LARGE PELAGICS RESEARCH CENTER
SEMI-ANNUAL PROGRESS REPORT

Report: #1 **Reporting Period: 11/01/2006 – 05/31/2007**

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 second six months:

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 six months we have been addressing these with good success. One area of prime concern was how to improve the volume sampled – for this we worked at doubling our depth of field by modifying the light trajectory. This change also necessitated a need for higher light output (and control on light intensity). Additional refinements include completion of switch over to fiber-optic data transmission, addition of small CTD to system, and other minor technical refinements (e.g. better heat transfer for light/camera, etc.).

We have researched an optical means of expanding our depth of field. By forcing the light to pass through a pin-hole and then a plano-convex lens, we were able to collimate the light rays (make them parallel). By focusing the light through a condenser *prior* to passing through the pinhole, we substantially increase the focal length thereby increasing the depth of field without lengthening the actual light

path (to do so would require much longer pods). Based on bench top tests, this change has resulted in at least a doubling of the depth of field (i.e. section of water parcel in focus) from 20 cm to at least 40 cm. That doubles the volume of water being imaged from 70 ls^{-1} to ~140 ls^{-1}. This change also required us to find a higher wattage light source – for which we have been pushing the envelope with respect to the current state of the art in LED technology. Fortunately, we found a source with some new higher wattage LEDs that we believe will meet our purpose.

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

During the first six months, the molecular identification technique was expanded to include Atlantic *Thunnus*, *Auxis* and *Coryphaena* species, in addition to its previous application with istiophorids. This choice of taxa includes those species with larvae that cannot be identified morphologically. We have continued to apply this technique to our large collection of pelagic larvae from the Straits of Florida. To date ≈2000 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.

2. Preliminary Data:

Obj. 1 – The best illustration of our success with ISIIS thus far is a set of images (see Figure 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.

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4. Difficulties: (Provide details for any delays in meeting milestones and how they are being resolved).

As mentioned in the last report, most of the difficulties we encountered were with respect to technical engineering details for the ISIIS or primer problems with the molecular sequencing. None have been critical as we have made good progress in both efforts. We remain on track.

One of the largest problems had to do with transitioning to fiber-optic cable for the towed vehicle. We had at UM/RSMAS ~ 2000 ft of fiber-optic cable and winch, but it was not set for the high data transfer rates that we required (it was only equipped with a modem for data transfer rather than optical/mechanical slip rings). This necessitated our initial field trials to be connected at set depths, with hand feeding of fiber optic cable (in addition to a mechanical, load bearing cable). As we reported earlier, we purchased the required optico-mechanical slip-rings, and these have now been added to the fiber-optic cable winch system (a large job). Transitioning to fiber optics is a learning experience for everyone (us and the ship's ET group). Field testing will be required to ensure that all bugs are out of the system; potential weak links are primarily the fiber terminations. These are very tricky to terminate (taking hours to terminate each fiber - 9μm). If done wrong (i.e. misaligning the fiber or breaking the fiber), data will not transfer. Another weak link that may occur is in the optico-mechanical slip-rings themselves – as with rotation, some signal loss is likely – the interplay between quality of termination, and optico-mechanical slip-ring transmission loss will need to be resolved.

5. 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, there were some problems with the fiber-optic winch system onboard the WRV Walton Smith, so this testing will have to occur this early fall.

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 new bugs are out of the system – we will run a full test/survey with the system by conducting a short survey of the SOF – tentatively planned for the late fall of 2007.

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6. 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. (in review). *In situ* Ichthyoplankton Imaging System (*IS*IIS): system design and preliminary results. Limnol. & Oceanogr. Methods.

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.

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.

Robert K. Cowen**. In situ Ichthyoplankton Imaging System (ISIIS)**

OCEANS '07 IEEE Aberdeen, Scotland. July 2007

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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 initiated an Invention Disclosure application to seek a patent for the ISIIS design.

7. 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

8. 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

8. Images and Captions:

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Figure 1. Launching (left) and recovery (right) of ISIIS during maiden voyage. Note twin pods which hold light source (left) and camera (right) with imaged area between the two pods (forward section). Credit: R.K. Cowen and G. Guigand, University of Miami, RSMAS.

Please note- we may be using some or all of these images for inclusion in a publication describing the ISIIS – therefore we would appreciate limited dissemination.

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Selected *in situ* images of fish larvae

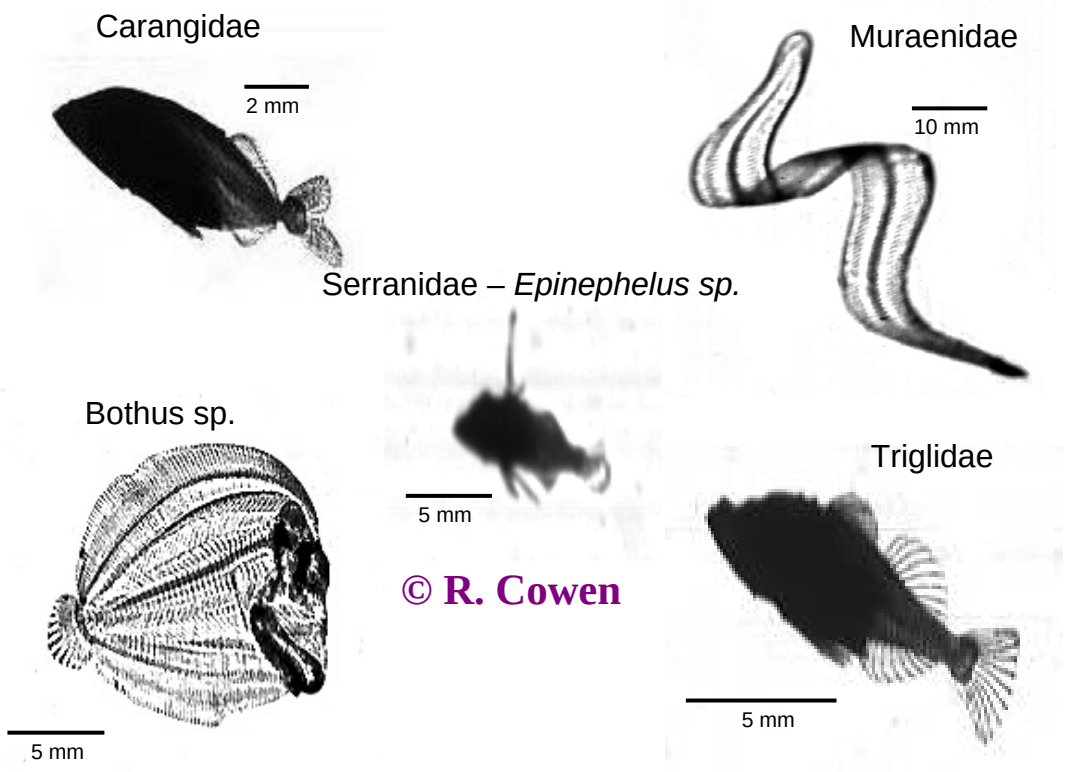

Figure 2. Example fish images from ISIIS taken while towing at 5 knots, and between 5 and 40 m depth. Sampling location was crossing the core of the Florida current east of Miami, FL. Credit: R.K. Cowen and C. Guigand, University of Miami, RSMAS.

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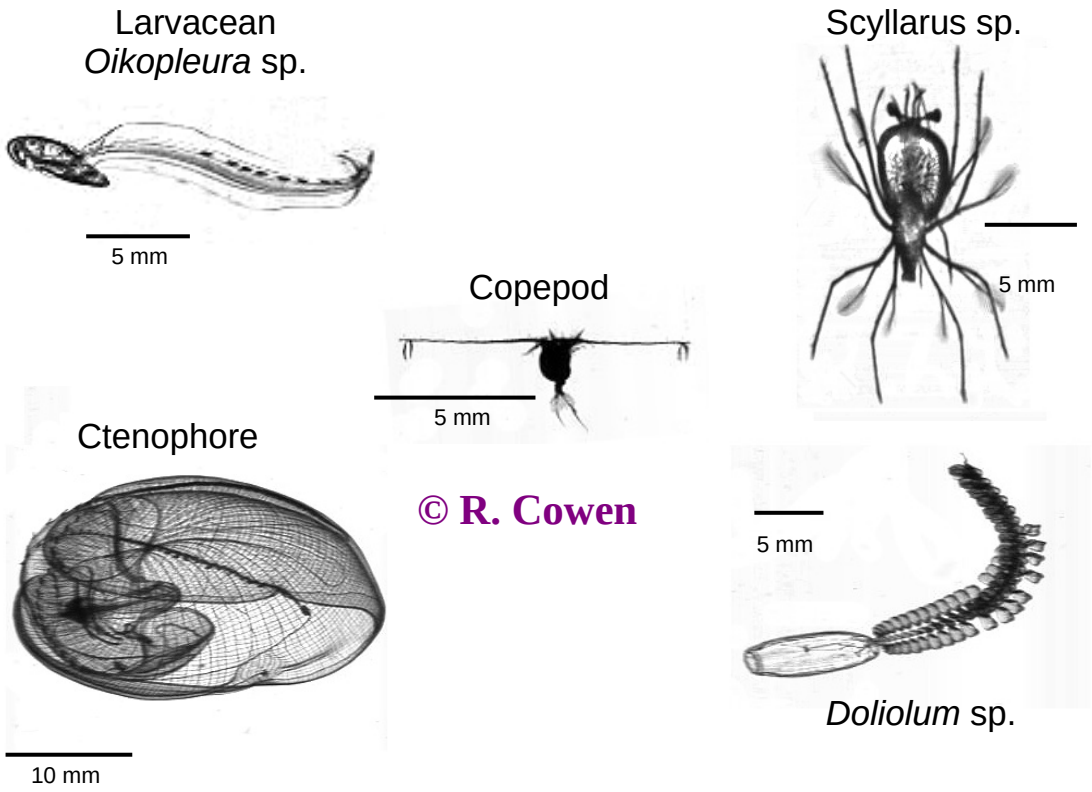

Figure 3. Example invertebrate plankton images from ISIIS taken while towing at 5 knots, and between 5 and 40 m depth. Sampling location was crossing the core of the Florida current east of Miami, FL. Credit: R.K. Cowen and C. Guigand, University of Miami, RSMAS.

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