LARGE PELAGICS RESEARCH CENTER
PROGRESS REPORT

Report: 3 **Reporting Period: 6/01/2007 – 6/30/2008**

Project Title: Technological development of a high resolution, rapid survey capability to identify spawning habitat of large pelagic fishes

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1. Purpose of the Project:

We proposed to improve the speed, accuracy and precision of ichthyoplankton surveys via the development and application of two technological advancements. Each is operable (and advances our capabilities) individually, but they are synergistically linked to maximize our ability to rapidly and quantitatively survey and identify spawning habitat of large pelagics based on early larval stages. Specifically – we propose to: 1) make operational a rapid, very high resolution imaging system capable of sampling sufficiently large volumes of water to accurately count larval fish *in situ*; and 2) establish a high-throughput DNA sequencing protocol to rapidly identify larval fish samples taken for validation and biological sample purposes.

2. Progress during the last year:

1) **Complete development of, and test *ISIIS* (*In situ* Ichthyoplankton Imaging System) for rapid survey of egg and larval scombroids**.

Following the initial building and successful testing of the prototype, we identified a series of refinement that were needed. During the last year we have made significant design modifications to: i) how the mirrors are held and adjusted (a critical alignment step for the accurate use of the instrument), ii) adjustment control of the light source, iii) light source itself, and iv) overall vehicle design. Each of these is described below.

Aligning the mirrors for proper light path is a critical step as even very minor mis-alignments can result in an image of poor quality or reduced field of view. We addressed this with an entirely new design that allows us to make very fine adjustments just prior to deployment (i.e. without the need to fully open and partially disassemble the system). This new design worked very well, with one critical exception. We built the prototype from molded plastic, but found that

large changes in temperature (such as going from AC lab conditions on the ship to sun heated deck conditions in the tropical sun) caused the plastic to warp, significantly altering the alignment and parallel nature of the light path. We will address this by machining the mirror support from aluminum, rather than using molded plastic. Otherwise, the design worked extremely well.

During the previous year we had addressed the issue of improving the volume (depth of field) sampled. Tied to that work was the need to reduce the initial light source size. As reported in our last progress report, we worked with a pinhole and light condenser. Since then, we have found a new light source that is very small and bright allowing us to do away with the condenser but also requiring more careful alignment, and hence 3-D adjustment capability. This new design has been tested with the new light source and is working very well, allows for light alignment in the lab, and then securing the setting prior to final instrument assembly for field deployment.

Finally, the towed vehicle has undergone a complete redesign to accommodate added instrumentation, undulation capabilities and minimize cable attachment (i.e. bridle) profile. This new design also significantly reduces the overall footprint of the instrument (see Figure 4).

2) **Develop high-throughput molecular sequencing of cytochrome B portion of mtDNA for all Atlantic scombroids**.

As reported in the last report – the molecular identification technique was completed following expansion to include Atlantic *Thunnus*, *Auxis* and *Coryphaena* species, in addition to its previous application with istiophorids. We have continued to apply this technique to our large collection of pelagic larvae from the Straits of Florida. To date ≈4000 larvae have been identified using this methodology. Ultimately this dataset will be used in a comparative study of the temporal and spatial distribution of spawning of pelagic species in the Straits of Florida. It has also been utilized in several more specific studies of larval distribution and feeding analyses. See citations below.

2. Preliminary Data:

Obj. 1 – The best illustration of our success with ISIIS thus far is a set of images (see Figures 2, 3 and 5 below) obtained in the Gulf Stream while towing the system at 5 knots! We estimated that our camera system was quantitatively imaging approximately 70 l s^{-1} which is equivalent to about 10% of that of a typical 1 m^2 plankton net. Our estimated densities measured from the camera were comparable to larval fish density estimates obtained for the same water mass, same time of year (but different years) collected with a 1 m^2 MOCNESS net (1200 larvae per 1000 m^3 vs. 950 larvae per 1000 m^3, respectively; SD= 180 and 200, respectively)**.**

Obj 2. – Since our initial report where we indicated that we have positively identified 1800 larval fish to species using the molecular techniques we developed, we have completed a study (sampled with NSF funding) on the larval assemblages of medium and large pelagic fish in the SOF (see Richardson et al, in review). This study is part of a series of papers that will build on our larval samples which are now well identified as a direct result of this project. The overall goals of that NSF funded study are to characterize the spawning patterns, larval habitat, and transport fates of large pelagics in the Straits of Florida – the initial emphasis was on billfish, but with the larval ID capabilities we now have via this molecular technique – we are able to expand the scope to include the full assemblage of scombroid and related fish found in this region. We have also utilized this approach to provide species identifications of species of the grouper (Serranidae) complex.

3. Plans for the next six months to year: (one paragraph):

Obj. 1. We are ready for field testing our refinements and this new optico-mechanical slip-ring. Our plan was to complete this testing in August 2007 associated with a cruise for a separate project that will also be sampling with our net system (MOCNESS). It is critical that both sampling approaches be spatially and temporally matched for critical comparison. However, as noted above, there were problems with the fiber-optic winch system onboard the RV Walton Smith and mirror adjustments. As these issues have now been overcome, full comparison sampling will occur this fall (2008) during a dedicated ISIIS cruise.

Obj. 2. We will continue to apply the high thru-put sequencing to our samples from the SOF as a check on the success of the method – to see if we encounter any unknowns or other sample processing problems.

Obj 3. – Once the full test/survey with the ISIIS system is complete, then full manufacture of the new towed vehicle will begin (with additional funding provided by the National Science Foundation).

4. Dissemination

Publications:

Richardson, D.E., J.D. VanWye, A.M. Exum, R.K. Cowen, D.L. Crawford. 2006. High-throughput species identification: from DNA isolation to bioinformatics. Molecular Ecology Notes doi:10.1111/j.1471-8286.2006.01620.x

Cowen, R.K., and C.M. Guigand. 2008. *In situ* Ichthyoplankton Imaging System (*IS*IIS): system design and preliminary results. Limnol. & Oceanogr. Methods. 6:126-134

Tsechpenakis, G., C.M. Guigand, and R.K. Cowen. (in press). Image Analysis Techniques to Accompany a new *In Situ* Ichthyoplankton Imaging System . IEEE

Richardson, D.E., J.K. Llopiz, C.M. Guigand and R.K. Cowen. (in review). Larval assemblages of large and medium sized pelagic species in the Straits of Florida.

Richardson, DE., RK Cowen, S Sponaugle. (in review). Importance of the Straits of Florida spawning ground to Atlantic sailfish (*Istiophorus platypterus*) and blue marlin (*Makaira nigricans*). **Fisheries Oceanography**

Marancik KE, DE Richardson, J Lyczkowski-Shultz, M Konieczna, and RK Cowen. (in prep.). Evaluation of morphological characters to identify grouper (Serranidae: Epinephelinae) larvae in Gulf of Mexico SEAMAP plankton collections using genetically identified specimens with a summary of decadal variation in relative abundance, distribution patterns, and spawning locations.

Workshops:

None

Conferences:

30th Annual Larval Fish Conference, Lake Placid, NY. Sept 2006.
Robert K. Cowen and C. Guigand. ***In situ* Ichthyoplankton Imaging System (ISIIS)**. [Plenary Talk]

Richardson, D.E., J.D. VanWye, A.M. Miyake, D.L. Crawford, R.K. Cowen. **High throughput species identification: from DNA isolation to Binformatics.** Poster Presentation. [Received BEST STUDENT POSTER Award]

Gulf and Caribbean Fisheries Institute (GCFI), Belize City, Nov 2006
Robert K. Cowen. **Advances in the study of billfish early life history**
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Large Pelagics research Center 1st PI meeting. April 2007/April 2008.
Robert K. Cowen**. In situ Ichthyoplankton Imaging System (ISIIS)**

OCEANS '07 IEEE Aberdeen, Scotland. July 2007

11th International Coral Reef Symposium, July 2008.
Robert K. Cowen. **Population Connectivity in Coral reef systems: Progress and promising new directions**.

Manuals, Protocols:

Species Identifier: A character-based sequence analysis script developed as a part of the molecular species identification portion of the project. The script is freely available from the author (D. Richardson) and has the following characteristics:

- Uses MATLAB and the freely available MbeToolbox.
- Produces results in a readily interpreted spreadsheet format.
- Entire plate of samples is analyzed with a single run of the program; no preprocessing of the sequence data is required.
- Incorporates base call quality scores (Phred scores) to provide a measure of the reliability of sequence data, and to allow more efficient unidirectional sequencing to be used.
- Identification of larvae based on a comparison 1) of the sample sequence to each voucher sequence across the entire sequence and 2) at diagnostic nucleotides separating pairs of voucher sequences.

Outreach Activities:

Patent, Copyright, Invention Disclosure Activity:

At the request of the University of Miami, Robert Cowen and Cedric Guigand have applied for a patent for the ISIIS design.

5. Collaborators and Personnel: (list collaborators and personnel working on this project, include terminal degree and institution not listed on the proposal).

Mr. Cedric Guigand, M.S., RSMAS/UM

6. Students: (list students receiving funding, degree type, anticipated graduation date, thesis or dissertation title)

David E Richardson - PhD Student planned graduation in Dec 2007.
Dissertation title: The Straits of Florida as spawning habitat for pelagic species