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CRUISE REPORT

VESSEL: *Oscar Elton Sette*, Cruise 03-03, Leg 2 (OES-04)

CRUISE PERIOD: 1-8 May 2003

AREA OF OPERATION: Kona coast off the Island of Hawaii (Fig. 1)

TYPE OF OPERATION: Surface net tow operations for billfish larvae and eggs 3-25 nmi off the Kona coast of the Island of Hawaii. Surface slick and non-slick tows were conducted with a 1.5-m diameter (2 square meter) ring net and a 1.8-m Issacs-Kidd (IK) trawl towed from the ship. Net operations including a 1-m neuston net, 50-cm egg net, and dipnetting were conducted from the safeboat independent of the ship. A grid transect of shallow conductivity-temperature-depth (CTD) casts were also conducted along the Kona coast.

ITINERARY:

1 May Embarked Robert Humphreys, John Hyde, Eric Lynn, Lianne Mailloux, Mike Musyl, Robert Nishimoto, and Francois Poisson. Departed Snug Harbor at 1430 and proceeded to Kailua-Kona, Island of Hawaii.

2 May Arrived off Kailua-Kona 0500 and commenced egg and larval billfish collections with surface IK and 1.5-m ring net tows from the ship. At 0800, embarked Andrew West from the Kailua-Kona pier and resumed 1.5-m ring net tows. Later in the morning safeboat operations began using a 1-m neuston net, a 50-cm egg net, and dipnets; all attempting to target surface slicks off the Kona coast. At nightfall, commenced nightly operations that included CTD casts and 1.5-m ring net tows for billfish eggs and larvae.

3-6 May Continued daily schedule of daylight surface 1.5-m ring net tows (no further IK tows) from the ship and 1-m neuston and 50-cm egg net tows and dipnetting from the ship's safeboat for billfish eggs and

larvae. Nighttime operations included shallow 150-m CTD casts followed by 1.5-m surface ring net tows.

7 May — Continued surface 1.5-m ring net tows from ship. Disembarked Andrew West at 0830 and at 1430 ended tow operations off the Kona coast and proceeded back to Snug Harbor, Honolulu.

8 May — Arrived Snug Harbor, Honolulu at 0800. Disembark Humphreys, Hyde, Lynn, Mailloux, Musyl, Nishimoto, and Poisson; end of cruise.

MISSIONS AND RESULTS:

A. Collect egg and larval billfish specimens in surface waters along the Kona coast of Hawaii.

A total of seventy-five 1.5-m ring net tows and one 1.8-m Issacs-Kidd tow were conducted during the cruise. During this cruise, tows targeted coastal sea surface slicks (when available) 3-25 nmi miles offshore of the Kona coast between Keahole Point (19^0 43'N latitude) to the north and Milolii (19^0 10'N latitude) to the south. Hauls were conducted from midship off the port J-frame. The 1.5-m ring net filtered the top 0.75-1.00 m of surface water including the neuston layer while the IK filtered the top 1 m. The safeboat worked independently of the ship and equipped with a 12-ft. angle iron cross-piece, allowed a 1-m neuston net (1 mm mesh) and a 50-cm egg net (0.5 mm mesh) to be towed simultaneously. When not towing nets, dipnetting from the safeboat was conducted while operating in surface slicks. Safeboat operations were limited to the morning daylight hours (0600-1200). Surface slicks are normally present only during the morning periods since offshore breezes produced by thermal release over the island disrupt slick occurrence by early afternoon. However, during most of this cruise, surface slicks were uncommon, even during morning hours.

The 1.5-m surface ring net was specifically used to collect billfish eggs (finer 0.5 mm mesh and slower 2.5 knot towing speed). This contrasts with previous Kona cruises where billfish larvae were targeted with 1.8-m IK surface tows (front 4/5 of net 5 mm mesh, remainder 0.5 mm mesh, allowing a faster 4.0 knot towing speed). Use of the 1.5-m ring net resulted in the occurrence of a large number of eggs varying in size from about 0.8 to 3.0 mm in diameter. Based on the only verified descriptions of fertilized billfish egg stages (that of Mediterranean swordfish characterized by heavily pigmented eggs 1.6-1.8 mm in diameter), each egg tow sample was first qualitatively sorted for egg size by pouring the entire tow contents through a column of graded sieves of 2.8 mm, 2.5 mm, and 1.0 mm mesh. The contents that passed through the two coarser sieves and accumulated on the 1.0 mm

sieve were immediately examined under magnification to remove any pigmented eggs suspected to be billfish. The remainder of each tow sample was then pooled and preserved for post-cruise examination.

Initially all pigmented eggs of a range of sizes were measured, photographed, and individually preserved in ethanol for subsequent shipboard PCR identification. As the PCR method began to yield positive identifications of billfish eggs, pigmented eggs between about 1.25 and 2.0 mm in diameter were selected. However, all remaining eggs were preserved in ethanol for possible post-cruise PCR testing. A total of 149 suspected billfish eggs were PCR tested during the cruise and yielded 62 positive billfish identifications. The majority of these identified eggs were swordfish (n = 54) and the remainder shortbill spearfish, *Tetrapterus angustirostis* (n = 8). This may be the first time that planktonic billfish eggs have been identified off Hawaii. This also represents the first known collections of planktonic eggs of shortbill spearfish, whose fertilized egg stages remain undescribed in the literature.

Net tows also collected larval billfish consisting of 11 swordfish and 76 istiophorid larvae. Istiophorid larvae are notoriously difficult to identify to species, particularly if damaged. All 76 istiophorid larvae were also PCR tested during the cruise and identified as shortbill spearfish (n = 72) and blue marlin, *Makaira nigricans* (n = 4).

Identified billfish eggs and larvae co-occurred among only 6 net tow stations; the remaining billfish eggs and larvae were collected separately from 12 and 25 other stations, respectively, of the 81 net tow stations conducted during the cruise. Thirty-six net tow stations yielded no billfish eggs and/or larvae. Billfish eggs were distributed 5 to 25 nmi offshore with the majority (87%) collected adjacent or between the 1,000 to 2,500 meter bathymetric contours. No co-occurrence of swordfish and shortbill spearfish eggs were present in the 18 tows for which billfish eggs were PRC identified. Only six of the above 18 tows had multiple captures of billfish eggs. Post-cruise processing of these net samples for billfish eggs continues; results discussed herein represent the initial at-sea examination for billfish eggs.

From these initial at-sea examinations of all samples, the highest number of billfish eggs occurred in one particular tow off south Kona (Station 53) which yielded 37 (PCR identified) swordfish eggs plus 2 swordfish and 17 (PCR identified) shortbill spearfish larvae. This was the only net tow that provided any evidence that billfish eggs might be encountered in patches or aggregations. Furthermore, this tow was conducted through a well-defined surface slick.

Typically, however, there were few opportunities to tow through surface slicks during the cruise. The presence of a southerly swell and a variable west to north wind were not optimal conditions for the formation and persistence of surface slicks.

B. Conduct DNA-based procedures for the identification of billfish eggs and larvae using a new PCR (polymerase chain reaction) protocol.

This was the third cruise (preceded by *Townsend Cromwell* cruises 01-06 and 02-03, Leg 2) where trials were conducted of a molecular-based method to identify billfish larvae at sea in near-real time. During the previous cruises, Eric Lynn of the Genetics and Physiology Group at the NMFS, SWFSC La Jolla Lboratory tested a PCR method published by Seinen Chow in 1994 that used an RFLP (restriction fragment length polymorphism) technique to identify all six Pacific billfish species. Much of the effort during these previous two cruises were made toward modifying Chow's PCR-RFLP protocols to work in a shipboard environment.

During this cruise, Eric Lynn was joined by John Hyde (Scripps Institute of Oceanography) who has developed a different set of primers and protocols for the identification of Pacific billfish species. This new technique uses a multiplex species-specific PCR designed to produce unique gel band patterns for each billfish species. This multiplex PCR technique has reduced identification time from 12 to 3 h. Furthermore, the lab bench space needed to conduct the PCR protocols has been substantially reduced and all related equipment is transportable within a single suitcase.

Prior to initial DNA extraction, all eggs to be tested were stored in individual vials of 95% undenatured ethanol. The entire egg was consumed during these procedures while for larvae, only one eyeball was used. Egg and tissue standards from known billfish species were periodically run with unknown egg samples to confirm results. The multiplex PCR technique provided more rapid identifications, ease of use, and shipboard adaptability which will allow future egg surveys to quickly adapt sampling schemes during the cruise.

C. Conduct nightly CTD casts at predetermined sites off the Kona coast.

A sampling grid of 15 Seabird CTD casts were conducted along four latitudinal transects off the Kona coast to acquire environmental data on the upper 150 m of the water column.

D. Miscellaneous oceanographic observations.

During transect runs between CTD stations, the ADCP display was monitored for changes in current flow. Throughout the cruise, a strong southeasterly current was recorded along the Kona coast. Further offshore, this current became slack at around 156°15'W longitude, and then reversed to a northerly direction west of this location.

Throughout the cruise, sea surface temperature (SST) and sea surface salinity (SSS) values of around 25.3°C and 34.5, respectively, were recorded on the thermosalinograph. Based on previous Kona cruises, when these SST and SSS conditions were encountered off Kona, swordfish larvae were most frequently collected in surface 1.8-m IK tows.

DATA COLLECTED:

The following forms, logs, charts, and data records were kept and given to the Pacific Islands Fisheries Science Center upon termination of the cruise. These include all data captured onto computer storage media during the cruise. All the records are filed there unless indicated otherwise in parentheses.

ADCP DOPPLER ping data files on CD-ROM*
CTD Station Data Log Sheet
Seabird CTD data files on CD-ROM*
Digital camera photos (JPG file format) on CD-ROM*
Marine Operations Log
Deck Log
Plankton, Eggs and Larvae #1 (all net tows)
Scientist's Log
SCS data files (raw & compressed) on CD-ROM*
XBT (SEAS) data files on CD-ROM*

*All data files together on the same (1) CD-ROM

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Attachment

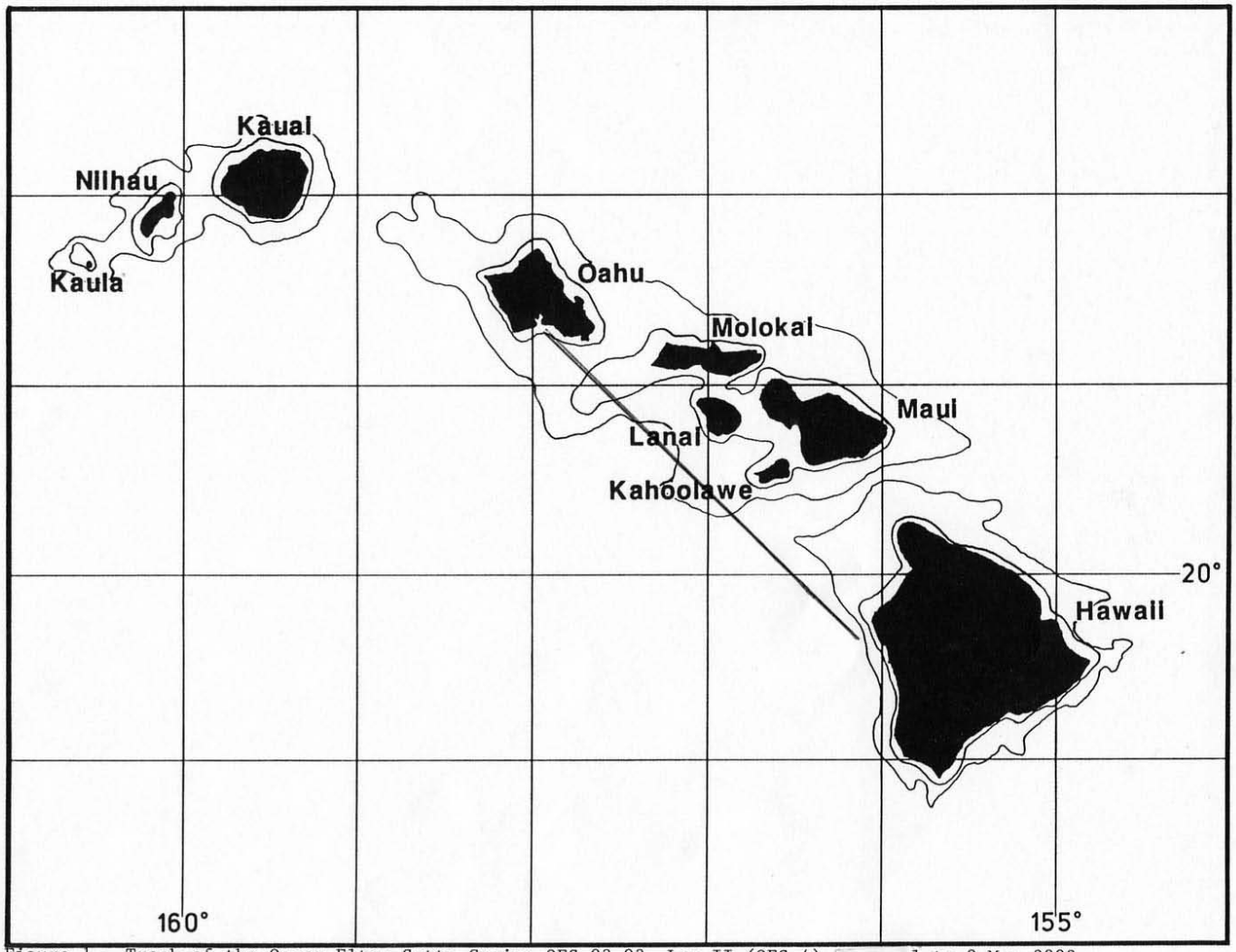

Figure 1.--Track of the Oscar Elton Sette Cruise OES-03-03, Leg II (OES-4) 1 to 8 May 2003.