Title
High-Throughput Molecular Identification of Fish Eggs and Larvae

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California Sea Grant College Program
Completion Report

Project Information

Year | **Grant No.:** NA10OAR4170060

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Title High throughput molecular identification of fish eggs and larvae

Project Leader

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Project Hypothesis

Ichthyoplankton surveys can reveal the location, timing and intensity of spawning activity for many fish species and are widely used to assess fisheries resources. However, the utility of these surveys is limited by the fact that many eggs and larvae cannot be identified to the species level using morphology alone. This project was motivated by the hypothesis that fish eggs and larvae can be more accurately identified by species specific DNA sequences than by morphology alone.

Project Goals and Objectives

The primary goal of this project was to develop a high throughput methodology for identification of fish eggs. To distinguish among 100 different species (as in the waters of the California Current), existing methods consist of morphological identification (often inaccurate for even some distantly related species) or molecular identification of eggs requiring sequencing each egg, resulting in high costs and low throughput. Our goal was to make molecular identification faster and more affordable.

Briefly describe project methodology

Fish species can be distinguished based on short sequences of mitochondrial DNA (mtDNA)that are called "DNA barcodes." Based on a DNA barcodes database for California marine fish (developed by P. Hastings and R. Burton with previous Sea Grant funding), we developed species-specific 25 base oligonucleotide probes. Using suspension bead array (Luminex) technology, we attached each probe to a different color bead (beads come in 100 colors so we can multiplex 100 probes in the assay). The target genes (mitochondrial COI and 16S from the barcodes database) were amplified from individual eggs and hybridized to the probes. The hybridized probes are detected via flow cytometry with the Luminex 100. The color of the beads to which the DNA binds uniquely is determined by the Luminex and identifies the egg.

Describe progress and accomplishments toward meeting goals and objectives.

A bead array consisting of 36 probes targeting 26 different species was successfully developed and validated by double blind tests. The array successfully distinguished a wide variety of species, including several pairs that cannot be distinguished by morphology (e.g., sanddab vs. halibut and white sea bass vs. barracuda). This work has now been published in a peer-reviewed scientific journal.
In addition, good progress has been made on using the array (and direct DNA sequencing) on a time series of samples collected from the CalCOFI program. Plankton samples obtained up to ten years ago have been sorted and fish eggs from various stations have been identified using the array. This retrospective work is continuing with renewed Sea Grant support.

PROJECT MODIFICATIONS: Explain briefly any substantial modifications in research plans, including new directions pursued and ancillary research topics developed. Describe major problems encountered and how they were resolved.

Our project has used Luminex technology that is almost a decade old. Recently Luminex released new instrumentation and we have received free long-term loan of the MAGPIX instrument and 75% discount on supplies to test this new machine in our fish egg identification project. The new approach can employ all our existing probes while speeding sample handling. The new machine is much smaller and more portable than the older version, making shipboard application much more feasible. We found that the MAGPIX instrument is a considerable improvement over the Luminex 100. It does, however have two limitations: 1) over 50 probes can be multiplexed at once (vs. 100), and the supplies for the new machine, at list price, double the cost of egg identification. This increase in price combined with dramatically lower prices now available for direct DNA sequencing (by third party vendors) means that except when shipboard analyses are conducted for adaptive sampling purposes, land-side sequencing is likely to be the method of choice where start-up capital for equipment and supplies is limiting.

PROJECT OUTCOMES: Briefly describe data, databases, physical collections, intellectual property, models, instruments, equipment, techniques, etc., developed as a result of this project and how they are being shared.

This project has produced the following primary outcomes:
1) We have found that fish eggs sorted from ethanol-preseved sampled over a decade old can be successfully identified using DNA-barcoding. Methods for DNA extraction, PCR amplification, sequencing and probe hybridization have been developed and verified.
2) Short species-specific oligo nucleotide probes can be developed based on the existing barcodes database.
3) The molecular identification procedure can reduce the frequency of errors inherent in morphology-based identification.

IMPACTS OF PROJECT: Briefly describe how this project has contributed to a discipline; to developing human resources; to developing physical, institutional or information resources; technology transfer; and society beyond science and technology. Please notify CASG of impacts that occur after your project ends; CASG may contact you after your project ends to learn about additional impacts that occur over time.

Although the approach has the potential for refining stock assessments and thereby influence resource management, sufficient data analyses have not yet been completed.

BENEFITS, COMMERCIALIZATION, AND APPLICATION OF PROJECT RESULTS: Please list any companies, agencies, organizations or individuals who have used your project results, scientific/technical advice, etc., and provide names, emails and phone numbers. Briefly describe how results were used and quantify results and socioeconomic benefits, if possible.

The methodologies developed are now being applied to two sets of samples: 1) existing (and future) CalCOFI ichthyoplankton samples covering the past 15 years, and 2) a new set of samples taken from the end of the SIO Pier. The later will be used to establish a baseline of spawning activity around the local MPA so future studies can document changes in the fish fauna in response to management efforts.

ECONOMIC BENEFITS generated by discovery, exploration and development of new, sustainable coastal, ocean and aquatic resources (i.e., aquaculture, marine natural products, foods, pharmaceuticals).

Issue-based **forecast capabilities** to predict the impacts of a single ecosystem stressor, developed and used for management (i.e., climate change, extreme natural events, pollution, invasive species, and land resource use).

As analyses of the ichthyplankton samples are extended, patterns of change mmay be correlated with environmental changes in a way that could enhance forecasting of fisheries resources.

Tools, technologies and **information services** developed (i.e., land cover data, benthic habitat maps, environmental sensitivity index maps, remove sensing, biosensors, AUVs, genetic markers, technical assistance, educational materials, curricula, training).

Publications (list in appropriate category below) Each listing should be a stand-alone bibliographic reference, including all authors' names. For each Publication type, specify title, authors, date and journal details, where appropriate (repeat headers as necessary).

Technical Reports

Title	Authors	Date

Conference Papers, Proceedings, Symposia

Peer-reviewed journal articles or book chapters

Journal Molecular Biology Resources **Issue Num** Volum **Page Num** 57-66 **Date** 2012

Title High-throughput molecular identification of fish eggs using multiplex suspension bead arrays. **Authors** Lani U. Gleason and Ronald S. Burton

Non-peer Reviewed Reprints

High Throughput Molecular Identification of Fish Eggs Using Bead Arrays	Lani U. Gleason and Ronald S. Burton	2012

Publications, Brochures, Fact Sheets

Books & Monographs

Handbooks, Manuals, Guides

Electronic publications: (non-print formats).

Maps, Charts, Atlases

Theses, dissertations

Newsletters, periodicals

Program reports (annual/biennial, strategic plans, implementation plans)

Educational Documents

Topical Websites and Blogs

Miscellaneous documents (not listed above).

MEDIA COVERAGE: Select 'Yes' or 'No'. If yes, describe any radio, TV, web site, newspaper, magazine coverage your project has received. Send original clippings or photocopies to the Sea Grant Communications Office.

Although filmed in 2007 during previous Sea Grant support, my public lecture entitled "DNA Forensics in Marine Ecology," (one of the Perspectives on Ocean Science series at the Birch Aquarium, 3/14/2007; 56 minutes) is still available and has now logged over 155,000 views. The lecture describes the origin of our Sea Grant supported research that led to the current project. http://www.ucsd.tv/search-details.aspx?showID=12106

MEDIA NOTES: Brief description of the type media coverage your project has received.

DISSEMINATION OF RESULTS: List any other ways in which results of your project have been disseminated. Indicate targeted audiences, location, date and method.

The primary concept involved in this work is that fish eggs (and any other fish tissues) in California marine waters can now be identified by DNA sequence. This implies that: 1) all fish species have different DNA sequences, and 2) all tissues and life stages of a given species have the same DNA sequence. Our outreach project seeks to teach these basic biological concepts to 5th grade students using a quick, hands-on laboratory experience. With our outreach partners, Ocean Discovery Institute (ODI), we developed an exercise where students use micropipettors to load standards and an "unknown" DNA sample on a prepared agarose electrophoresis gel (provided by Life Technologies Corp, Carlsbad, CA) and determine the identity of the unknown by comparison to the standards. Over 500 students from underserved San Diego County schools participate in the exercise annually; the ODI staff do the presentation with input and materials from the Burton lab - with Sea Grant support for the PI, Sea Grant trainee, and molecular biology samples.

WORKSHOPS AND PRESENTATIONS: A brief description of location, date, time, topic, number of attendees and name of presenter.

none

COOPERATING ORGANIZATIONS: List those (e.g., county or state agencies, etc.) who provided financial, technical or other assistance to your project since its inception. Describe the nature of their cooperation.

Federal Organizations

NOAA's Southwest Fisheries Science Center Ichthyoplankton Lab

Regional Organizations

State Organizations

Nongovernment Organizations

Outreach partner: Ocean Discovery Institute

International Organizations

Industry Organizations

Luminex Corp., Austin, TX

Academic Organizations

Sea Grant Organizations

Other Organizations

INTERNATIONAL IMPLICATIONS: Does your project involve any colleagues overseas or have international implications?

The molecular identification methodologies developed in this project could be applied to ichthyoplankton surveys in neighboring regions including Mexico and Canada.

AWARDS: List any special awards or honors that you, or any co-project leaders, have received during the duration of this project.

KEYWORDS: List keywords that will be useful in indexing your project.

PATENTS: Please list any patents or patent licenses that have resulted from this project, and complete the patent statement form available on the web site.

NOTES: Please list any additional information in the notes area

FOR ALL STUDENTS SUPPORTED BY THIS GRANT, PLEASE LIST:

Volunteer Count 1

Graduate Student Info