Development of a novel genetic tool for rapid identification of red drum (Sciaenops ocellatus) eggs
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Abstract

Red drum, Sciaenops ocellatus, is an economically important finfish found in the Atlantic Ocean from Massachusetts through the Gulf of Mexico and is a common recreational fish along South Carolina's shore. S. ocellatus does not sustain a commercial fishery, and fundamental knowledge about its life history needed to accurately determine stock is lacking. Egg production indices can estimate a species' spawning stock biomass when information about breeding adults is relatively unknown. Egg identification for S. ocellatus and related species typically entails time-consuming microscopy that relies on morphological differences for species-level identification. These morphological distinctions may not become apparent until 24 hours after hatching.

Sandwich hybridization assay (SHA) is a molecular method that allows for species or taxa-specific identification by direct detection of unpurified and unamplified large subunit (LSU) ribosomal RNA (rRNA). SHA has been successfully used to identify and quantify numerous planktonic taxa including harmful algal bloom species, zooplankton, invertebrate larvae, and bacteria. SHA utilizes two oligonucleotides, a signal probe and species or taxa specific capture probe, conjugate and enzyme substrate that produces a colorimetric response that can be accurately determined. Results from sequencing of LSU rRNA for S. ocellatus and other closely-related Sciaenids indicated low genetic divergence, so probe design efforts are currently focused on the more variable ITS region.

Approach

Sandwich Hybridization Assay (SHA)

- LSU rRNA-targeted DNA probes
- No amplification or purification of sample homogenate needed
- Optical density quantifies LSU rRNA (Figure 2)
- Used with a range of planktonic species and taxa (e.g., Scholin et al., 1996; Goffredi et al., 2006)

Figure 2. SHA schematic. A capture probe, anchored on a solid support medium, hybridizes to LSU rRNA. Next, a biotinylated signal probe hybridizes to the rRNA at a location near the capture binding site, creating an LSU rRNA sandwich. The digoxygenin-labeled signal probe binds to an avidin and horse-radish peroxidase (HRP) conjugate, which binds to a HRP substrate, producing a colorimetric response that can be measured at 450 and 650 nm.

Probe Development

- Sequenced LSU rRNA of red drum and closely related species. Results showed less species variability than expected, so efforts are focused on the more variable ITS region for species-specific capture probe design.
- Ability to quantify eggs will be assessed by generating standard curves using laboratory spawned eggs.
- The probe’s range and level of detection will be evaluated.

Other Considerations

- Effects of egg viability on the assay signal (Figure 3).
- Ontogeny - laboratory-spawned red drum egg samples are collected and analyzed over the developmental period prior to hatching

Citations


Renkas, B. 2010. Description of periodicity and location of red drum (Sciaenops ocellatus) spawning in Charleston Harbor, South Carolina. Masters Thesis, College of Charleston, SC.


Acknowledgements

I appreciate all of the assistance from SCDNR Inland Fisheries Division, particularly Bill Roumillat for his technical skills, advice, and help with field work. Another thank you goes to the SCDNR Murrells Inlet facility for providing red drum eggs for this study. Thank you to Dr. Dianne Greenfield and Dr. Steve Arnott without whom this project would not be possible. Thank you to my committee: Dr. Allan Roumillat for his support and guidance, and Dr. William J. Jones and the Environmental Genomics Core Facility for RNA sequencing capabilities and direction. This project is funded from South Carolina Sea Grant Award #M387.