

## **Pelagic Fish Egg Abundance and Mortality Estimation by CUFES and Real-Time Machine Vision**

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### Introduction

Assessment of the distribution and abundance of fish eggs in the sea is necessary to estimate fish spawning stock size and habitat. Knowledge of stock size is needed by fisheries managers to control catch. Knowledge of spawning habitat is needed to better manage fished stocks in an ecosystem context, and for policy formation. Our primary objective was to improve the methods used to assess the distribution and abundance of fish eggs. We did so by using machine vision, involving video and computers, to image, identify, and count eggs of target fish species collected with CUFES, the Continuous, Underway Fish Egg Sampler, in real time. The result is REFLICS, the Real-time FLOW Imaging and Classification System. Our secondary objective was to use this technology to investigate the spawning and mortality of eggs of pelagic fish, including the Pacific sardine (*Sardinops sagax*) and northern anchovy (*Engraulis mordax*) off Southern California.

### REFLICS

Fish eggs, and other particles and bubbles, flow in water between the concentrator and sample collector in CUFES (<http://cufes.ucsd.edu/text/descr.htm>). REFLICS images those objects and each is classified, e.g., as a sardine egg, copepod, bubble, or other type. Our system consists of a flow tube, line-scan video camera, illuminator, computer, and custom software (Fig. 1). Water with particles is backlit and continuously imaged with the line-scan camera. The computer acquires digital data from the camera and processes it with custom software. REFLICS software (1) produces 2D images from sequential line scans; (2) creates a background image from multiple, sequential images; (3) segments objects that differ from the background image in a specified manner (Fig. 2); (4) classifies segmented objects into two or more pre-defined classes, based on features extracted for each object (e.g. size, shape, and intensity). At present, we have tested REFLICS with CUFES at sea in April 2003 and 2004 on Scripps research ships performing tasks 1-3. Task 4, while now able to run in real time, has been to date been performed ashore on data acquired at sea.

Figure 1. REFLICS. Water from CUFES concentrator (not shown) passes upward through the flow cell at  $20 \text{ l min}^{-1}$  and on to CUFES sample collector (not shown). Particles are back lit by the illuminator and imaged by the line-scan camera. Data are transferred from camera to computer for real-time acquisition and analysis.

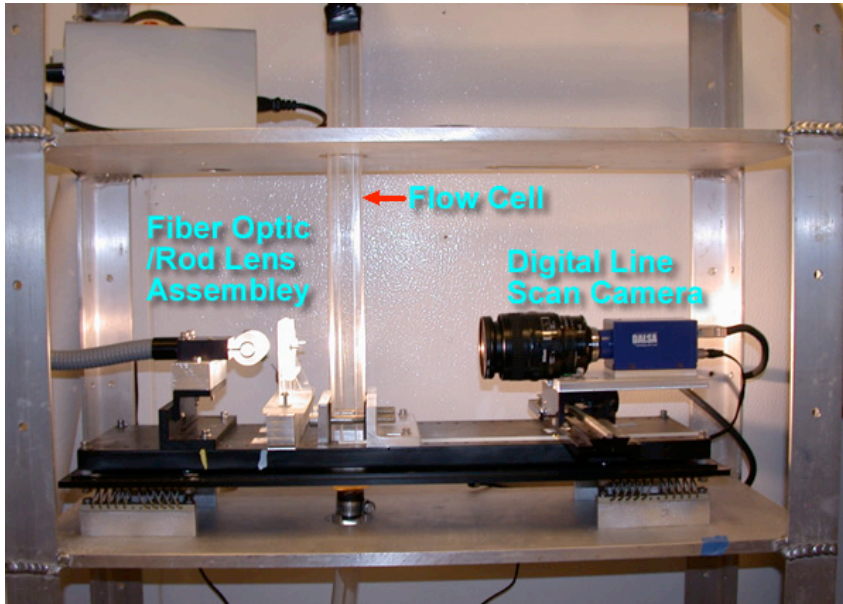
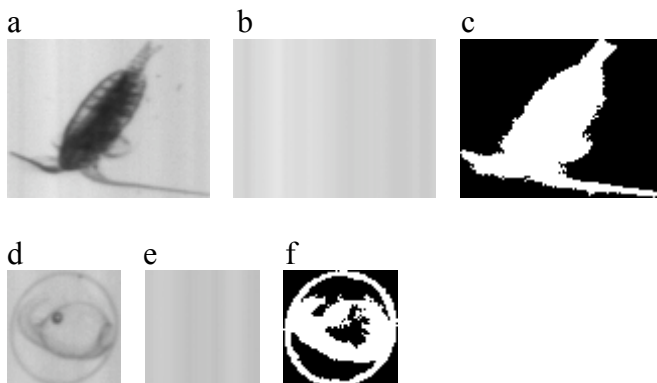


Figure 2. Segmented (a, d), background (b, e) and binarized (c, f) images of a calanoid copepod (a-c) and Pacific sardine egg (d-f). The segmented image occupies a box the size of which is determined by the object's difference from the background, size, and intensity. The binarized image is created from the grayscale image and used in subsequent classification. These images were acquired off Southern California in April 2004. Scale: copepod is  $\sim 1 \text{ mm}$  long, sardine egg  $\sim 1.4 \text{ mm}$  diameter.



Several challenges have been confronted and overcome. We initially proposed to use an expensive hardware board to acquire and process, in real time, data from the camera. Instead, custom REFLICS software was written to obviate the need for this hardware. Real-time operation of REFLICS did not prove possible until PC processor speed, together with enhancement of our custom software, enabled so. Success of REFLICS also depends on a robust and accurate classifier. To date, we have employed used Classification and Regression Tree (CART) classifiers, embedded in the REFLICS software. REFLICS software has been written to be flexible and, in particular, to allow use of different feature sets and classifiers. We continue to improve and test our classifier. CARTs are fast and moderately accurate, but other classifiers may prove better suited for our purposes. Importantly, however, REFLICS performed well during an April 2003 and 2004 egg surveys. April 2003 data yielded results for the distribution and abundance of fish eggs in CUFES consistent with data from counting of the same eggs by trained experts at sea. In addition, valuable data for other plankton types, e.g. copepods, are produced by REFLICS. Data analysis continues.

### Fish Egg Dynamics

CUFES was used to investigate the dynamics of patches of eggs of Pacific sardine and northern anchovy. Patches of eggs off Southern California were discovered using CUFES and sampled 3-5 times at relatively high resolution. Eggs were identified, staged, and counted ashore at SIO. Cohorts (individuals of the same age) of eggs were evident in patches of both species and spatial statistics were computed. Anchovy eggs were more variable in their distribution than sardine eggs at the scales sampled. The vertical distribution of eggs of both species was both observed in nature and predicted using egg buoyancy measured in lab experiments, physical conditions, and a model. Euphausiids, dominant predators of pelagic fish eggs, and eggs of anchovy were distributed in a complimentary manner during 1953-1959 and 1995-2002, consistent with the hypothesis of fish egg mortality is due to euphausiid predation. This work comprised the doctoral dissertation of K. Alexandra Curtis, now a postdoctoral investigator at Memorial University in St. John's, Newfoundland, Canada.

### Future

We attained our proposed objectives. However, prior to use by others, REFLICS must be enhanced as follows. First, a robust and accurate classifier must be developed to successfully identify and enumerate eggs of target species in CUFES. This is a task all users of REFLICS must perform in order to adapt it for use with specific regions and taxa. Second, REFLICS must be made user-friendly, including operation of both hardware and software. For example, installation and use of REFLICS hardware must yield reproducible images of calibration objects, to ensure correct classification by REFLICS software. REFLICS is powerful yet moderate in cost. It must now be made easy to use.

## Contributors

Professors Checkley and Cosman, as well as, initially, Professor Mohan Trivedi (ECE), were co-PIs for this project. Graduate students included Ben Ochoa and Stephen Krotosky (ECE, REFLICS software) and Alex Curtis (SIO, fish egg dynamics). Jesse Powell was Staff Research Associate (SIO, REFLICS hardware and lab and sea testing). Hubbs Sea World fish provided live eggs of white seabass (*Atractoscion nobilis*) for REFLICS development and testing in the lab.