delta Smelt (DSM), the California threatened Longfin Smelt (LFS), and the non-native Wakasagi (WAG)
- These smelt are difficult to distinguish as at early life stages- misidentifications can lead to incorrect abundance and distribution estimates

Methods

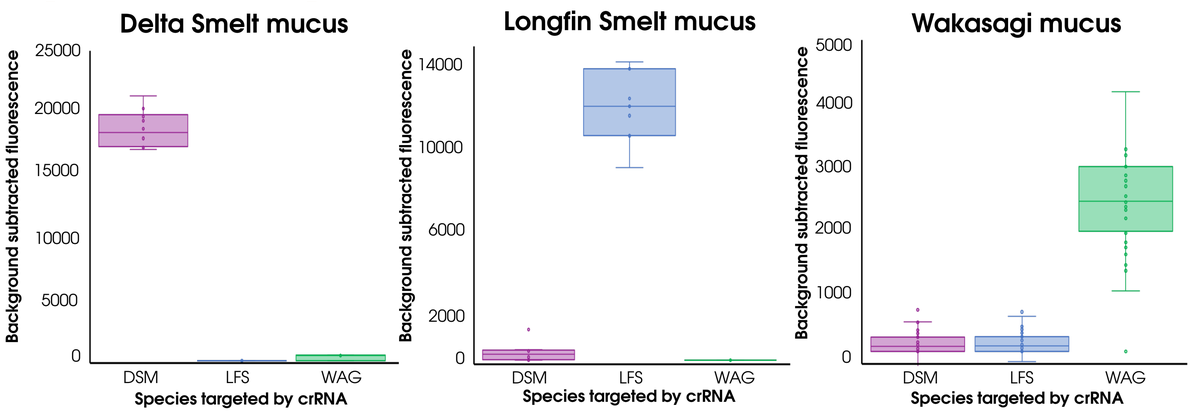


Just swab a fish, swirl in PBS buffer, add a few microliters to the one-pot SHERLOCK reaction & insert tube in fluorescence reader

- A region of Cytochrome-b containing species-specific SNPs was used for primer and crRNA design
- Traditional, minimal, and non-extraction methods were tested for DNA input
- Assays were validated with genomic DNA
- A qPCR machine was used to measure fluorescence values

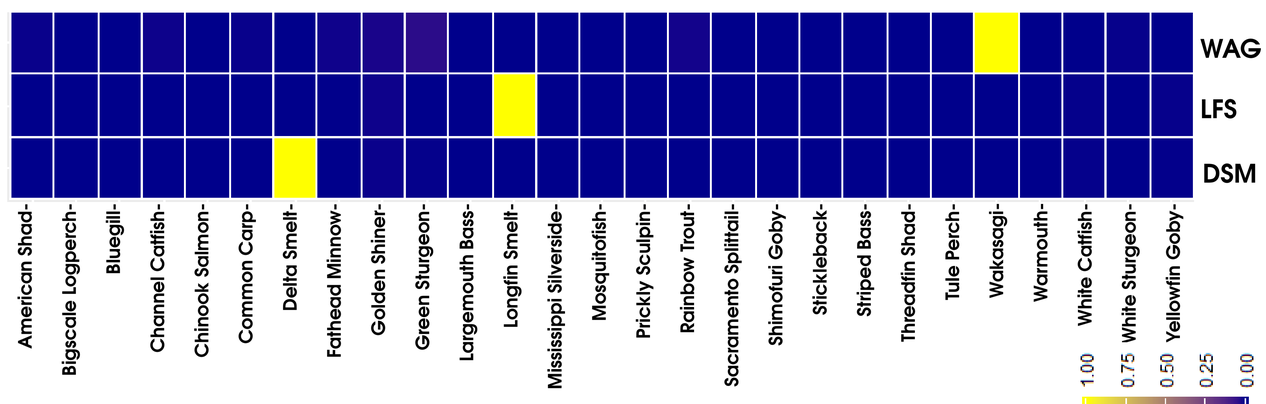
• NO DNA EXTRACTION REQUIRED • DIAGNOSTIC TEST • RESULTS IN UNDER AN HOUR

Specificity



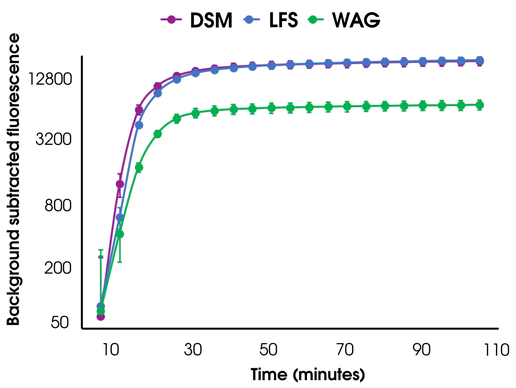
Osmerid species specificity based on fluorescence readings after 1 hour. Mucus swabs (without DNA extraction) from each target were tested against all three species-specific crRNAs. For target species, N=10 (DSM), N=7 (LFS), and N=39 (WAG) and ranged from 3 – 10 for each non-target species

Species-specific identification with each row representing a crRNA and each column representing a common fish species found in the San Francisco Estuary. Fluorescence values are the background subtracted averaged from two biological replicates per species followed by normalization

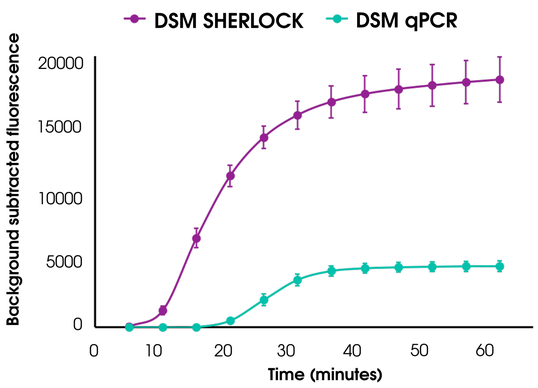


• NO CROSS-SPECIES AMPLIFICATION

Speed and Sensitivity



SHERLOCK time course. Fluorescence of species-specific crRNA combined with 20 ng DNA from each target species (measured every 5 minutes over a 110 minute time course). 3 biological replicates averaged per species and error bars are 1 S.D. error bars



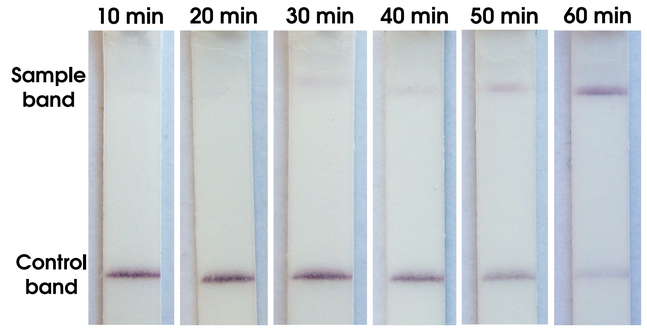
Comparison of DSM SHERLOCK and qPCR time course. The qPCR reaction also used 20 ng DNA as template and amplified the same Cyt-b region as SHERLOCK by using a TaqMan assay. Fluorescence was measured every 5 minutes for 60 minutes. 3 biological replicates averaged with 1 S.D. error bars

Field deployablity

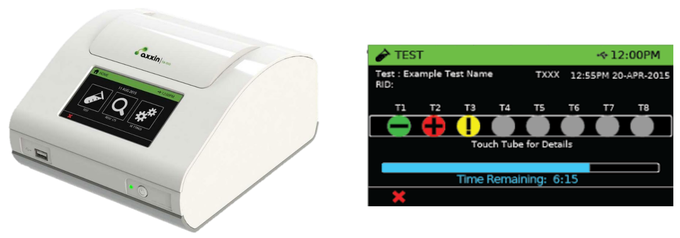
• EASY-TO-INTERPRET RESULTS

via lateral flow or portable fluorescence reader

- One-pot reactions are incubated at 37°C and then run on a lateral flow strip
- No expensive equipment needed
- Results cannot be seen quite as rapidly



Delta Smelt mucus time series



- One-pot reactions are loaded directly into the reader with a clear pos/neg results window
- 8 or16 samples can be processed simultaneously
- Small with rechargeable battery and hard case

• REAL-TIME DATA COLLECTION IN THE FIELD

Conclusions

• PUBLICATION IN MOL. ECO. RES. https://onlinelibrary.wiley.com/doi/full/10.1111/1755-0998.13186

- Has the potential to revolutionize management and monitoring practices (save both time and money)
- We are currently optimizing the field deployability for use by non-molecular biologists, with minimal training required
- This technique can be applied to an expansive range of organisms and across many fields of conservation biology

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