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CRUISE REPORT

VESSEL: *Oscar Elton Sette*, Cruise 04-09 (OES-18)

CRUISE PERIOD: 20-25 July 2004

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

TYPE OF OPERATION: Surface net tow operations for billfish larvae and eggs 2-25 nmi off the Kona coast of the Island of Hawaii. Daylight surface slick and non-slick tows were conducted with a 1.8-meter Issacs-Kidd (IK) trawl net (net entirely 0.5 mm mesh) towed from midship. Safeboat operations independent of the ship included tows of dual 0.5-m egg nets (with 0.5 mm mesh) and dipnetting of billfish larvae within slicks. A grid transect of shallow conductivity-temperature-depth (CTD) casts was also conducted along the Kona coast.

ITINERARY:

20 July	Embarked Robert Humphreys, David Ambrose, Dan Curran, John Hyde, Ellen Jacobson, Eric Lynn, and Lianne McNaughton. Departed Snug Harbor at 1300 and proceeded to Kailua-Kona, Island of Hawaii.
21 July	Arrived off Kailua-Kona at 0800 and embarked Andrew West from the Kailua-Kona pier. Afterwards, commenced egg and larval billfish collections with surface IK tows from the ship. At midmorning, began safeboat net tow operations using dual 0.5-m egg nets for eggs and larvae and dipnetting for larvae. At nightfall, commenced nightly CTD casts to 150 meters depth at predetermined sampling sites.
21-23 July	Continued daily schedule of daylight surface IK tows from the ship and morning safeboat net tow operations using dual 0.5-m egg nets for eggs and larvae and dipnetting for billfish larvae. Also continued nightly CTD casts to 150 meters depth at predetermined sampling sites.

24 July — Conducted daylight surface IK tows from the ship and morning safeboat net tow operations using dual 0.5-m egg nets for eggs and larvae and dipnetting for billfish larvae. At 1500, disembarked Andrew West at Kailua-Kona pier, discontinued net tow operations, and proceeded back to Snug Harbor, Honolulu.

25 July — Arrived Snug Harbor, Honolulu at 0730. Disembarked Humphreys, Ambrose, Curran, Hyde, Jacobson, Lynn, and McNaughton; 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 thirty-three 1.8-meter Issacs-Kidd (IK) tows were conducted; all tows were made with a 0.5-mm mesh nylon net. Tows targeted coastal sea surface slicks, when present, at distances of 2-25 nmi offshore of the Kona coast between Kailua-Kona (19^0 37'N latitude) to the north and near Milolii (19^0 12'N latitude) to the south. Tows were conducted for 1 hr alongside the ship off the port side J-frame. The IK net filtered the top 1-1.25 m of surface water including the neuston layer during tows except for four subsurface tows which filtered water at a depth of 1.5-3.0 m below the surface. The safeboat worked independently of the ship and equipped with a 12-ft. angle iron crosspiece, allowed dual 0.5-m egg nets (nets with 0.5-mm mesh) to be towed simultaneously for 30 min per tow. When not towing nets, dipnetting from the safeboat was conducted while operating in surface slicks. Safeboat operations were limited to the morning daylight hours (0600-1300). Surface slicks are normally present only during the morning periods since offshore breezes produced by thermal release over the island typically disrupt slick occurrence by early afternoon. During much of this cruise, surface slicks were present by midmorning, dissipated by the afternoon, and sometimes reappeared in the late afternoon-early evening prior to sundown.

The 1.8-meter IK 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 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 IK net with 0.5-mm mesh resulted in the occurrence of a large number of eggs varying in size from about 1.0 to 3.0 mm in diameter. Each 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 and 1.0-mm mesh. The contents that passed through the top 2.8-mm sieve 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 in 95% ethanol for post-cruise examination.

Based on last year's cruise (OES 03-03, Leg 2), we initially felt capable of visually identifying swordfish and shortbill spearfish eggs based on their larger sizes and distinct pigment patterns but were uncertain what blue marlin eggs might look like. Therefore, all

pigmented eggs 1-2 mm in diameter were measured and photographed prior to subsequent destructive sampling via shipboard multiplex polymerase chain reaction (PCR) identification. It was not until the final sampling day of the cruise (24 July 2004) that we were able to confirm the presence of blue marlin eggs in our sample and become aware of their smaller size (1.25-1.30 mm diameter) and dark pigmenting over the oil globule and partially over the yolk. A total of 83 suspected billfish eggs were PCR tested during the cruise and yielded 23 positive billfish identifications. Additionally, 37 eggs were visually identified as swordfish after initially confirming via multiplex PCR identification that a previous subsample of 10 eggs, also visually identified as swordfish, were correct. A total of 60 billfish eggs were identified during the initial sorting of tow samples at sea. The majority of these identified eggs were swordfish (n = 47), then blue marlin, *Makaira nigricans* (n = 8), and shortbill spearfish, *Tetrapterus angustirostis* (n = 5). This represents the first known collections of planktonic eggs of Pacific blue marlin, whose fertilized egg stages remain undescribed in the literature. For this cruise, the multiplex PCR technique was expanded by John Hyde to include markers to identify wahoo, *Acanthocybium solandri*, mahimahi, *Coryphaena hippurus*, and the pompano mahimahi, *C. equiselis*. During the cruise, one wahoo and three pompano mahimahi eggs were identified via multiplex PCR.

Net tows (both 1.8-meter IK and the dual 0.5-meter egg nets towed independently from the ship's safeboat) also collected numerous (n = 292) larval billfish consisting of 5 swordfish and 287 istiophorid larvae. Istiophorid larvae are notoriously difficult to identify to species, particularly if damaged. Two hundred fifteen of the 287 istiophorid larvae collected during the cruise were PCR tested at sea and identified as blue marlin (n = 191), shortbill spearfish (n = 10), and 14 istiophorid larvae that did not yield a species identification. The latter unidentified istiophorid larvae are probably the result of poor DNA extraction and/or PCR amplification and need to be resampled and run a second time.

Identified billfish eggs and larvae co-occurred in only 2 net tow stations; the remaining billfish eggs and larvae were collected separately from 3 and 24 other stations, respectively, of the 37 net tow stations conducted during the cruise. Eight net tow stations yielded no billfish eggs and/or larvae. Billfish eggs were distributed 2.5 to 5.0 nm offshore with the majority (57 of 60 eggs) collected along or just seaward of the 1,000 fm bathymetric contour. No co-occurrence of swordfish, shortbill spearfish, or blue marlin eggs were present in the 5 tows for which billfish eggs were PRC identified. All swordfish eggs (n = 47) were collected from a single tow while all shortbill spearfish eggs (n = 5) were collected from two tows (3 and 2 eggs, respectively). All swordfish and shortbill spearfish eggs were collected 5 nmi off the southern portion of the Kona coast. All blue marlin eggs (n = 8) were collected from two tows (5 and 3 eggs, respectively) taken 5.0 and 2.5 nmi off the coast from Keauhou to Kealakekua. All blue marlin and shortbill spearfish eggs (4 tows) were collected from surface and subsurface tows targeting surface slicks; the single tow which produced all 47 swordfish eggs was not directed along a slick. All blue marlin and shortbill spearfish eggs were collected between 0630 and 1115; swordfish eggs were all collected midafternoon (1415-1515).

For billfish larvae, blue marlin predominated; highest catches occurred in early morning (0600-0800) and late afternoon (1600-1900) tows. A large proportion of these larvae were small (≤5 mm total length). These smaller larvae had not been encountered during previous Kona cruises because of the larger mesh sizes of the nets used. Although only four subsurface IK tows (net mouth at 1.5-3.0 m depth) were conducted during the cruise, these tows yielded 71 of the 287 istiophorid larvae collected. Surface tows targeting surface slicks did not appear to catch significantly greater amounts of billfish larvae compared to nonslick tows. Post-cruise processing of these net samples for billfish eggs and larvae has begun and will undoubtedly yield more billfish eggs and larvae that were overlooked during our initial at-sea processing.

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

This was the fourth cruise (preceded by *Townsend Cromwell* cruises 01-06 and 02-03, Leg 2, and OES cruise 03-03, Leg 2) where trials were conducted of DNA-based methods to identify billfish larvae and eggs at sea in near-real time. During this cruise, John Hyde (Scripps Institute of Oceanography) added an additional set of three unique markers to detect wahoo, mahimahi, and pompano mahimahi in addition to the markers developed last year to differentiate all six species of Indo-Pacific billfishes. This technique uses a multiplex species-specific PCR designed to produce unique gel band patterns for each of the nine species. Processing time for this assay is now 3 hours. Furthermore, the lab bench space needed to conduct these PCR assays on the ship 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 first photographed and then placed in individual 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 provided 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. Initial examination of the raw data indicated that sea surface temperature ranged from 27.1°C to 27.9°C along the Kona coast while sea surface salinity varied from 34.52 to 34.63. The mixed layer was deep (~100 meters) at most of the CTD stations. The ADCP unit was not operational during the entire cruise so data on current direction and speed with depth was unavailable.

D. Miscellaneous oceanographic observations.

Trichodesmium concentrations were frequently noted within surface slicks, and surface tows targeting such slicks usually produced clogged nets that required high pressure spraying of the plankton net to clear this algae from the nylon mesh.

Exclusive use of the 0.5-mm mesh plankton net during this cruise greatly reduced the catch of istiophorid larvae ≥10 mm TL. Alternately, the much higher catches of istiophorids <10 mm TL is probably a function of the much finer mesh and lower towing speeds used during this cruise. The total catch of billfish larvae from this cruise almost equaled the entire catch of billfish larvae from the previous eight cruises that used the much coarser meshed (5 mm) IK net.

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.

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)
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

SCIENTIFIC PERSONNEL:

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Appendices attached

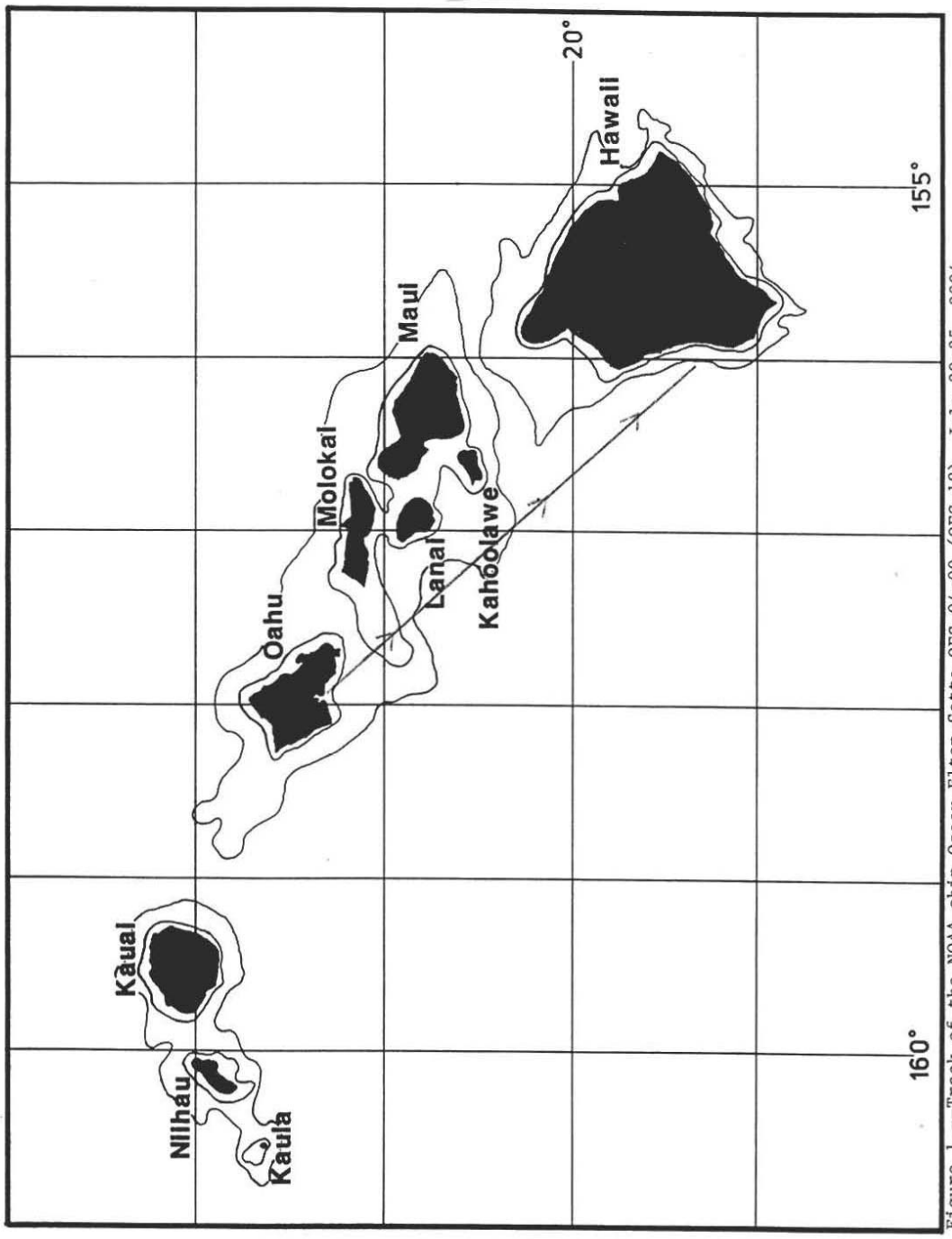

Figure 1.--Track of the NOAA ship Oscar Elton Sette OES-04-09 (OES-18), July 20-25, 2004.