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Biological Research Conducted during 2002-2003 In Support of Swordfish Stock Assessment[1]

Robert L. Humphreys. Jr., Michael Musyl, and Edward E. DeMartini
Pacific Islands Fisheries Science Center
National Marine Fisheries Service, NOAA
Honolulu, Hawaii 96822-2396 U.S.A.

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At the Swordfish Working Group session of the Third Meeting of the Interim Scientific Committee for Tuna and Tuna-Like Species in the North Pacific Ocean (ISC3), held during the 25-26 January 2002, in Nagasaki, Japan, several biological research topics were identified for further study that encompassed swordfish age and growth, movement, and stock structure. Age and growth studies centered on estimates based on otolith increment analysis and the evaluation of possible regional growth differences. Movement studies examined movement patterns and inferred behaviors based on conventional tag recaptures and electronic tags using archival and PSAT tag technologies. Stock structure work required additional tissue samples of young swordfish from specific areas. Additionally, sampling and analysis of meristics was to be conducted to ascertain whether distinct geographic differences exist that could serve as stock indicators. In this report we update and summarize: (1) the age and growth of juvenile Hawaii swordfish based on otolith increment analysis and compare these results to juveniles from the western North Pacific aged by Sun et al. (2002); (2) movement patterns from recent PSAT tagging around Hawaii; and (3) meristic counts based on juvenile swordfish collected over a broad latitudinal expanse within the central North Pacific. Also reviewed are related studies on the feasibility of otolith elemental composition as indicators of nursery site origin and the at-sea identification of swordfish eggs collected from plankton tows using a new PCR protocol developed for shipboard use.

1. Age and Growth

An age and growth study based on the enumeration of presumed daily growth increments within the sagittal otoliths of young swordfish is near completion. The objectives of this study were to determine the early growth rate of swordfish and to corroborate the size-at-age 1 results of a concurrent age and growth study based on second anal fin ray sections. The size range of specimens (n=48) utilized in this study ranged from 5.3 cm to 150.4 cm lower jaw fork length (LJFL.) This is equivalent to 3.7 cm to 133.0 cm eye-to- fork length (EFL). Larvae 5.3-6.1 cm LJFL (3.7-4.2 cm EFL) were collected from neuston tows off the Kona coast of the Island of Hawaii. Late larvae to early juveniles 19.9-44.0 cm LJFL (14.5-35.7 cm EFL) were taken from predator stomachs and by surface dip-netting while larger juveniles 66.5-150.4 cm LJFL (54.6-133.0 cm EFL) were caught on pelagic longline sets conducted in central North Pacific waters in and adjacent to the Hawaiian Archipelago.

An otolith preparation technique was developed to allow the enumeration of the entire series of sagittal otolith increments from core to tip of rostrum. Overlying otolith material along this path was selectively removed by applying minute amounts of dilute acid from a fine tip brush. Acid application ceased when the internal increments appeared directly at the surface. Processed sagittae were similarly etched with EDTA, cleaned in ethanol, carbon coated, and then

examined using SEM. This technique provided the longest increment pathway between core and edge to be used for increment enumeration. However, with increasing age, growth along the rostrum tip narrows in width and internal increment widths decline to <0.5 microns. For each sagitta, a series of overlapping digital photos was made from the core region to the tip of the rostrum. Increment counts were made directly from these photos using NIH Image software.

The growth curve based on 48 young swordfish spanning 5.3-150.4 cm LJFL (3.7-133.0 cm EFL) was better estimated using a three parameter polynomial function compared to a two parameter power function (Fig. 1). The polynomial function provided a better predictor of size at age for swordfish <180 days old. The estimated size at 365 days (age 1) was 112 cm and 110 cm LJFL (97 and 95 cm EFL), based on the polynomial and power function, respectively. These age 1 size estimates were considerably larger than the increment based age 1 (at 365 days) size estimate of 94 cm LJFL reported in Sun et al. (2002) for swordfish caught off Taiwan.

Additional individuals for ageing will be selected to fill the size at age gaps at 30-60 cm LJFL and 90-100 cm LJFL in Fig. 1.

Otolith increment validation studies on adult swordfish have yet to be successfully conducted. No tag recaptures of oxytetracycline injected adult swordfish have occurred around Hawaii. Larval swordfish collected from plankton tows off Kona, Hawaii, rarely yield live larvae. Efforts to keep alive the few live larvae collected in these tows have been unsuccessful beyond 2 days. For billfish species, the assumption of daily periodicity of otolith increment formation is usually evaluated based on circumstantial evidence. This includes comparison of the known temporal spawning period (based on larval catches) with the backcalculated distribution of birthdates (derived from otolith increment enumeration). Based on surface plankton tows conducted for billfish larvae off Kona, Hawaii, the known annual occurrence of swordfish larvae is from April through October. Of the 48 swordfish larvae and juveniles aged thusfar, 90% (43 of 48) of the backcalculated birthdays coincide with the April-October period.

2. Meristic Variation of Juvenile Swordfish in the central North Pacific

Differences in meristic characters of teleosts among latitudinally distant nursery regions may originate from the separate or combined influences of genetic and environmental factors. Observed meristic differences between fishes in distant nursery areas may be indicative of stock separation (Pawson and Jennings, 1996). In terms of environmental influence, meristic differences in vertebral number tends to be higher in cooler waters than warmer waters (Jordan's rule) and may also be expressed through like variation in fin ray elements (Lindsey 1988). A study to examine the possibility of using meristic variation as a natural marker of natal spawning sites was conducted on young-of-year (YOY) juvenile specimens (47-99 cm EFL, n=217) collected from equatorial to temperate waters within the central North Pacific. We assumed that these YOY juveniles remained latitudinally adjacent to their natal sites. Juvenile specimens were obtained as by-catch from Hawaii based tuna longline vessels. Meristic counts in this study were limited to total dorsal and anal fin elements. Although the fin rays comprise separate first and second dorsal and anal fins in adults, the rays within the dorsal and anal fins typically appear to be continuous in YOY juveniles examined and were therefore recorded as total counts. Meristic data were grouped geographically within latitudinal bands (0-7^{o} N, n=29; 13-16^{o} N, n=38; 20-22^{o} N, n=39; and 28-34^{o} N, n=34, and all specimens 0-37^{o} N, n=214) to represent areas associated with the equator, North Equatorial Current, main Hawaiian Islands, the Subtropical Transition Zone, and the entire central North Pacific, respectively. The geographic extent of this

comparison also approximately coincides with the maximum latitudinal range of swordfish spawning (surface waters $\geq 24^{o}$ C) in the central North Pacific. The distribution and mean values for both dorsal and anal fin elements showed no significant differences among these latitudinal groupings, even when the two most distant sample groups are compared (Fig. 2). No meristic counts for YOY juvenile specimens from other regions in the Pacific were available. Published meristic counts for total dorsal and anal fin elements, however, are reported by Potthoff and Kelley (1982) for larvae and juvenile specimens (1-69 cm eye standard length) collected from the western Atlantic specimens (Gulf of Mexico, Carribean, and Florida) (Fig. 2). The distribution and means for both meristics are indistinguishable from those derived from the central North Pacific. Results suggest that these meristics offer little future promise as a natural marker of natal or nursery site origin.

3. Elemental Fingerprinting of Swordfish Otoliths

In collaboration with Steve Campana (Bedford Institute of Oceanography, Nova Scotia, Canada), a preliminary study of the trace element composition of whole sagittal otoliths was conducted for YOY juvenile swordfish. Specimens (62-79 cm EFL, n=23) were collected from four nursery sites at different latitudes within the central North Pacific. Our objective was to assess whether a distinct elemental composition (fingerprint) occurs in juvenile otoliths from these nursery sites. The existence of geographically distinct elemental fingerprints could serve as natural markers embedded in the juvenile portion of adult otoliths. Such markers could help determine the origin and interchange of adults captured on the major swordfish fishing grounds, typically at higher latitudes. Detection of otolith trace element concentrations was determined using isotope dilution-inductively coupled plasma mass spectrometry (ID-ICPMS); assays were conducted at the National Research Council Laboratory in Ottawa, Canada. Based on earlier assays of adult swordfish otoliths, elements present above the limits of detection of the spectrometer and used in this study were Mg, Zn, Sr, Ba, and Pb. Elemental concentrations were similar to those of other marine fish otoliths. For ID-ICPMS of the juvenile otoliths, left and right sagittae were pooled for each fish prior to dissolution in isotope enriched nitric acid. Juvenile swordfish specimens were collected within a narrow swath of longitude ($160\text{-}165^{o}$W); latitudinal sites within this swath included equatorial waters ($0\text{-}1^{o}$N, n=12), waters immediately south of Hawaii (17^{o}N, n=2), west of the main Hawaiian Islands (22^{o}N, n=7)), and in temperate waters north of Hawaii (31^{o}N, n=2). Concentrations of Mg, Zn, and Pb displayed similar levels of concentrations among these latitudinal sites. The concentrations of Sr and Ba, however, varied significantly with greater latitudinal separation. The concentration of Sr declined with increasing latitude while Ba concentrations showed a reverse trend. Using discriminant analysis, Sr and Ba concentrations significantly differed (Fig. 3) between the two larger sample size sites ($0\text{-}1^{o}$N and 22^{o}N sites). Discriminant analysis of the two other sites (17^{o}N and 31^{o}N) with low sample size (n=2) was not significant. These results point out the potential promise rather than the direct application of the geographic variation in the swordfish elemental fingerprint. Other lines of investigation are needed before this technique can be used to attempt assignment of individual adults to their nursery area of origin. To sample only the core area of the sagittal otolith which corresponds to the natal and early juvenile stage, a probe based mass spectrometry technique using laser ablation (LA-ICPMS) sampling will need to be tested. Additional YOY juveniles collected from sites outside the central North Pacific (western Pacific off Japan and French Polynesia) will be included in these tests to assess whether distinct otolith elemental

fingerprints exist for juveniles associated with these distant nursery areas. Furthermore, the temporal stability of elemental fingerprints needs to be established by testing samples over several years from the same nursery area(s). Currently, several years of otolith samples collected around Hawaii are available to address this issue.

4. Update of Results from PSAT Tagging

In March-April 2001, NMFS Honolulu scientists conducted the first PSAT tagging of swordfish in the vicinity of Hawaii. These tags are capable of recording vertical and horizontal movements by recording pressure (depth), geolocation (based on changing light levels), and ambient temperature. PSAT tags release and transmit data to a satellite when its pre-programmed pop-off date is reached. PSAT tags also release and transmit data if no pressure change is recorded for four consecutive days (tag has presumably been "shed" and is free-floating) or if the tag reaches a depth of 1200 m (the fish has presumably died and sunk through this depth). The PSAT tags were attached to a barbed nylon dart tip with heavy monofilament line and implanted into the dorsal musculature using a long wooden tagging pole.

Twenty-eight swordfish have been PSAT tagged through the year 2003. Nine of 28 PSAT tags reported their data earlier than their pre-programmed release dates. Data from these 9 tags represent in aggregate 527 days at liberty (range, 5-190 days, avg. 59 days). Horizontal start-end movements for these nine fish appear in Fig. 4. Because of deep crepuscular dive patterns, estimates of geolocations (from changes in ambient light-levels to calculate dawn/dusk) were not possible in a majority of cases. However, incorporation of SST into Kalman filter algorithms (Sibert et. al., 2003), whereby satellite-derived SST measurements are matched to those given by the PSAT, may help geoposition the tag. The premature shedding of PSATs is common for billfish. For example, Sedberry and Loefer (2001) reported a 40% shedding rate of swordfish in the western Atlantic. The cause(s) of this "early-release" problem with PSATs has not been identified but a new "speargun" head may promote better retention by increasing surface area of the tag anchor. Repetitive dives to 700-800m (ca. 70-80 atmospheres) may help explain the high non-reporting rate (i.e. tags weaken and fail over time due to pressure breech). Until these problem(s) can be rectified, the potential of PSATs to provide new insights into long term horizontal movement patterns and possible stock separation in swordfish remains limited.

5. At-Sea Identification of Swordfish Eggs Collected from Plankton Tows Using a Species-Specific PCR Assay

Colleagues at the NMFS Southwest Fisheries Science Center and the Scripps Institution of Oceanography have developed a species-specific multiplex PCR assay capable of identifying the early life stages of swordfish including the five Indo-Pacific species of Istiophoridae that co-occur in Hawaiian waters. This single-step PCR assay allows billfish species identification within 3 hours of sample acquisition. Although developed in the laboratory, this technique was tested at sea in May 2003 during a NMFS research cruise onboard the *Oscar Elton Sette* off Kona, Hawaii. A 1.5 meter ring net with 500 micron mesh plankton netting was towed through the surface layer in areas of previous larval swordfish catches. A total of 54 swordfish eggs were identified out of 148 suspected billfish eggs that were removed from plankton catches and assayed using the multiplex PCR technique. Swordfish eggs were similar in appearance (Fig. 5) to previous descriptions of artificially spawned eggs in the Mediterranean (Yasuda et al. 1978).

Swordfish eggs were collected in surface waters 5 to 25 nautical miles off the Kona coast over a bottom depth of 1,000 to 2,500 meters. This area overlaps the distribution of previous larval swordfish captures off Kona and suggests that spawning activity could have occurred in the immediate vicinity. Future swordfish egg collections using this molecular identification technique are planned off Kona in an effort to define the spatial and temporal dynamics of spawning in this region and to characterize the oceanographic features associated with swordfish spawning habitat.

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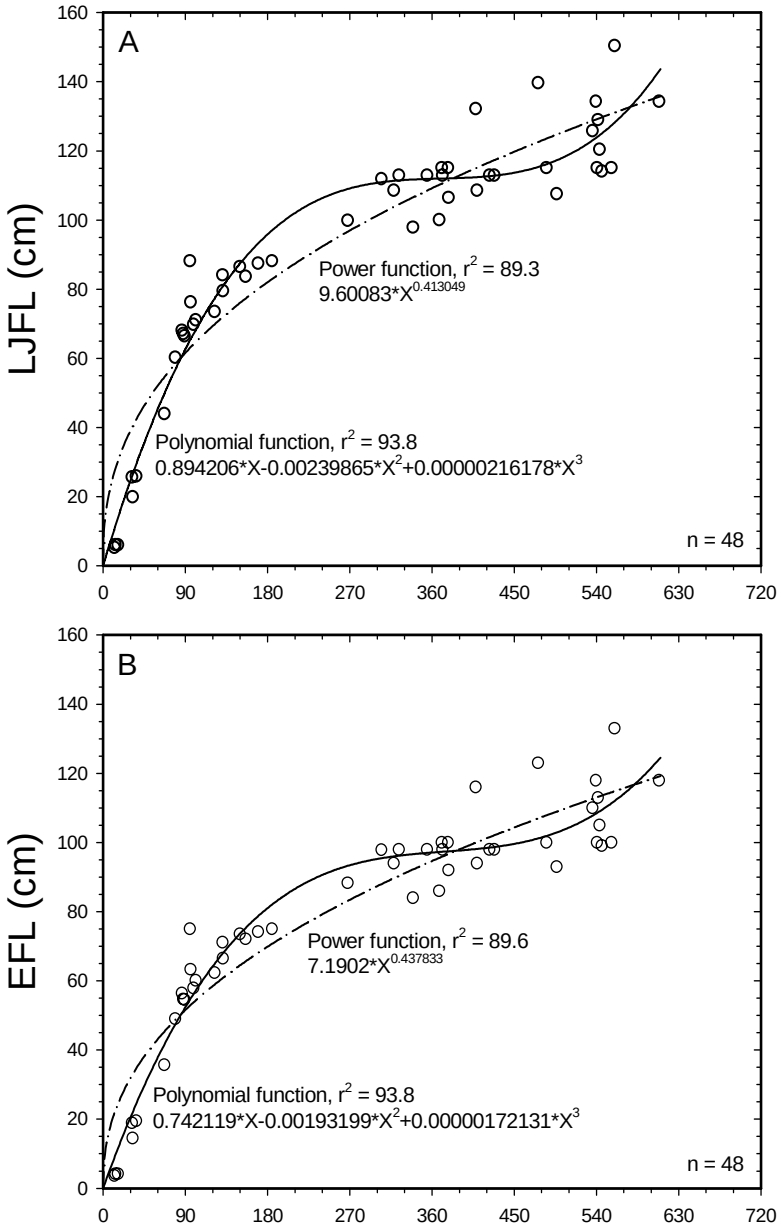

Figure 1. Age-length plots of otolith increment counts on juvenile swordfish from the central North Pacific near Hawaii. Ages at length are plotted for both (A) LJFL and (B) EFL. Polynomial and power functions are fitted for both.

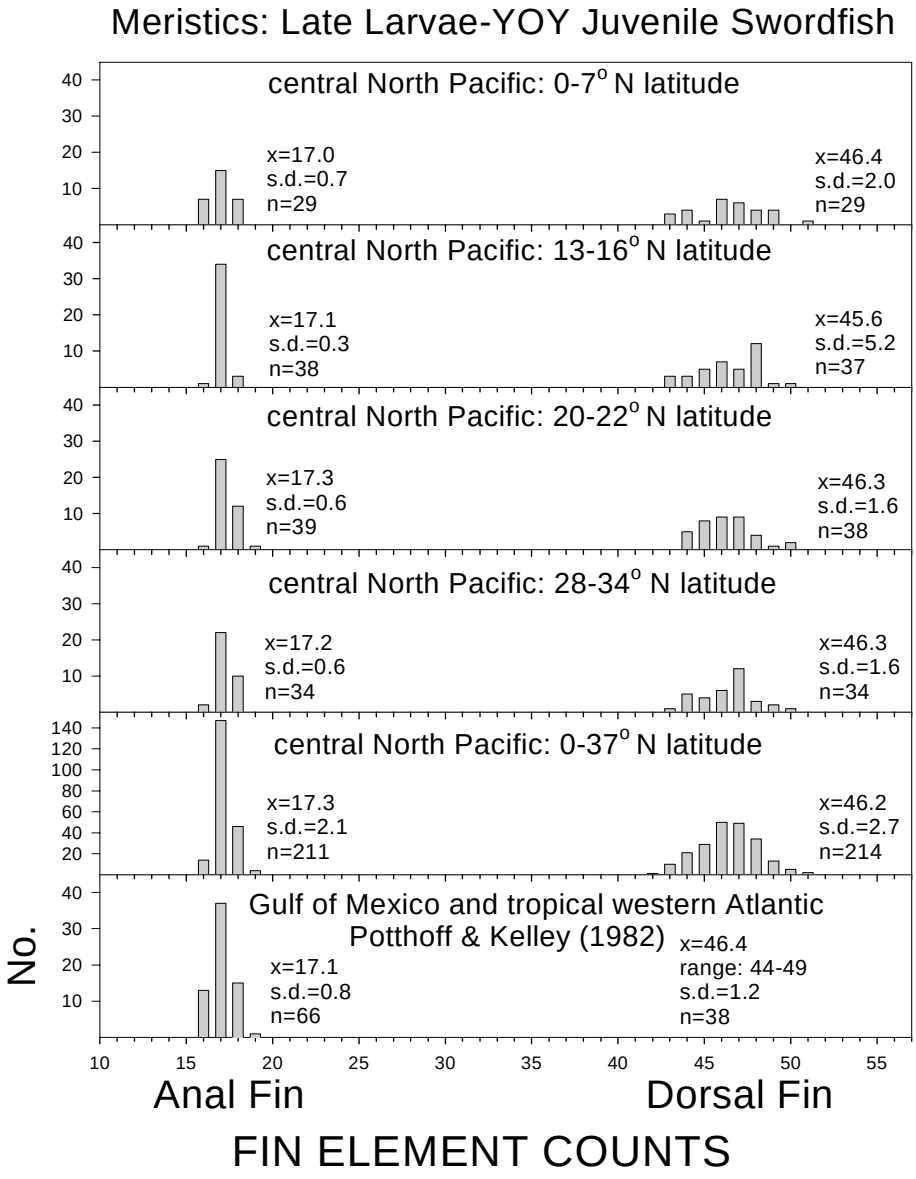

Figure 2. Distribution of total anal and dorsal fin element counts from YOY juvenile swordfish by latitudinal sites within the central North Pacific. Results from published Atlantic counts (in Potthoff and Kelley, 1982) are provided for comparison.

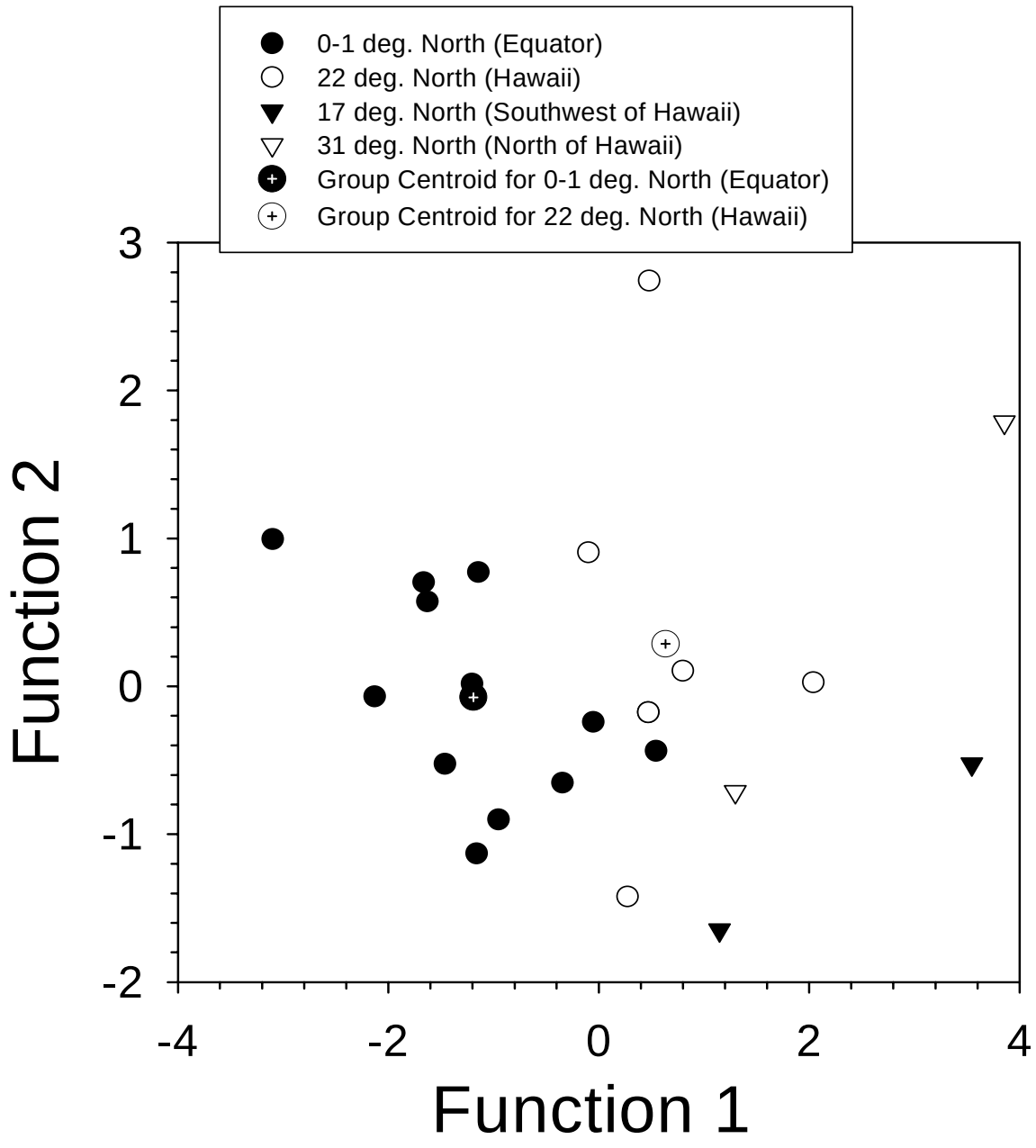

Figure 3. Plot of first two canonical variates from discriminant analysis of otolith elemental composition data for YOY juvenile swordfish collected from different sites within the central North Pacific.

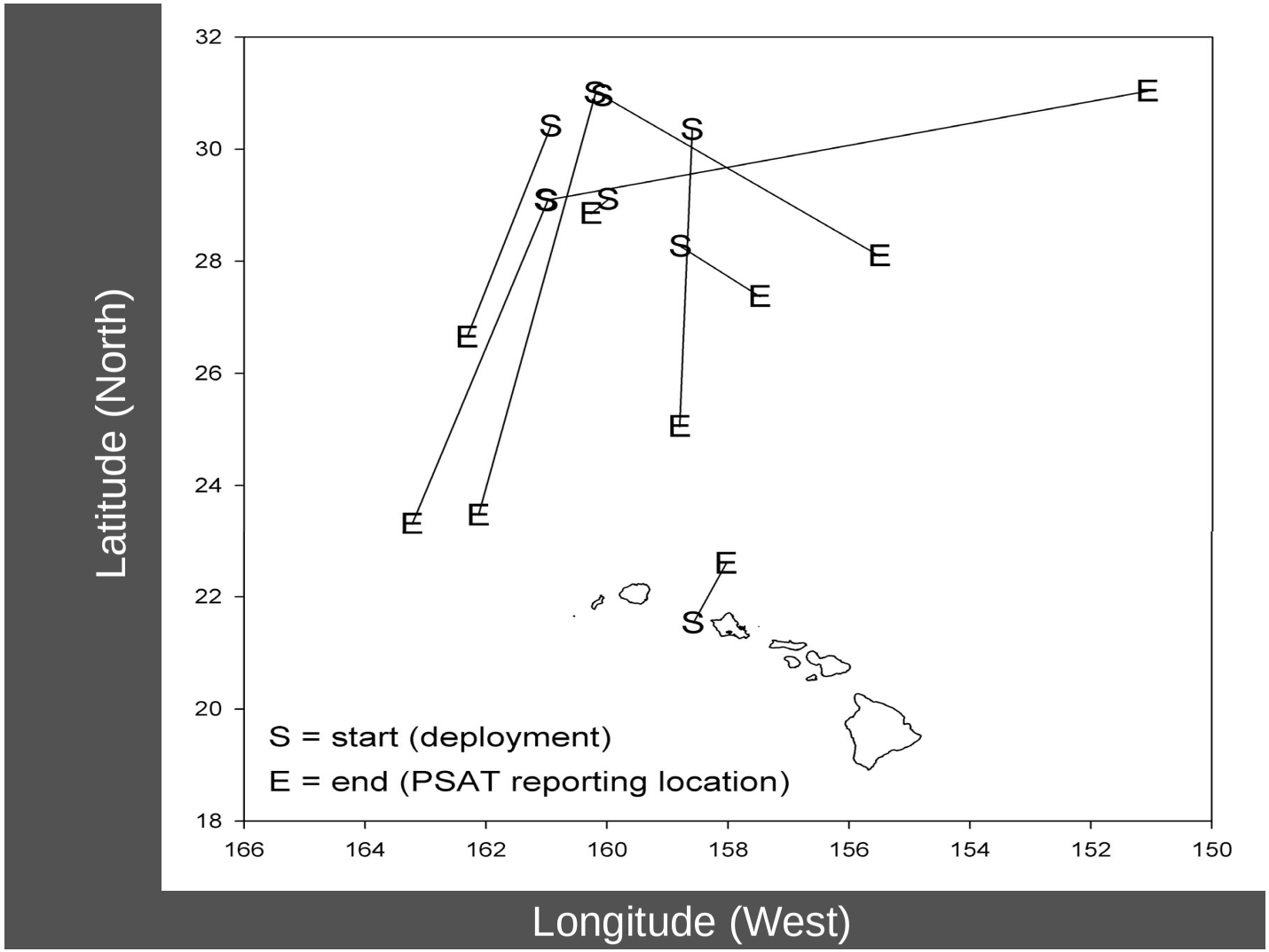

Figure 4. Start and end positions for the 9 swordfish PSAT tags that were tagged in the vicinity of Hawaii and prematurely shed.

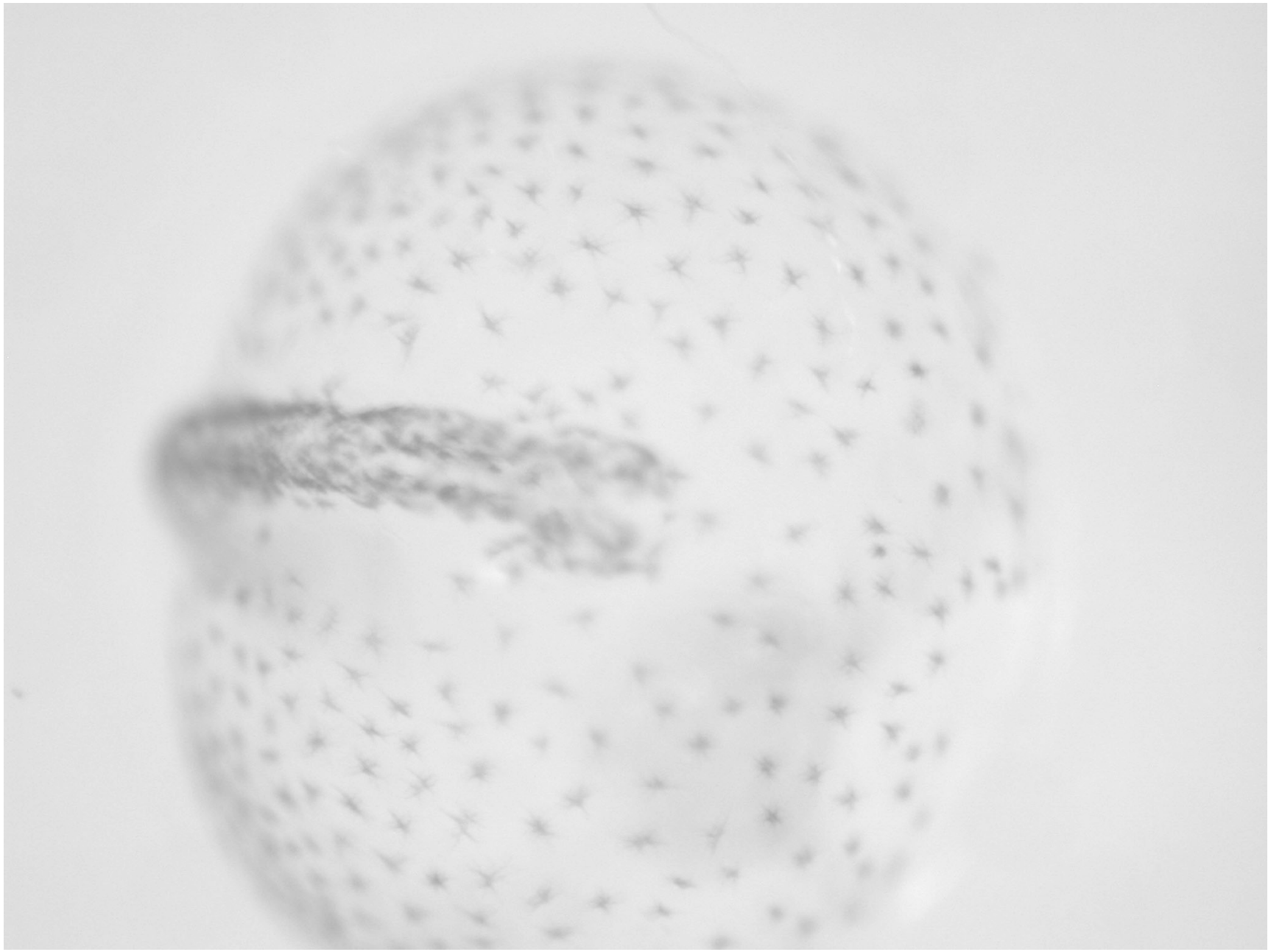

Figure 5. Photomicrograph of late stage (1.70 mm diameter) swordfish egg in seawater prior to preservation. Species identity of this egg was later confirmed as swordfish using the multiplex PCR assay.