

**Updated Biological Research
in Support of Swordfish Stock Assessment¹**

Edward E. DeMartini, Robert L. Humphreys, Jr., and James H. Uchiyama

Honolulu Laboratory, SWFSC
National Marine Fisheries Service, NOAA
Honolulu, Hawaii 96822 U.S.A.

January 2002

¹A working document submitted at the Third Meeting of the Interim Scientific Committee for Tuna and Tuna-Like Species in the North Pacific Ocean (ISC), January 2002, Nagasaki, Japan. Document not to be cited without permission of the authors.

Updated Biological Research in Support of Swordfish Stock Assessment

Edward E. DeMartini, Robert L. Humphreys, Jr., and James H. Uchiyama
Honolulu Laboratory, SWFSC
National Marine Fisheries Service, NOAA
Honolulu, Hawaii 96822 U.S.A.

The Swordfish Working Group at the Second Meeting of the Interim Scientific Committee for Tuna and Tuna-Like Species in the North Pacific Ocean (ISC2), held January 15-23, 1999, in Honolulu, Hawaii identified several priority research topics: (1) an evaluation of age estimates being generated by different fisheries laboratories conducting ageing studies of swordfish, and (2) the need for continuing multidisciplinary studies (tag-recapture and archival/PSAT tag programs to assess movements, geographical comparisons of growth rates, genetics and meristics) to elucidate swordfish stock structure within the Pacific. To date no laboratory has conducted the large-scale conventional tag-recapture study necessary to address the movements issue. In this report we: (1) summarize the results of an experiment conducted to evaluate bias among laboratories in estimating swordfish ages using anal fin ray cross-sections; (2) provide a preliminary characterization of length-at-age for swordfish from four of the five fisheries regions sampled by the five participating laboratories and a comparison of weight-at-length (condition) for fish from two regions with suggestive differences in length-at-age; (3) provide preliminary data on meristics of larval-juvenile swordfish collected near Hawaii; and (4) update movement patterns data obtained from recent tag-recapture and PSAT tagging around Hawaii.

1. Inter-Laboratory Calibration Test

Five Pacific fisheries laboratories (CICESE, IFOP, NMFS/Honolulu, NRIFSF, and the Institute of Oceanography National Taiwan University) prepared fin ray specimens for swordfish caught in their respective fishery regions and later read fin ray preparations for swordfish from all five areas. Initially, each laboratory prepared one or more cross-sections for the second ray of the first anal fin, using standard protocols (Ehrhardt et al., 1996; Sun et al., 2002) for 25 swordfish of both sexes and a range of body sizes and months of capture, as available from various fisheries. Specimens were sent to the Honolulu Laboratory, where they were first examined by dissecting microscopy. Twenty of the most readable specimens from each series of 25 were selected for preparation of digitized images. Each specimen was assigned a unique random number to cloak the source of samples. Two image files (one color, one grey scale) were prepared for each specimen. Images were prepared using a Sony DKC-5000 digital camera and macro-zoom lens with autofocus. Each color image (maximum 1520 x 1144 pixels; provided to help determine edge type) was slightly enhanced with the nonlinear brightness-contrast adjustment in Adobe Photoshop for NIH Image. A 2-mm line segment was added to each image for reference. A copy of each color image file was converted to grey scale without further processing, so that each laboratory could process images using their own image analysis system. All image types were saved as TIF files. Each series of 200 files (one color plus one grey scale file for each of 100 specimens) was formatted for Windows PC and copied onto a CD.

Image files for all 100 specimens were then distributed among all of the laboratories for reading. Each of the five laboratories completed one or more readings of all specimens whose images were deemed readable by that laboratory. Each laboratory read ages without reference to data on fish size, sex, or date and region of capture. Age estimates were then sent to the Honolulu Laboratory, where they were analyzed. A single age estimate per laboratory was used to describe specimen age; if a laboratory provided more than one age estimate per specimen, we used the mode of that laboratory's multiple estimates for the specimen. Draft summary results were distributed to all laboratories in early December 2001, with individual laboratory identities hidden (labeled as "A", "B", "C", "D" and "E").

Of the total 100 test specimens, size (eye-to-fork length, EFL) data were available for 80 specimens from four of the five laboratories. The distribution of lengths among each laboratory's series was broad, with somewhat greater numbers of larger fish for the Chilean and Hawaii-based fisheries (Figure 1).

There were a total of 83 specimens for which all five laboratories were able to provide an age estimate. However, consensus (100% age agreement among the five laboratories) occurred for only seven of these 83 specimens and only 14 additional specimens had 80% agreement among laboratories. Thus, there was a cumulative total of only 21 specimens with agreement greater than or equal to 80%. This was considered too small a sample size, so we proceeded with a lower standard of agreement for further analyses. There were 38 specimens with three identical counts, hence a cumulative total of 59 (21 + 38) specimens for which a 60% or greater majority (at least three of the five laboratories) agreed. These 59 specimens were used to provide a "majority agreement standard" (MAS) against which each laboratory's age estimates could be compared.

Mean Average Percent Error (APE) was 21% and the mean Coefficient of Variation (CV) was 28%, based on the 83 specimens for which five counts (five independent lab readings) were available. According to information available at the European Fish Ageing Network (EFAN) website (www.efan.no), our test swordfish APE and CV values are about average for this type of exercise. We consider this acceptable given the obviously greater-than-average level of difficulty involved in reading swordfish fin rays.

Inter-laboratory Bias.--For the evaluation of inter-laboratory variability (bias among labs), we examined each laboratory's readings independently for fish from all five geographic-fishery regions pooled. Analyses used two (mode, median) central tendency metrics. Primary analyses used the mode of each of the 59 MAS specimens; supplementary analyses used the median age estimate for each 5-value set of the 83 specimens with counts from each laboratory.

The age estimate provided by each laboratory for each of the 59 MAS specimens was first regressed against the agreed MAS age mode for each respective specimen (Figure 2). Ancova (with MAS as covariate and laboratory as class variable) was used to evaluate bias among labs. The ability to detect real differences among laboratory readings using these data (statistical power) was high. The percentage variance explained by each component regression variable was > 90% for 4 of the 5 laboratories ($r^2 = 93-98\%$ using the MAS mode as independent variable; $r^2 = 93-97\%$ using the median of all 83 specimens with five counts).

If all five laboratories' data are included in the analysis, significant heterogeneity among the slopes precludes a formal test of intercepts in ancova. This was caused by a single laboratory's readings (Laboratory C) differing significantly in slope as well as intercept

(Figure 2) from the other four laboratories' readings.

If the ancova is rerun excluding Laboratory C, the regressions for the remaining four laboratories do not differ among one another in either slope (ancova: slopes equal at $P > 0.70$) or intercept (laboratories equal at $P = 0.47$; Figure 2). We therefore conclude that: (a) the remaining four laboratories equivalently aged the sample fish from all five areas and (b) only Laboratory C aged fish in a manner inconsistent with the other four laboratories. Laboratory C appears to have generally underestimated fish ages, with the degree of underestimation increasing with fish size and age (Figure 3). We suggest that Laboratory C re-evaluate their viewing and counting methods and make certain that their future readings are more consistent with the MAS of the other laboratories when estimating ages of swordfish from their fishery region.

2. Possible Geographic Variation in Growth Rate

For the evaluation of possible regional variation in growth rates, we examined the age estimates from all five laboratories pooled for fish from each of the five regions. This analysis was complemented by an examination of available weight-at-length data for fish from two regions with large differences in length-at-age estimates.

Growth rate comparisons.--If eye-to-fork length (EFL) is related to the median of all five laboratories' age estimates for the respective fish and the data are partitioned by geographic region of capture, differences among regions become apparent. These differences are substantiated by ancova; analysis was limited to fish from only four fisheries because length data were unavailable for CICESE specimens. The slopes of the EFL versus median age relations do not differ, but intercepts do differ significantly among regions. This seems to be the case if all age estimates (including several influence points for fish ≥ 8 -yrs-old) are used in the analysis. Patterns become more demonstrably true (slopes equal: $P > 0.07$; intercepts [regions] different: $P < 0.001$) if these five influence points are deleted (Figure 4). Deleting the largest and oldest fish also reduces doubts about an assumed linear fit between length and age at small sizes and young ages.

Significant variation in the model intercepts is due to differences in length-at-age for fish from two groups of regions (Hawaii-Chile and the western North Pacific). Fish from the Hawaii-based and Chilean fisheries were in general aged by all laboratories as younger at a given body length than were Taiwan-NRIFSF specimens. These small sample sizes do not warrant any definite conclusions. At present all we can say is that the data suggest that readers from all five laboratories have, without knowledge of capture location and body size of specimen, independently assigned consistently different "ages" to swordfish of given size from the various regions. Since we lack validation of presumed age-related features, our observations may or may not reflect different growth rates among regions. Our exercise has merely detected different numbers of presumed age-related features present on same-sized swordfish from the various regions. We recommend that this analysis be repeated, after laboratories have completed size-at-age characterizations for swordfish from their respective fishery region using the suitably large sample sizes provided by production-scale, length-at-age characterizations. This could be accomplished by including a comparison of growth rates of swordfish among Pacific regions as a discussion topic in the last of the published production-scale studies.

Weight-at-Length.--If we speculate that regional differences in growth rate in fact exist, we would predict that weight-at-length relations might differ in parallel. Faster growth should relate

to heavier weight-at-length (better condition), so we compared available weight-at-length data for fish from two area-fisheries (Hawaii, Taiwan) with large differences in presumed age-at-length. Consistent with our prediction, swordfish from the Hawaii fishery ($n_1 = 165$) were heavier (whole weight) at a given body length than swordfish ($n_2 = 227$) from the Taiwan fishery (ancova: slopes ns different at $P = 0.20$; intercepts [= area-fisheries] different at $P < 0.0001$). The greater weight-at-length of Hawaii fish was especially pronounced (13-16% heavier) for young fish less than about 120 cm lower jaw-to-fork length (LJFL) (Figure 5). This confirmative prediction supports our recommendation to further compare length-at-age for swordfish among regions, as these are the first empirical life-history data, consistent with expectations based on recent genetic characterizations (Chow et al., 1997; Reeb et al., 2000), which suggest stock structuring for swordfish within the Pacific.

3. Preliminary Meristics Data for Post-larval and Juvenile Swordfish from Hawaii

A preliminary examination of total dorsal and anal fin ray elements were conducted on post-larval (5-27 cm EFL) and young-of-year (YOY) juvenile specimens (47-99 cm EFL) to evaluate the possible use of these meristics to distinguish swordfish stocks. The working hypothesis is that differences in meristic characters of young swordfish among nursery regions may originate from the combined influences of genetic and environmental factors. Such differences between fishes in distant nursery areas may indicate stock separation (Pawson and Jennings, 1996). Our initial effort was to examine the variation in meristics of young swordfish collected in the vicinity of the Hawaiian Archipelago. Larvae <5 cm EFL were not examined since accurate meristic counts would have required use of staining and clearing methods that would hinder any future molecular analysis of these specimens. Instead, post-larval specimens (5-27 cm EFL, $n=35$) were examined; most of these were collected from the stomachs of mahimahi, *Coryphaena hippurus*, obtained from a local fish market in Honolulu. YOY juveniles (47-99 cm EFL, $n=115$) obtained as by-catch from Hawaii tuna longline vessels were also examined.

Meristic counts were limited to total dorsal and anal elements. Although first and second dorsal and anal fins are separate in adults, rays within the dorsal and anal fins appeared continuous on the post-larvae and YOY juveniles examined and were therefore recorded as total counts. A greater range of total dorsal elements was found in both size groups compared to total anal elements (Figure 6). Despite the disparity in sample sizes, the distribution of total dorsal elements among post-larvae was slightly skewed toward higher counts. The distribution of anal counts shows little difference between the two size groups. The current lack of young specimens from other regions in the Pacific precludes an assessment of regional differences including a comparison among-regions of within-region variation.

4. Updated Results from Tag-Recapture and PSAT Tagging

A total of 607 swordfish have been tagged in the region of Hawaii since 1990. These tags have been deployed by both NMFS Honolulu scientists and cooperating commercial longline fishermen. Since the last (January 1999) ISC Swordfish Working Group Meeting, only one additional tagged swordfish has been recaptured thus far, for a total of 6 tag-recaptures (1% recapture rate)(Figure 7). This latest tag-recaptured fish was at liberty for 18 months and was

recovered 247 nautical miles SSE of its tagging location (Figure 7). Of the total 607 swordfish tagged, 21 swordfish were also injected with oxytetracycline for age validation study if recovered. No recapture of an oxytetracycline-tagged fish has been reported.

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 a pre-programmed pop-off date is reached. These tags also release and transmit data if no pressure change is recorded for four consecutive days (indicating that the tag has presumably been "shed" and is free-floating) or if the tag reaches a depth of 1200 m (indicating that the fish has presumably died and sunk through this depth). The PSAT tags which we used were attached to a barbed nylon dart tip with heavy monofilament line and implanted into the dorsal musculature using a long wooden tagging pole. Eight swordfish were successfully tagged. Six of the eight tags released earlier than their pre-programmed release dates. The straight-line distance between tagging and tag-shedding for three of these six "early release" swordfish appear in Figure 8. The remaining two swordfish with long term release dates are presumably still alive and recording meaningful data. The early release of 6/8 PSAT tagged swordfish is similar to that Sedberry and Loefer (2001), who observed a 40% shedding rate of PSATs for western Atlantic swordfish. The cause(s) of this "early-release" problem with PSATs has not been identified. Until this problem can be rectified, the potential of PSATs to provide a new understanding of long term horizontal movement and possible stock separation remains limited.

Literature Cited

- Chow, S., H. Okamoto, Y. Uozumi, Y. Takeuchi, and H. Takeyama. 1997. Genetic stock structure of the swordfish (*Xiphias gladius*) inferred by PCR-RFLP analysis of the mitochondrial DNA control region. *Mar. Biol.* 127:359-367.
- Ehrhardt, N.M., R.J. Robbins, and F. Arocha. 1996. Age validation and growth of swordfish, *Xiphias gladius*, in the northwest Atlantic. *ICCAT Col. Vol. Sci. Pap.* 45(2):358-367.
- Pawson, M.G. and S. Jennings. 1996. A critique of methods for stock identification in marine capture fisheries. *Fish. Res.* 25:203-217.
- Reeb, C.A., L. Arcangeli, and B.A. Block. 2000. Structure and migration corridors in Pacific populations of the swordfish *Xiphias gladius*, as inferred through analysis of mitochondrial DNA. *Mar. Biol.* 136:1123-1131.
- Sedberry, G.R. and J.K. Loefer. 2001. Satellite telemetry tracking of swordfish, *Xiphias gladius*, off the eastern United States. *Mar. Biol.* 139:355-360.
- Sun, C.L., S.P. Wang, and S.-Z. Yeh. 2002 (in press). Age and growth of the swordfish *Xiphias gladius* L. in the waters around Taiwan using anal fin rays. *U.S. Fishery Bulletin*.

Figure Captions

Figure 1. Length-frequency distributions of swordfish specimens used to estimate age. Data are available for only four of the five fishery regions; no length data were available for the eastern North Pacific specimens provided by CICESE.

Figure 2. Straight lines describing the least-squares fitted relation between each laboratory's best age estimates and the modal value of all laboratories' best estimates (for the 59 MAS specimens with $\geq 60\%$ agreement among laboratories).

Figure 3. Scatterplot and fitted straight line of the same relation depicted in Figure 2, for Laboratory C only. The individual data plotted are the age estimates made by Laboratory C. The dashed reference line represents a hypothetical 1:1 relationship; comparison with Laboratory C indicates Laboratory C's general tendency to underestimate ages above age 2.

Figure 4. Fitted straight lines describing the relation between eye-to-fork length (EFL, cm) regressed on median age (of the age estimates provided by all 5 laboratories pooled), for swordfish from each of four geographic regions-fisheries. (CICESE data are excluded because no length data were available).

Figure 5. Fitted power curves to whole weight (kg)-at-length (LJFL, cm) relation for Hawaii- and Taiwan-caught swordfish. Individual fish data are available and plotted for Hawaii specimens only.

Figure 6. Frequency distributions of meristics for total dorsal and anal elements among the post-larvae and YOY juvenile swordfish examined from Hawaii. Upper graphs display the meristic distributions of YOY juveniles and lower graphs the meristics for post-larvae.

Figure 7. Tag and recapture locations and straight-line distance of all six swordfish tagged with conventional tags and recaptured from waters near and distant from the Hawaiian Islands.

Figure 8. Preliminary horizontal movement tracks for three of six PSAT tagged swordfish from Hawaii in which the PSAT released earlier than the pre-programmed release date.

Figure 1

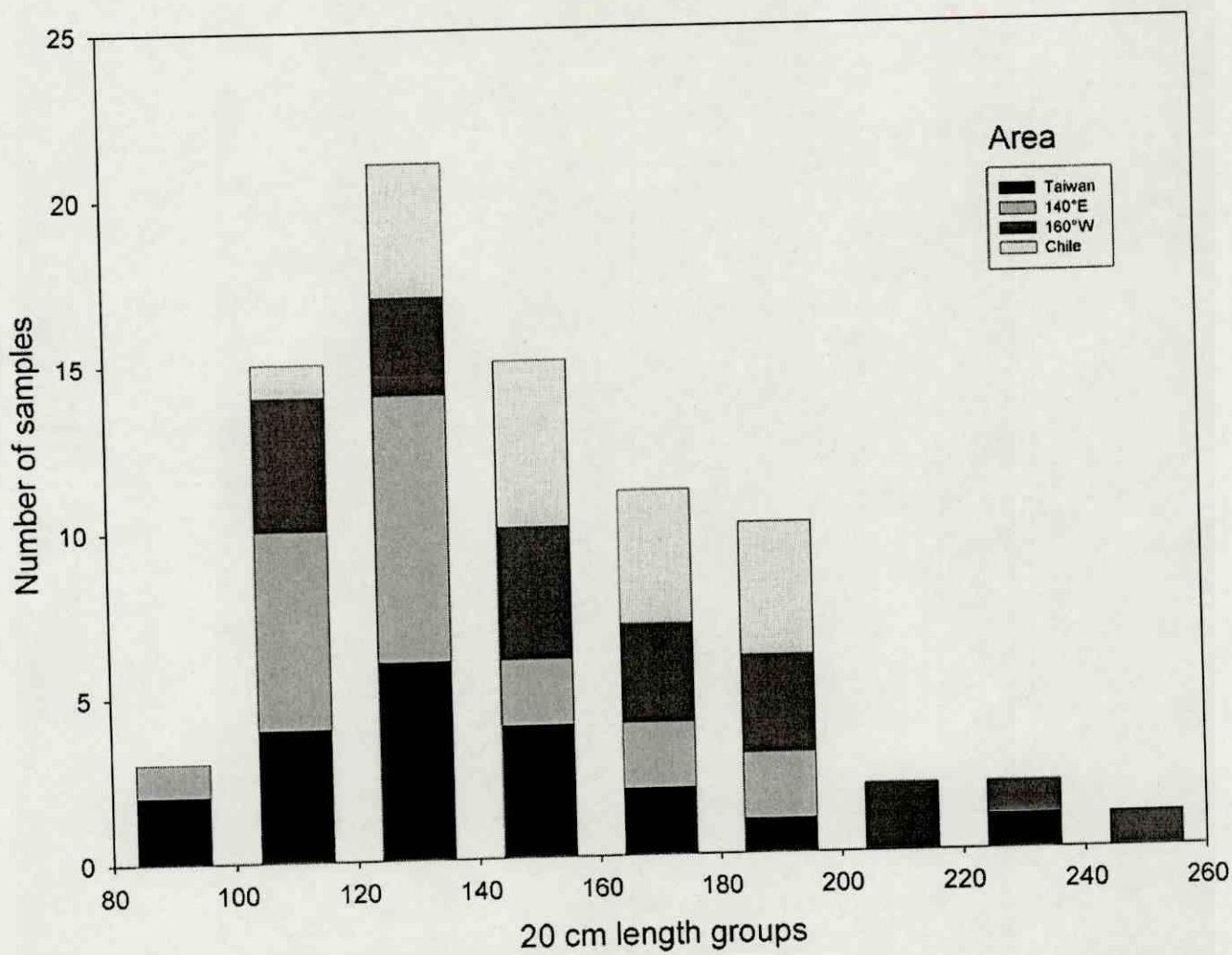


Figure 2

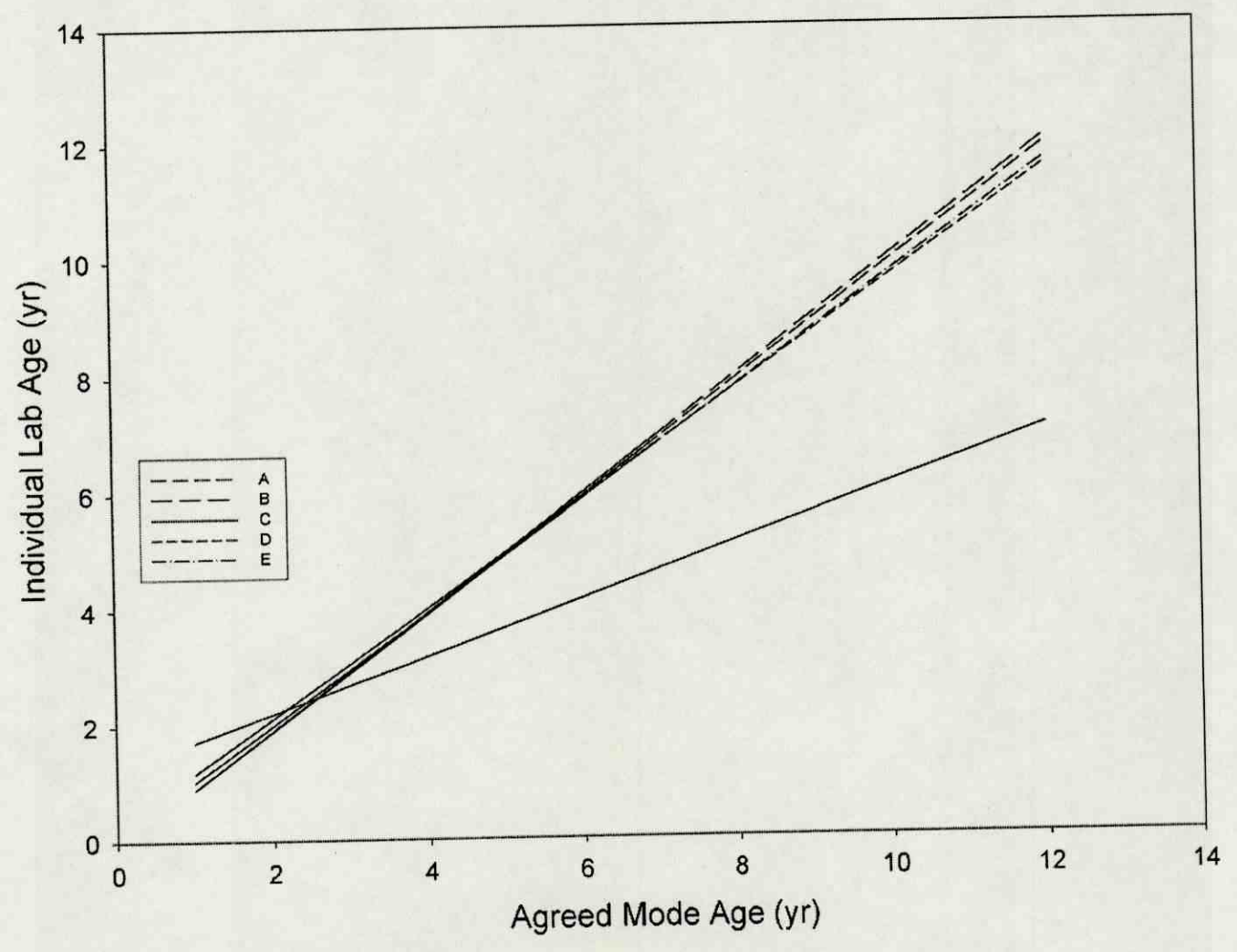


Figure 3

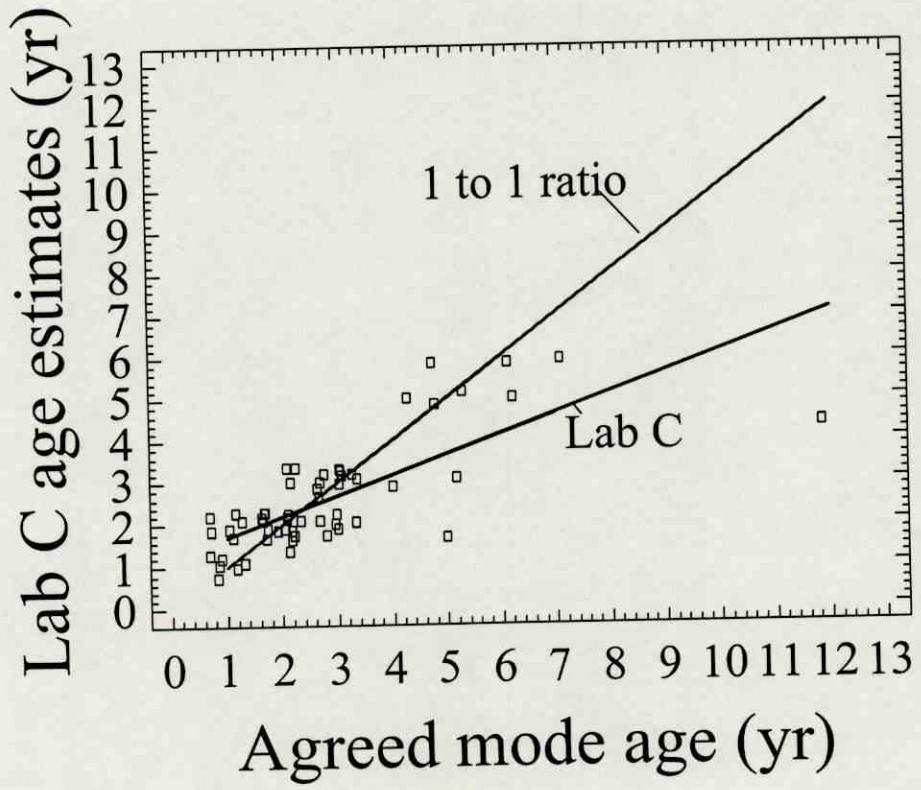


Figure 4

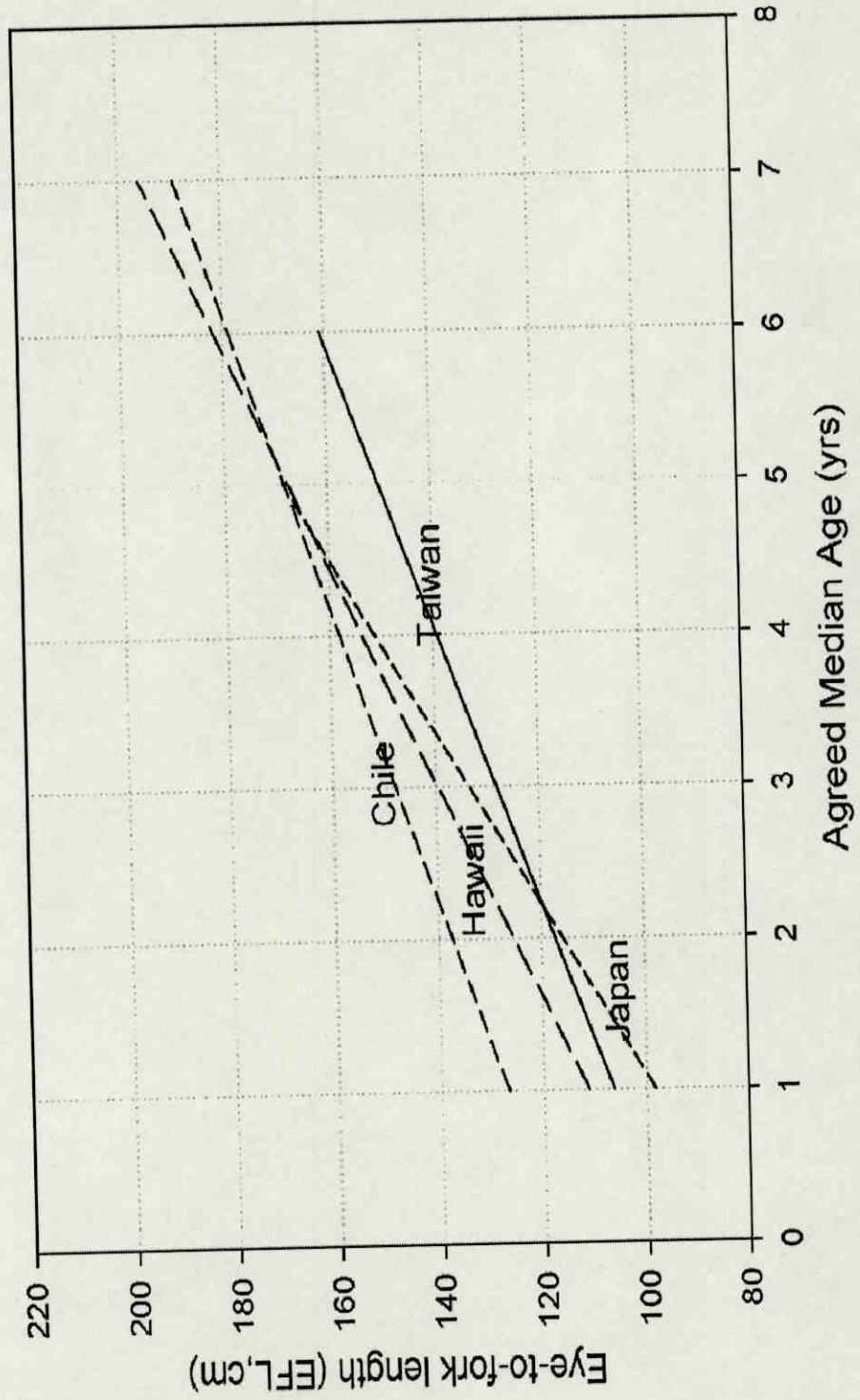


Figure 5

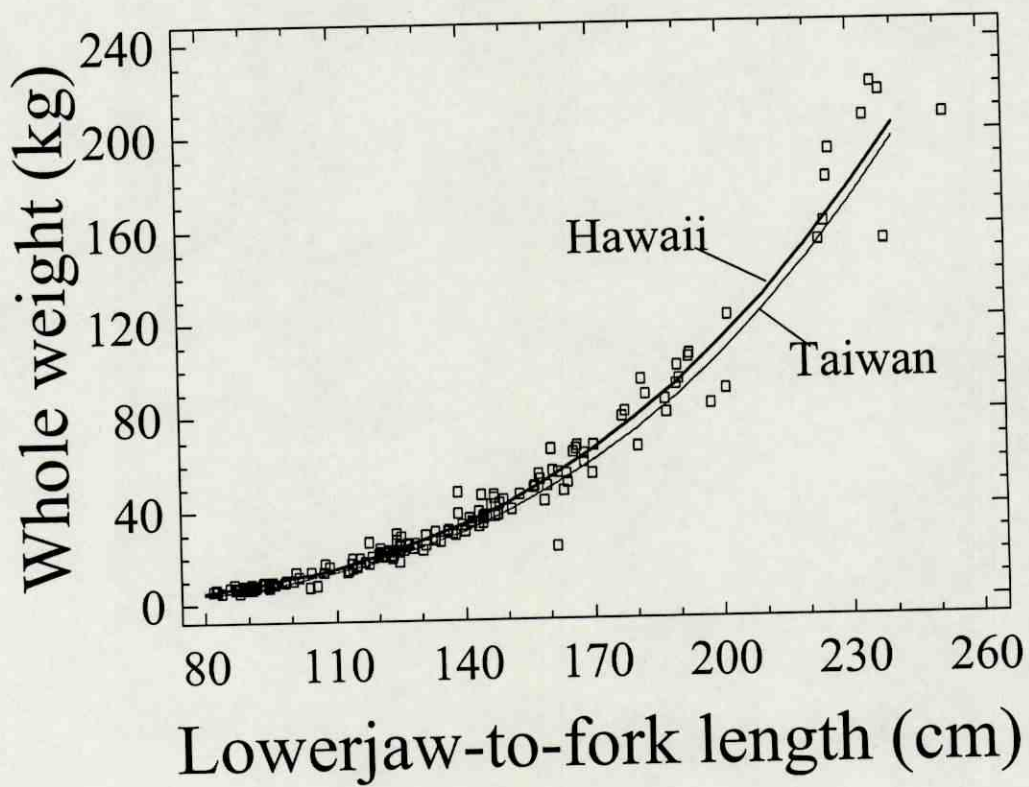
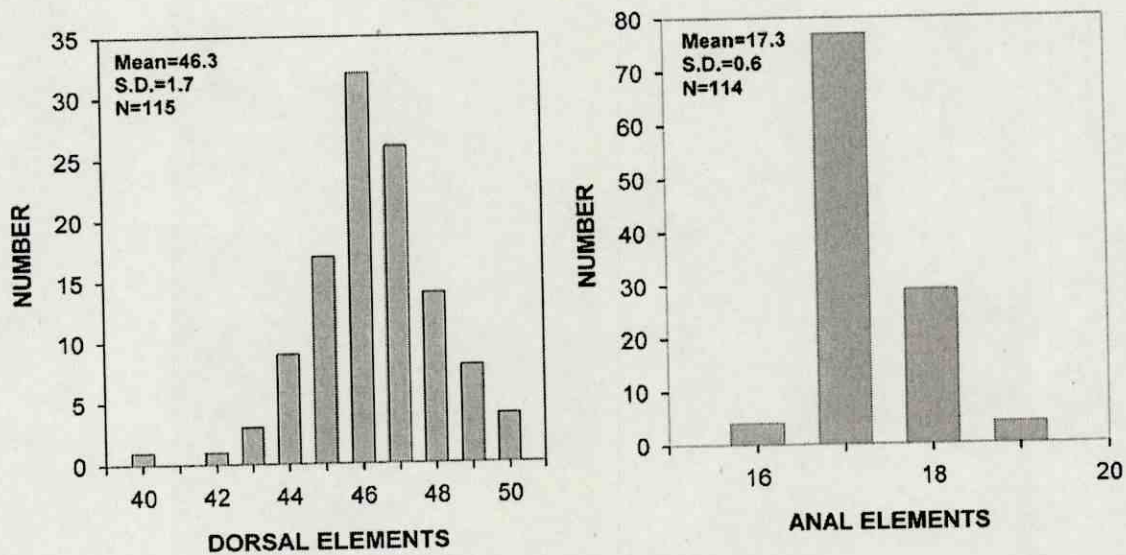


Figure 6

SWORDFISH MERISTICS-HAWAII

YOY Juveniles (47-99 cm EFL)



Post-Larvae (5-27 cm EFL)

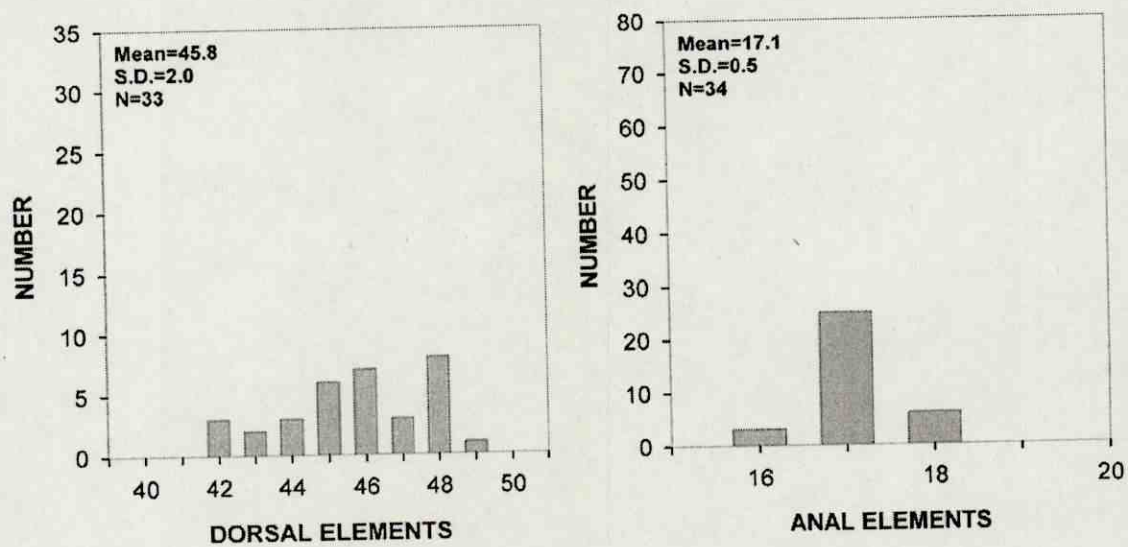


Figure 7

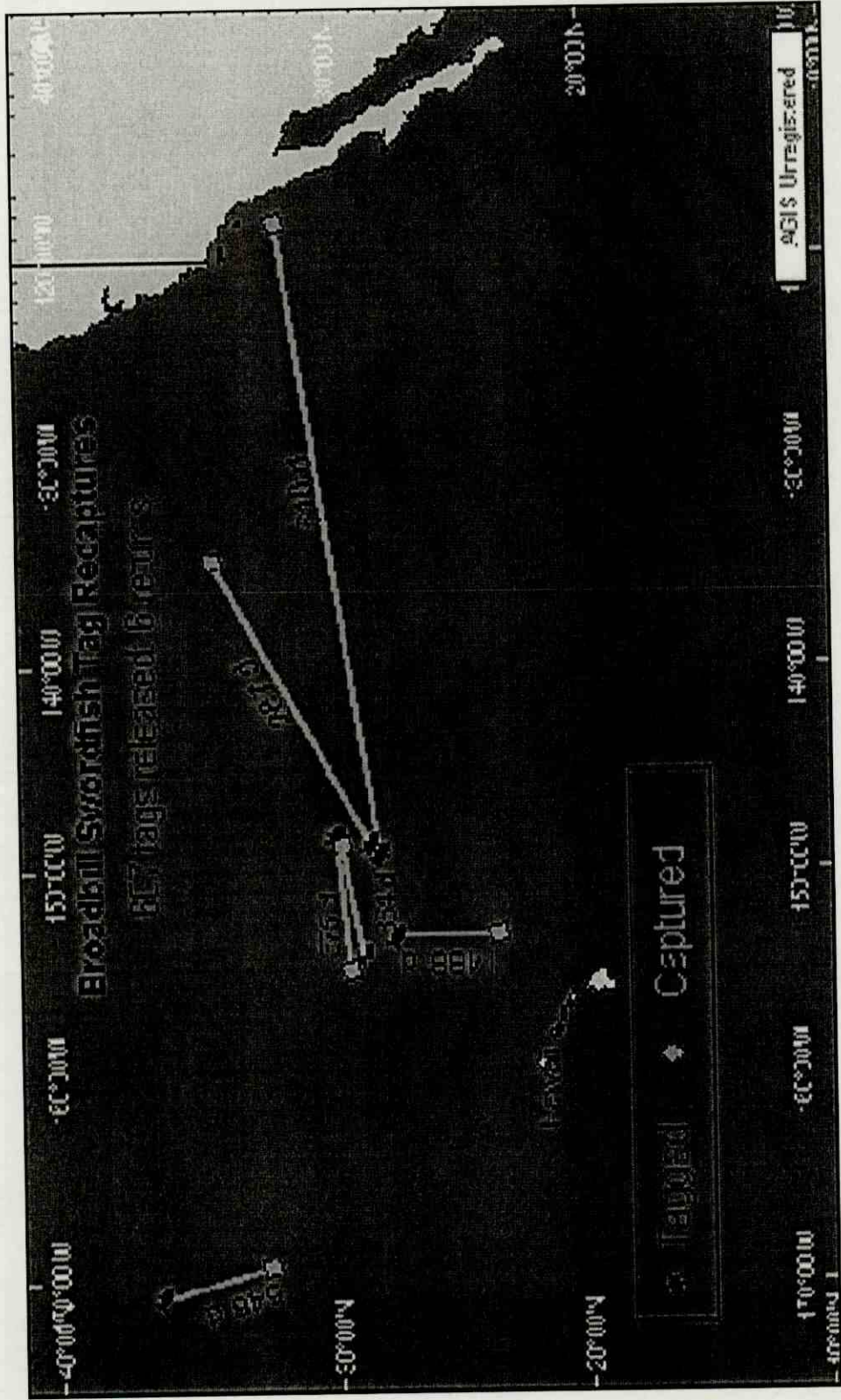


Figure 8

