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Project Title: Application of Otolith-Based Methods to Distinguish Nursery Areas of Juvenile Swordfish (Project #657780)

Funding Agency: Hawaii Fisheries Disaster Relief Program (FDRP)

Fishery Targeted: Hawaii-based Pelagic Longline Fishery

Award Received: Original Amount Awarded (October 2006) - $55,583
Revised Amount (March 2008) - $47,583

Actual Amount Spent: $45,000 (estimated; to be confirmed by JIMAR Administration)

Project Objectives:

1. Collect samples (30-50 per region) of age 0+ juveniles from additional regions to extend sampling coverage across the North Pacific Ocean.

2. Remove and process otolith sagittae, and analyze trace elemental composition of individual sagittae from area adjacent to core and outer edge using LA-ICP-MS.

3. Section and process otolith lapilli and record the internal microstructure and serial microincrement pattern via scanning electron microscope (SEM).
How Project Objectives Were Met:

1. Collect Samples
Prior to this project, otoliths had been obtained from age 0+ juvenile swordfish collected from the Main Hawaiian Islands (n=240), the Equatorial Central Pacific (n=38), French Polynesia (n=34), and coastal Ecuador (n=98).

During the course of this project, heads of age 0+ juvenile swordfish were obtained from coastal Japan, the Subtropical Convergence Zone north of Hawaii, and the Indian Ocean. These additional specimens were collected for this project at the request of the principal investigators by scientists from the National Research Institute for Far Seas Fisheries in Shimizu, Japan.

2. Extract, Process, and Analyze Otoliths for Trace Element Composition
All three otolith pairs (sagittae, lapilli, and asterisci) were extracted from swordfish heads received from coastal Japan, the Subtropical Convergence Zone north of Hawaii, and the Indian Ocean. Due to their minute size and location within the braincase, one or both pairs of otoliths types were occasionally not available due to damage or loss during extraction. Sagittal otoliths are the largest of the three otolith types and were used exclusively for trace element analysis during this project (Figure 1). One to two sagittal otoliths per fish were extracted from heads collected from coastal Japan (n=50), the Subtropical Convergence Zone north of Hawaii (n=40), and Western Indian Ocean (n=24).

Individual sagittal otoliths from a total of 240 swordfish were successfully prepared for analysis. Prepared otoliths represented six different nursery areas within the Pacific consisting of the Main Hawaiian Islands (n=48), the Equatorial Central Pacific (n=35), French Polynesia (n=32), coastal Japan (n=35), the Subtropical Convergence Zone north of Hawaii (n=35), and coastal Ecuador (n=35). An additional sample of prepared otoliths (n=20) was from the Western Indian Ocean. Sagittal otolith preparation was tedious and time consuming with some loss due to sample destruction during preparation. Thin frontal sections were prepared on all sagittae in order to remove overlying otolith material on either side and expose the inner core and larval stage series of daily growth increments (first 60 increments) out to the outer otolith margin (Figure 2).

Prepared otoliths were subsequently decontaminated in the clean room facilities at the University of Hawaii, HIG Lab 419 or at Oregon State University’s (OSU) W.M. Keck Collaboratory for Plasma Spectrometry (Keck).

Trace element composition was conducted on the 240 prepared sagittal otoliths by laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS) at the OSU Keck facility. Otoliths were analyzed for the presence of 12 trace elements (B, Mg, Al, Si, P, Mn, Fe, Cu, Zn, Sn, Ba, Pb) as well as Ca and Sr. Analysis was conducted using a VG PQ ExCell inductively coupled plasma mass spectrometer equipped with a DUV193 excimer laser for ablating minute samples from each otolith. Laser ablation sampling on each otolith consisted of three radial transects (250-300 μm in length) outward from the otolith core and another two transects of similar length along the outer margin of each otolith (Figure 3). The laser was configured to sample otoliths using a 50 μm spot size operating at 8 Hertz with the beam moving along each transect at 3 μm/s. The laser removed otolith material to a depth of approximately 20 μm. The principal investigator (Humphreys) received training and subsequently operated the Keck LA-ICP-MS.
instrument during routine analysis runs. Otolith analysis runs were conducted by Keck staff when Humphreys was unable to travel to the facility.

3. Preparation and Analysis of Lapilli Internal Microstructure
A total of 36 otolith lapilli from three different regions (n=12 each from coastal Japan, the Main Hawaiian Islands, and coastal Ecuador) were prepared using grinding and polishing procedures similar to those developed for sagittae to produce thin frontal sections. Analysis of these sections is on hold until LA-ICP-MS data is analyzed.

**Difference Between Work Anticipated and Work Actually Completed:**

1. Collect Samples
The anticipated sample size (n=50) available for each of the seven different regions sampled was not achieved except for coastal Japan, the Main Hawaiian Islands, and coastal Ecuador. Opportunities to obtain samples from eastern Australia and Samoa were sought but proved unsuccessful. The sample collection from the Western Indian Ocean was unanticipated but may prove to be important as a potential “outgroup” for comparisons with the other regional Pacific samples. Unfortunately, fish sample size collected from this region (n=24) was small. Swordfish heads collected from coastal Japan and the Western Indian Ocean were not collected until late in the project (November 2007-January 2008).

2. Extract, Process, and Analyze Trace Element Composition
Although a few otoliths were lost or destroyed during extraction, these few losses are always anticipated when working with fragile and minute otoliths such as those of billfishes.

The project originally anticipated 400 sagittal otoliths to be analyzed instead of the 240 analyzed. Otolith sample sizes were smaller than expected for some regions, particularly for the Equatorial Central Pacific and French Polynesia. Another unanticipated factor was the large amount of processing time devoted to the preparation of polished sagittal otolith sections for LA-ICP-MS analysis. Relatively simple transverse sections were originally planned but these did not produce enough available surface area to conduct multiple laser ablation sampling using the large laser spot size (50μm diameter) employed. Instead, frontal sections were prepared for all sagittae and allowed the entire dorsal surface of this otolith to be available for multiple sampling transects. Although these preparations proved optimal for replicate trace element analysis, successful frontal section preparations required 2-3 hours per sagitta. The highly 3-dimensional shape of sagitta and the need to carefully grind off overlying otolith material without removing the core and larval stage growth increments beneath required a processing rate much slower than anticipated.

Instrument operations using the Keck LA-ICP-MS were relatively trouble-free although the time needed to run samples was underestimated given the need to run standards, calibrate and tune the instrument each day, downtime during sample changes, and the occasional operator error (Humphreys). Due to first of the fiscal year travel restrictions, travel to OSU was unavailable during October 2007 through January 2008. Since the majority of the LA-ICP-MS analysis runs were conducted late in the project (March-June 2008), there has been no time to analyze the processed and raw data files as yet.
3. Preparation and Analysis of Lapilli Internal Microstructure

A limited number (n=36) of lapilli were successfully prepared for examination. Ground and polished frontal sections of lapilli required less time (~1.5 hours per otolith) to complete than sagittae. Further work on lapilli was put off during this project in order to give full priority to the preparation of sagittae for LA-ICP-MS analysis.

Difficulty was encountered in preparing lapilli sections that allowed both the core and larval increment series to be exposed along a common exposed surface plane. It was anticipated that these sections would be examined using scanning electron microscopy (SEM). However, SEM is designed to image features at the surface and has little “depth of field” capability. The core and larval stage increments within the lapilli required examination using compound microscopy. The acquisition of apochromatic microscope objectives during this project will provide requisite depth of field capabilities for examining the internal core and larval increment structures (Figure 4).

Differences in Expected and Actual Costs:

A revised spending plan and budget was submitted to JIMAR in March 2008. The amount budgeted for LA-ICP-MS was reduced from $30,000 to $24,000 due to the projected decrease in the planned number of otoliths samples to be analyzed.

A total of $12,000 was budgeted for SEM instrument time at the UH Biological Electron Microscope Facility. This was reduced to $1,000 in March 2008 but remained unspent upon completion of this project. The $3,000 budgeted for shipping samples was also unused. The $14,000 in unused mony was used to buy needed equipment (horizontal laminar flow hood, apochromatic microscope objectives, and a new polisher/grinder machine) specified in the March 2008 revised spending plan for this project. Our current understanding is that all other money budgeted for this project (except the $1,000 of SEM time) was used.

Publications, Posters, Brochures, and Other Informational Material Published with Project Funding:

None
Figure 1. Paired sagittae (top), lapilli (middle), and asterisci (bottom) of a juvenile swordfish. Length of the sagittae here are about 2 mm.

Figure 2. Example of a ground and polished thin frontal section of a juvenile sagittal otolith prepared for LA-ICP-MS analysis. The core is the small black dot located at the point where the lines converge (photo 300x).
Figure 3. Otolith in Fig. 2 after undergoing LA-ICP-MS sampling. The width of the laser ablation transects are 50 μm and 250 μm long. The three transects in the center all began at the core; the other two transects are aligned along the outer margins (photo 300x).

Figure 4. Ground and polished frontal section of a lapillus showing the central core and series of larval stage increments radiating outward (photo 600x).