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CRUISE REPORT[1]

VESSEL: *Oscar Elton Sette*, Cruise 06-05 (OES-41)

CRUISE PERIOD: 24 April-1 May 2006

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

TYPE OF OPERATION: Operations conducted off the Kona coast included daytime surface net tows targeting billfish eggs and larvae 2-14 nmi off the Kona coast of the Island of Hawaii using a 1.8-m wide Isaacs-Kidd (IK) trawl and morning safeboat operations to dip-net and observe billfish larvae in surface slicks. Night operations consisted of performing conductivity-temperature-depth (CTD) casts at predetermined transect positions and conducting acoustic Doppler current profiler (ADCP) transects along and off the Kona coast. A cooperative agreement between Syd Kraul and Pacific Islands Fisheries Science Center (PIFSC) was again arranged in an attempt to raise IK-collected billfish eggs to hatching and first feeding at Kraul's rearing facility on the Island of Hawaii.

ITINERARY:

24 April — Embarked Robert Humphreys, Rogelio Armas, David Liittschwager, and Michael Musyl. Departed Snug Harbor at 1050 and "hove to" off the south shore of Oahu in order to conduct a practice ship-to-air rescue operation with a U.S. Coast Guard helicopter. Upon completion, the ship proceeded directly to Kailua-Kona, Island of Hawaii.

25 April — Arrived off the Kona coast at 0700 and embarked at 0800 Eric Lynn, Catherine Purcell, Russ Vetter, and Andrew West from the Kailua-Kona pier. After embarkation, the ship transited to the south Kona coast and at 1122 began surface tow operations for billfish eggs and larvae. At nightfall, commenced CTD casts to 150 meters depth at predetermined sampling sites.

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26-29 April	Continued daily schedule of daylight surface IK tows from the ship and morning safeboat operations to observe and dip-net billfish larvae in surface slicks. Nightly CTD casts at predetermined sampling sites and ADCP transects were conducted during 26-28 April. Embarked Roy Eisenhardt from the Kailua-Kona pier on the afternoon of 27 April. Disembarked Andrew West at Kailua-Kona pier on the afternoon of 29 April.

30 April — Conducted daylight surface IK tows from the ship; no morning safeboat operations were conducted. Ended surface tow operations at 1222 and in the early afternoon disembarked Roy Eisenhardt, Eric Lynn, Catherine Purcell, and Russ Vetter at Kailua-Kona pier. Afterwards, proceeded back to Snug Harbor, Honolulu.

1 May — Disembarked via the safeboat Humphreys, Armas, Liittschwager, and Musyl at Snug Harbor, Honolulu around 0600; end of cruise.

MISSIONS AND RESULTS:

A. Collect billfish eggs and larvae in surface waters along the Kona coast of Hawaii.

A total of forty-four 1.8-meter wide Isaacs-Kidd (IK) tows were conducted; all tows were made with a 10-m length, 0.5-mm mesh nylon net. Tows were conducted at distances of 2-14 nmi offshore of the Kona coast between Kailua-Kona (19^0 38'N latitude) to the north and near Milolii (19^0 11'N latitude) to the south. Tows were conducted for 1 h alongside the ship off the port side J-frame. The IK net filtered the top 1 m of surface water including the neuston layer during all 44 surface tows.

The 1.8-meter IK net was specifically used to collect billfish eggs (finer 0.5-mm mesh and slower ~3.0 knots towing speed). This contrasts with previous Kona cruises prior to 2003 where billfish larvae were targeted with 1.8-meter IK surface tows using nets with a substantially larger mesh size (front 4/5 of net 5-mm mesh, remainder 0.5-mm mesh, allowing a faster 4.0-knot towing speed). Use of the 1.8-meter wide IK with a 0.5-mm mesh net resulted in the occurrence of fish eggs varying in size from about 0.7 to 2.5 mm in diameter. For the majority of the tows, the entire sample was examined for billfish eggs and larvae. The contents of each tow were concentrated and examined under dissecting microscopes for the presence of billfish eggs and larvae. Billfish larvae and suspected eggs were removed for photography and identification via the multiplex-PCR assay. The remainder of the contents and any portion of the tow catch that could not be examined were preserved together in 95% undenatured ethanol. These ethanol-preserved tow samples will be examined later in the laboratory.

Based on results of previous cruises, we felt capable of visually identifying swordfish and shortbill spearfish eggs based on their different sizes and distinct pigment patterns. However, during this cruise we encountered swordfish eggs in a more advanced stage of development and somewhat different in appearance than previously. These advanced stage eggs (embryo circumscribed the egg to nearly 360°) had pigmented chromatophores over the yolk, embryo, and oil globules as previously observed but the intensity of the starburst-shaped chromatophores was substantially less and rather hard to detect. The shape of these chromatophores appeared to be stretched similar to what occurs when a mark on the surface of a balloon is stretched after expansion of the balloon. The identity of these advanced eggs was confirmed at sea based on the multiplex-PCR assay.

A total of 41 eggs were run using the at-sea multiplex-PCR assay to confirm the species identities of suspected billfish eggs and discount the identity of other eggs with somewhat

similar appearance but of non-billfish origin. During this cruise, egg abundances were recorded for the following species; swordfish (n = 33 from 9 tows), shortbill spearfish (n = 5 from 1 tow), wahoo (n = 4 from 3 tows), and mahimahi, *Coryphaena hippurus* (n = 3 from 3 tows). Most of these egg captures were collected from tows off the south Kona coast (below 19° 18´N latitude). Blue marlin eggs were not recorded during this cruise.

Net tows using the same 1.8-meter IK also collected larval stages of billfish (n = 27) consisting of swordfish (n = 10 from 8 tows), shortbill spearfish (n = 16 from 13 tows), and 1 yet unidentified istiophorid larva. Shipboard multiplex-PCR analysis was conducted to identify all (except one) istiophorid larvae collected. Unlike last year's cruise (OES 05-07; 26 May-2 June 2005), no larvae of blue and striped marlin were recorded. For both swordfish and shortbill spearfish larvae, positive tows occurred almost exclusively along the south Kona coast (below 19° 18´ N latitude). Swordfish and shortbill spearfish larvae were collected throughout the day (0700-1800). Post-cruise processing of net tow samples will probably yield a few additional billfish larvae (pre-flexion stage) that were overlooked during our initial at-sea processing.

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

Prior to initial DNA extraction, all eggs to be multiplex-PCR tested were first digitally photographed under a dissecting microscope and then placed in individual microcentrifuge tubes of Chelex solution for DNA extraction. The entire egg was consumed during these procedures while for larvae, only one eyeball was extracted. Egg and tissue standards from known billfish species were periodically run with unknown egg and larvae samples to confirm results. The multiplex PCR technique provides rapid identifications (3 h processing), ease of use, and shipboard adaptability which allows our billfish egg collection efforts to quickly adapt sampling schemes during the cruise.

With the net collection, digital imaging, and shipboard PCR identification, we were able to resolve that the advanced stage eggs (embryos circumscribed eggs to nearly 360°) that looked different from other swordfish eggs collected from previous cruises were indeed swordfish. Furthermore, we were able to detect eggs of *Coryphaena hippurus* and resolve that their egg diameters (1.56 mm) are slightly less than that of swordfish, and that *C. hippurus* eggs also differ in having scale-like patterns and point-like pigmentation on the yolk adjacent to the embryo. The changes in appearance of swordfish eggs with development and the current lack of described egg stages for sailfish and striped and black marlin indicate the continued need for shipboard multiplex-PCR testing during these cruises.

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. Initial examination of the raw data indicated that sea surface temperature (SST) ranged from 24.7°C to 25.5°C along the Kona coast while sea surface salinity (SSS) varied from 34.56 to 34.67 psu. The more distant offshore CTD stations recorded the cooler SSTs and

more saline SSSs compared to the inshore stations. The mixed layer was between 120 to 135 meters deep as recorded from the CTD profiles.

Near surface current directionality as recorded by the shipboard ADCP indicated a slow (0.1-0.2 m/s) southerly current immediately adjacent to the Kona coastline. This current switched to a northerly direction some 5 to 10 nmi offshore with swift current speeds >0.5 m/s.

During the first two working days (April 25-26) off Kona, the thermosalinograph (TSG) was operational but not transmitting data to the shipboard computer system (SCS) unit. On the second day (April 26) a SeaBird SBE-39 sensor unit was attached at the footrope entrance of the IK net to record temperate and pressure (depth) during these tows. Late in the day on April 26, a disconnected cable was found and the data stream of SST and SSS values from the TSG to the SCS unit was restored. Thereafter, SST and SSS data were available during the remainder of the cruise.

D. Cooperative arrangement between Syd Kraul and PIFSC to rear billfish eggs in captivity.

Because of the lack of abundant catches of billfish eggs during this cruise and after consulting with Syd Kraul via telephone, it was decided not to transfer any of the billfish eggs caught in the surface IK tows for shoreside rearing. Instead, several of the swordfish eggs were placed in small plastic bottles half-filled with seawater and were made to float within an aquarium tank. The three swordfish eggs that were reared all hatched on the day of capture; two of the hatchlings were immediately preserved in 95% ethanol. The other hatchling survived to the next day and was also preserved. The later hatchling was supplied with newly hatched brine shrimp but showed no feeding behavior. This hatchling floated in the water column of the plastic bottle and only infrequently displayed a brief twitch of the tail.

D. Miscellaneous activities and observations.

Aboard this cruise were two photographers on assignment for National Geographic to produce a story on the surface microfauna off the Kona coast. David Liittschwager photographed pristine specimens of larval fish and invertebrates collected from our surface IK tows, and dip-netting from both surface slicks and around a night-light. A canopied shelter was assembled in the fantail that allowed Liittschwager to set-up his photographic equipment in a semi-sheltered environment. Roy Eisenhardt videotaped shipboard operations, laboratory processing of the net tow catches, and video-interviewed members of the scientific filed party regarding their research and its connection to fisheries and management.

From April 26 to April 29, the *Sette*'s safeboat was deployed from 0700 to 1100 to collect and observe billfish larvae within surface slicks. Andrew West and Russ Vetter dip-netted istiophorid larvae that were presumed to be shortbill spearfish, although species identifications were not confirmed via the multiplex-PCR assay. These dip-netted istiophorid larvae were held temporarily onboard the safeboat and then released back into the water. Their swimming behavior was recorded both visually and on underwater

videotape by West and Vetter. Although some of the slicks contained a considerable amount of flotsam, particularly plastics and plant parts, few istiophorid and virtually no swordfish larvae were observed. However, visual sightings of larval and juvenile-staged exocoetids and coryphaenids were common within these same slicks.

Russ Vetter and Eric Lynn (Southwest Fisheries Science Center, La Jolla) used viable eggs of swordfish and shortbill spearfish to measure oxygen consumption using a microrespirometry system developed by Anders Bang and Peter Grønkjær of the Institute of Biological Sciences, University of Aarhus, Denmark and Unisense S/A, Aarhus, Denmark. The use of oxygen consumption for the study of eggs and yolk sac larvae has been limited by the technical limits of polarographic oxygen electrodes and the simple difficulties of manipulating tiny, rapidly developing organisms in small chambers. For species such as billfishes, whose eggs cannot be spawned in the laboratory, the problems are compounded by the unpredictable and low availability of field caught eggs, the variance in egg stage, and the short study times for working with developing eggs and larvae. The system developed by Peter Grønkjær's Lab is designed to allow accurate measurements of single eggs, and the oxygen electrode can be moved from chamber to chamber so that multiple eggs can be measured at the same time. Using this system, Vetter and Lynn were able to obtain the first oxygen consumption measurements of eggs and hatchlings of swordfish and the eggs of shortbill spearfish. Since billfish eggs from a single tow are often in a similar developmental stage, the advantages of working concurrently on up to eight eggs rather than working sequentially was clear given the rapid developmental rates.

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 data files on DVD-R*
CTD Station Data Log Sheet
Seabird CTD data files on DVD-R*
Marine Operations Log
Deck Log
Plankton, Eggs and Larvae #1 (all net tows)
SCS data files (raw & compressed) on DVD-R*
Metadata files on DVD-R*

*All data files together on the same (1) DVD-R

SCIENTIFIC PERSONNEL:

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Roy Eisenhardt, Photographer, Independent
David Liittschwager, Photographer, Independent
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Attachments

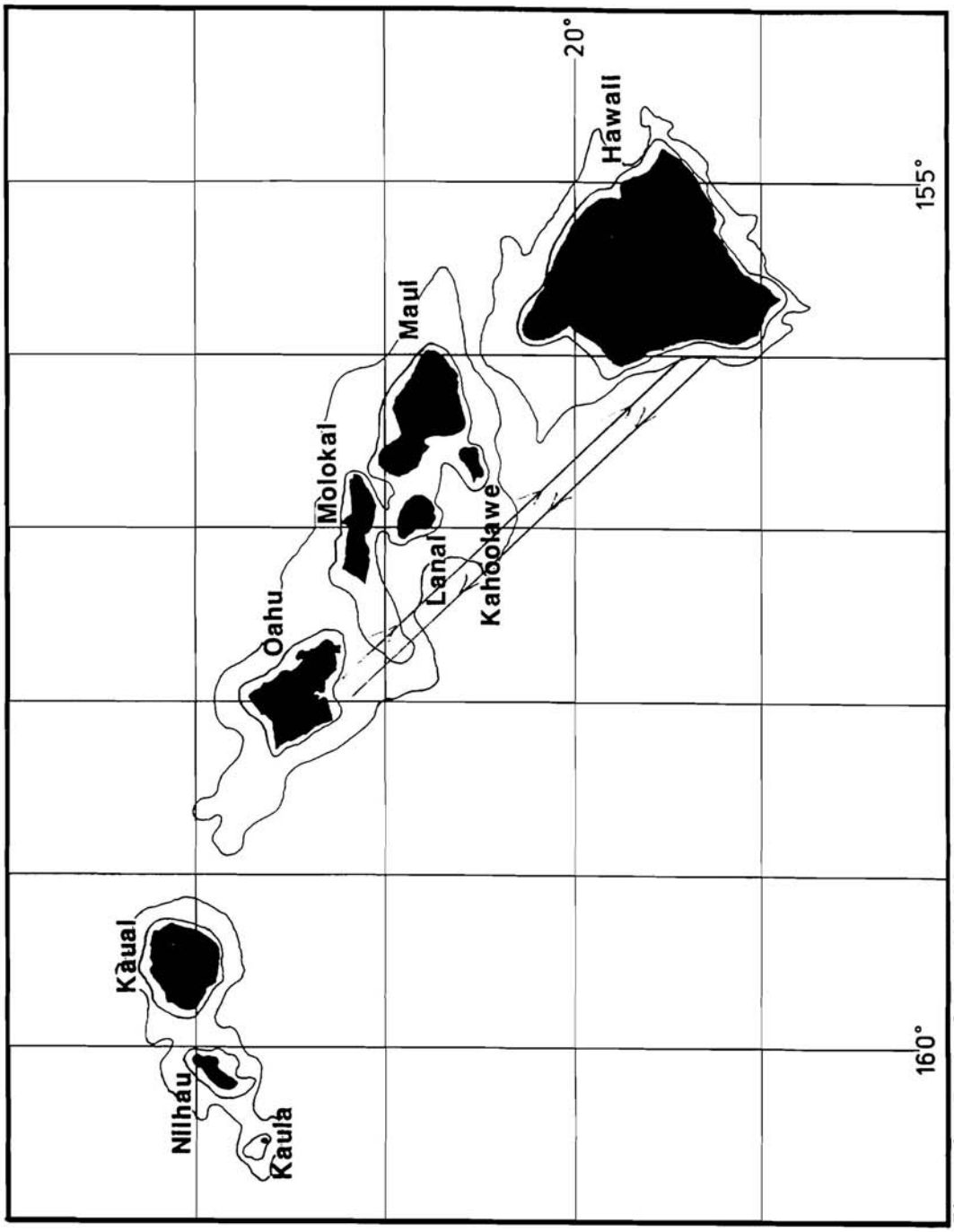

Figure 1.--Track of the NOAA ship Oscar Elton Sette OES-06-05 (OES-41), April 24 to May 1, 2006.