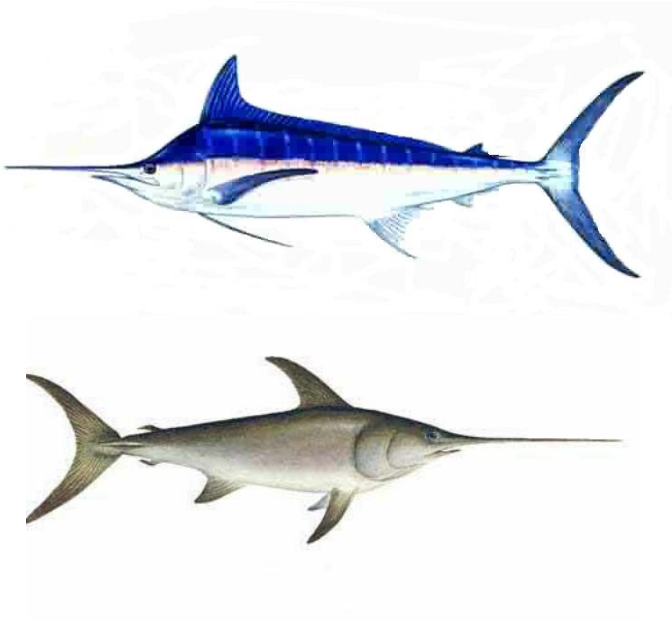
ISC/08/BILLWG-2/06

Draft ISC Billfish Research Plan: Research of Future Age & Growth and Length at 50% Reproductive Maturity Studies

ISC Billfish Working Group



[1]Working document submitted to the ISC Billfish Working Group Workshop, June 11-19, 2008, Abashiri, Hokkaido, Japan. Document not to be cited without authors' written permission.

DRAFT ISC BILLFISH RESEARCH PLAN
Research on Future Age & Growth and Length at 50% Reproductive Maturity Studies

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INTRODUCTION

The six billfish species inhabiting the North Pacific and under consideration for future ISC assessment and management include swordfish *Xiphias gladius*, striped marlin *Kajikia audax*, blue marlin *Makaira nigricans*, black marlin *Makaira indica*, sailfish *Istiophorus platypterus*, and shortbill spearfish *Tetrapturus angustirostris*. These species are all apex predators of the open ocean and are primarily taken as commercial bycatch (except for swordfish and sometimes striped marlin) by a variety of gears including longline, surface gillnet, harpoon, troll, and purse seine. Billfish caught in these fisheries may be processed at sea to remove head, fins, and/or viscera prior to stowing the remaining carcass either frozen or on ice prior to arrival at port. Common characteristics of billfishes include a presumed expansive home range, lack of dense schooling behavior, attainment of a large adult size, apparent longevity, rarity of available juveniles and largest adult sizes, and there inability to be propagated or survive in captivity. These biological characteristics coupled with their post-capture processing in most fisheries have limited the ability of researchers to collect desired samples in numbers sufficient to investigate pertinent life history questions. Success in studying these species will require improved collaboration on the part of researchers in order to leverage all available assets toward the collection of needed data and samples. It will be necessary to further develop our current ISC partnerships between participating countries and management organizations in order to forge the cooperative regional studies needed to advance life history research pertinent to future billfish stock assessment needs. Two of the primary life history topics currently in need for pending stock assessments include age & growth and length at 50% reproductive maturity.

OBJECTIVE 1: AGE & GROWTH STUDIES

Develop length-at-age growth curves and longevity estimates for each billfish species, as needed for stock assessments, within the three regional areas (western, central, and eastern) of the North Pacific. The three species of immediate interest for pending ISC stock assessments are swordfish, striped marlin, and blue marlin.

For swordfish, sex-specific length-at-age growth curves are available for swordfish from all three regions in the North Pacific (Castro-Longoria 1995, Sun et al. 2003, DeMartini et al. 2007). Unless stock assessment scientists need to update these published studies, the current priority will be to initiate age & growth studies for striped and blue marlin in the North Pacific.

For striped marlin, age & growth studies are needed for the western and central region of the North Pacific. In the eastern region where the only previous North Pacific age & growth study has been completed (Melo Barrera 2003), there is a need to update the

existing length-at-age growth curve through additional analysis of hardparts from very large striped marlin (>225 cm EFL) which were not available during the Melo Barrera et al. (2003) study. Collection of these large specimens will require the effort of fishery observer's onboard eastern tropical Pacific purse seine vessels.

For blue marlin, sex-specific age & growth studies are lacking for the central and eastern North Pacific regions. Previous experience in aging hardparts among the three priority species indicates that achievement of sex-specific length-at-age curves for blue marlin will be very difficult. These difficulties include the typical presence of less distinct growth rings within fin ray sections, the wider size distribution from which samples are needed, and the difficulty involved in acquiring needed hardparts from rare captures juveniles and large adult females.

PREVIOUS AGE & GROWTH STUDIES IN THE NORTH PACIFIC

Of the three high priority billfish species, swordfish and blue marlin are both sexually dimorphic (females attain larger size and faster growth rate) while striped marlin is apparently not. Therefore, sex-specific length-at-age growth curves are required for studies of swordfish and blue marlin. Black marlin is the only other known billfish species that also exhibits sexual dimorphism similar to swordfish and blue marlin.

For swordfish, sex-specific length-at-age growth curves are available from studies published by Sun et al. (2002) for Taiwan, by DeMartini et al. (2007) for Hawaii, and by Castro-Longoria (1995) for the Pacific coast off Mexico.

For striped marlin, the only sex-specific length-at-age study previously conducted in the North Pacific is the study by Skillman and Yong (1976) which fitted von Bertalanffy growth curves to length data (converted from weight) obtained from the Hawaii longline fishery. The only hardparts based age & growth study (utilizing sections of the 4th dorsal fin ray) was conducted by Melo Barrera et al. (2003) off Mexico in which sexes were pooled to construct a single growth curve. Efforts by the ISC Billfish Working Group to develop a stock assessment for striped marlin have revealed the presence of a very large size group (225-300 cm EFL) not available during the study by Melo Barrera et al. (2003). No other published growth curves exist for the North Pacific. A more recent hardparts based length-at age curve has been reported by Kopf et al. (2005) based on a study conducted in the Southwestern Pacific. The Kopf et al. (2005) age & growth study is currently being updated with a greater size range of samples from the Southwestern Pacific that has become available. Large sized individuals (similar to the eastern Pacific) occur seasonally off New Zealand.

For blue marlin, sex-specific length-at-age curves are only available from the length-frequency based Hawaii study reported in Skillman and Yong (1976). Another Hawaii-based age & growth study reported in Hill et al. (1989) detected faster growth rates among females but did not develop length-at-age growth curves from their results. Completion of a recent doctoral dissertation by Tamaki Shimose (National Research Institute of Far Seas Fisheries, Shimizu, Japan) contains a study of blue marlin age & growth from samples collected from Japan's southernmost Yonaguni Island. Results of

the age & growth portion of this study may become available in the near future. No other hardparts-based age & growth studies of blue marlin exist for the North Pacific.

POTENTIAL COLLABORATORS

For the striped marlin study, potential collaborators include life history scientists from the Fisheries Research Institute and National Taiwan University (Taiwan), National Research Institute for Far Seas Fisheries (Japan), Pacific Islands Fisheries Science Center, Life History Program (Hawaii, USA), Centro Interdisciplinaro de Ciencias Marinas, (B.C., Mexico), and Charles Sturt University (Australia).

For the blue marlin study, the list of potential collaborators will likely be the same as the above listing.

RESEARCH TASKS

Successful completion of the proposed age & growth studies will require the successful completion of a series of coordinated research tasks that include sampling, data compilation and sample storage, hardpart extraction, sample preparation, age estimation protocols, reference standards, and age validation/corroboration. The duties involved in each of these tasks will be elaborated on with respect to the proposed age & growth studies for striped and blue marlin.

Field Sampling

Length-Stratified Sampling

Length-stratified sampling, in which a pre-determined number of samples are collected from each length interval per unit of time, provides the best strategy for collecting samples across the full length range and is advantageous for the development of length-at-age growth curves. Sampling can be stratified into 5 cm size intervals over the available size distribution. The collection of hardparts (both otoliths and fin rays) from 5-6 fish per 5 cm length class per month for at least one year, and preferably over two spawning seasons, would likely provide a suitable sample size for developing sex-specific (or pooled sex) length-at-age growth curves. If possible, sampling should be obtained at port as well as at sea through fishery observers, research cruises, and/or cooperating fishermen. Depending on the region, samples should be collected from different fisheries since particular length intervals may be better represented in some fisheries.

The rarity of encountering specimens of some length intervals, such as young-of-year and very large individuals, requires that collections of these specimens be conducted opportunistically and may necessitate developing new collection procedures or fishermen incentives. This will be particularly important for the collection of otoliths and first dorsal fin samples from the rare large-sized striped marlin (>225 cm EFL) taken in the eastern tropical Pacific purse seine fishery. These specimens should be sampled at every opportunity by fishery observers or cooperating fishermen onboard these vessels.

Species Identification

Field identification of billfish should be based on published taxonomic guides and illustrations, such as those provided in Pepperell and Grewe (1996) for Indo-Pacific billfishes. If very small, very large, or damaged specimens are encountered that make identification difficult, a series of photographs that includes the entire body taken from above is desired. If possible, a 1 cm square fin clip or piece of muscle tissue can be retained for later DNA-based identification (Hyde et al. 2006)

Measurements

Length measurement on billfish should be conducted using a 1- to 2-meter sliding caliper that can measure to the nearest cm and is equipped with at least 15 cm long caliper jaws. Length measurements (to the nearest cm) should include eye-fork length (EFL) or lower jaw fork length (LJFL) and preferably both measurements whenever possible. Cleithrum-fork length measurements may also be required in fisheries where heads have been previously removed prior to sampling.

Otoliths

Since billfish otoliths are minute and difficult to extract in the field, the head should be saved for later otolith removal in the lab. To reduce the portion of the head containing the otoliths, the head can be trimmed by applying a vertical cut immediately in front of the eyes and another vertical cut down along the posterior-most margin of the preoperculum. The lower jaw can also be removed. This procedure can be applied for all billfish species including swordfish.

1st Dorsal Fin

The complete anterior portion of the first dorsal fin (first ~10 fin rays) should be removed as a single unit by slicing parallel along both sides of the fin base taking care to retain the entire base portion (condyles) of the fin rays. This will ensure that fin rays #3-6, of particular interest for aging, will be contained within the sample. Care should be taken to ensure that the entire anterior portion of the fin (including the small 1st fin ray) is retained with the fin as a single unit so that the sequential order of fin rays remains intact.

1st Anal Fin

The same procedures for removing the first dorsal fin can be applied to the removal of the 1st anal fin. Typically this fin is collected only for swordfish studies and is preferred over the use of first dorsal fin rays. Anal fin ray #2 is of particular interest for aging swordfish. Similar care should be taken to ensure that the entire anterior portion (including the small 1st fin ray) is retained.

Sample Labels

Each sample should contain an individual label that at a minimum includes date collected, species, fish length (in cm), sex, location, collector's name, and a unique identifying sample number or code. The label should be water resistant, label data recorded in permanent ink, and the label and sample placed together in a sealed plastic bag.

Data and Sample Storage

Aside from the data recorded on sample labels, collection data also needs to be recorded on a separate data form during sampling. Depending on resources, this could be manually recorded on data sheets or electronically recorded on a variety of data recording devices. As with the sample labels, it is important to include the correct measurement units and type of length measures conducted.

Samples of trimmed heads (containing otoliths) and fin rays are best stored frozen until hardparts can be extracted in the lab. Fin clips and/or muscle tissue for DNA-based billfish identification can be stored in 95% ethanol, in DMSO, or kept frozen.

Sample Extraction and Preparation

Otoliths

The minute size of billfish otoliths and their placement within the large and bony head requires the need to develop experience over a number of samples before consistent success in otolith extraction is achieved. Extraction of otoliths requires that the frozen head sample be first thawed. Once thawed, another vertical cut is made immediately behind the eyes. This exposed surface should now be oriented so that it is facing upwards. Another cut is now applied along the top of the head (front to back) just under the frontal bone to expose the top of the braincase. Brain tissue should be carefully removed to reveal the left and right bottom sides of the brain cavity. The three pairs of otoliths (sagittae, lapilli, and asterisci) are located within an interconnected labyrinth membrane on either side. Extensions of this labyrinth membrane are situated and interconnected within two small, cave-like cavities on each side. The sagitta and asteriscus are located together in an arrow-shaped membrane (sacculus) that lies in a depression just to the side of these cavities and can be easily separated and lost from the rest of the membranous labyrinth. The lapillus is located in another interconnected portion of the labyrinth (lagena). Radtke (1983) noted that the sagittae are especially difficult to extract from sailfish, striped and black marlin; swordfish sagittae are easiest to extract. Sagittae are the otoliths of greatest utility in billfish age & growth studies. Once extracted, otoliths should be manipulated under magnification in order to carefully release them from their membranous chamber. Otoliths should be cleaned in a drop of distilled water and manipulated using fine tipped art brushes. Otoliths can then be stored dry in clean plastic vials prior to preparation.

Preparation of billfish sagitta for daily growth increments (DGIs) typically involves embedding each sagitta in an epoxy resin followed by a transverse section through the core using a low speed diamond edged saw. The resulting section may require some light grinding to reveal the core area, and is then polished and examined under light microscopy. However, due to the narrow widths of these DGIs, sections require further treatment (decalcification with 7% EDTA and carbon or gold coating) to allow observation under scanning electron microscopy (SEM) (Radtke 1983). An alternate SEM preparation technique used by the PIFSC, Life History Program involves removal of overlying otolith material through selective acid etching to reveal the entire series of DGIs. This technique was successfully applied in the recent age & growth study reported in DeMartini et al. (2007).

1st Dorsal and 1st Anal Fin Ray Elements

It is recommended that fin rays not be boiled or cooked to remove various adhering tissues. These tissues are best removed by first thawing the sample. Each fin ray, starting sequentially from the first fin ray, can be cut free. Starting at the top of each fin ray, the adhering tissue can be slowly torn away by pulling down toward the fin ray base. If properly thawed, the adhering tissue will be torn free. Any remaining tissue can be lightly scrapped with a plastic knife. Scraping off adhering tissues with a metal knife is not recommended as the outer margins of these spines may be damaged and the specimen compromised. The order of the fin rays should be retained and the specimens allowed to air dry or dry inside a dehydrator for 24 hours.

Previous age & growth studies on marlins and sailfish have typically utilized either the 3rd, 4th, 5th, or 6th dorsal fin ray within the first dorsal fin. Swordfish studies have almost exclusively used the 2nd anal fin ray within the first anal fin. The selection of a particular fin ray has been based prior testing for clarity of internal growth rings and minimization of internal core vascularization (re-working of bony material) which increases with age. Standard approaches toward the preparation of transverse sections through these fin rays are documented in Erhardt et al. (1996) for swordfish and in Prince (1984) for marlin. Fin ray sections are either stored dry or mounted on a glass slide and coated with a clear mounting media.

Age Estimation Protocols

Otoliths

External ridges present along the rostrum of the sagittal otoliths of swordfish and blue marlin are reported in Radtke (1983) and Hill et al. (1989) respectively. These features were thought to represent annual growth marks but are difficult to detect and their appearance typically ambiguous due to overgrowth of otolith material. Other researchers have not been able to duplicate the use of these features. Studies to evaluate the presence of presumed internal annual growth marks in billfish sagittal otoliths have also been unsuccessful. Success in utilizing billfish otoliths for age & growth has been restricted to age estimation methods based on the enumeration of internal daily growth increments (DGIs). This method has been successfully applied in aging juveniles and young adults of swordfish (Megalofonou et al. 1995, DeMartini et al. 2007), blue marlin (Prince et al. 1991), and black marlin (Speare 2003). This technique typically requires the examination of otoliths using SEM. Furthermore, it is time-intensive and largely limited to aging fish <2.5 years old due to the difficulty in preparation and the sub-micron widths of DGIs prior to age 2. However, the value of this technique is the ability to better characterize the pattern of early rapid growth in young billfish. It also serves to corroborate the validity of interpreting age 1 and age 2 annual growth rings observed in fin ray sections where matching otolith counts from the same young individual are available. For DGI enumeration, standard protocols involve the use of a second reader, the use of blind counts, and methods to determine the acceptable level of count variations between readers (Campana 1992, Campana and Jones 1992).

1st Dorsal and 1st Anal Fin Ray Elements

The use of fin ray sections to age billfish based on presumed annual growth rings is the standard "production" method used in virtually all billfish age & growth studies. Standard protocols for analyzing these presumed annual growth rings include the application of objective protocols for locating the focus of each section, recognition of annual growth rings from false rings, measurements of annual growth ring radii, marginal increment and edge type analysis, and age assignment corrected for vascularization. The methods and protocols of Erhardt et al. (1996), Prince et al. (1988), and Sun et al. (2002) are typically applied. Marginal growth from the outermost growth ring in fin ray sections is analyzed to determine the time of year associated with annual growth ring deposition. A more complete documentation of these protocols based on current age studies of striped and white marlin is currently being developed by Kopf and Drew (*in prep.*). These protocols will be applied to future fin ray based age and growth studies proposed for striped and blue marlin and were previously used by ISC collaborators in Taiwan and Hawaii during there respective age and growth studies on swordfish.

Reference Standards

Collaboration between regional age & growth laboratories is important in establishing and re-enforcing agreed standards for the interpretation and measurement of growth rings. Such collaborations should also serve as a forum to discuss and reach consensus on unanticipated problems that may appear during the course of these studies. Visitation of researchers to other laboratories and /or the convening of an age & growth workshop would help toward ensuring consistency in conventions, methodologies, and age interpretations between all aging laboratories. Development and compilation of a digital reference library of sectioned fin rays would allow for periodic training "refreshers" for age readers in different regional labs. This would assist age readers in remaining consistent in their interpretation of growth rings. Furthermore, routine digital imaging of all fin ray sections will allow more opportunities for readers to electronically share images with readers in other labs rather than just within labs. This will provide additional opportunities to check for consistency in age readings between laboratories. In this manner, observed regional differences in length-at-age growth curves will be less likely attributable to differences in methodologies between laboratories.

Age Validation/Corroboration

Problematic to any research on billfish age & growth is the issue of validating fin ray and otolith based age estimates. The minute size of billfish otoliths negates application of radiometric and bomb radiocarbon techniques that have revolutionized age & growth studies of demersal and benthic fisheswhich typically possess large otoliths. Billfish tag-recapture studies typically have dismal recapture rates at 1-2%. However, those rare recapture events associated with reliable length measurements at release and again at recapture have provided very valuable data in support of derived length-at-age growth curves (DeMartini et al. 2007). Arguably, individual tag-recapture events associated with reliable length measures provide much more important information on growth rate than could ever be provided on movement. Although tag-recapture studies that apply oxytetracycline (fluorescent banding) or strontium chloride (check mark) are preferred, these types of tagging studies usually require the presence of researchers. However, tag-and-release studies that focus only on the application of conventional tags on smaller

individuals (whose age can be better estimated based on length) may allow for increased participation by trained fishers and may result in greater number of tagged and measured fish. There is no short-term solution to the billfish validation problem. However, a long-term conventional tag-and-release program that focuses on obtaining reliable lengths at tag release and upon recapture has already proven its utility in support of age & growth studies.

PROJECTED COST

Projected costs assume that head and fin ray samples are obtained free-of-charge. Projected costs also assume that participating laboratories will not have to invest in new microscopes, low-speed saws, and storage freezers. Project cost does not include labor.

Consumable supplies: $5,000 USD
[Sampling equipment, microscope slides, mounting medium, saw blades, SEM stubs, etc.]

SEM time for otolith increment analysis: $5,000 USD
[$50/hour for 100 hours]

Project Cost per species per lab: $10,000 USD

ESTIMATED TIMELINE

The first three tasks will overlap to varying degrees with each other.

Sample Collection (continuous): 1.5-2 years
Sample Preparation: 1-1.5 years
Sample Interpretation and Analysis: 1.0 years
Development of Report: 0.5-1 year
Time Interval between start of Sample Collection to Development of Report: 3.5-4 years

OBJECTIVE 2: SEX-SPECIFIC MATURATION STUDIES

Develop sex-specific estimates of length at median (50%) reproductive maturity for the three priority billfish species (swordfish, striped and blue marlin) in each of the three regional areas (western, central, and eastern) within the North Pacific.

The assumption regarding this objective is that the histological based approach to determining sex-specific length at 50% maturity is superior to other techniques that rely on GSI approaches. Stock assessment scientists need to discuss this point and confirm whether length at 50% maturity based on histology is preferred for applying maturation data to their expected assessment models or whether other reproductive indices (such as GSI) are preferred or just as useful.

PREVIOUS MATURATION STUDIES IN THE NORTH PACIFIC

Reproductive maturation studies on swordfish have been conducted in the western, central, and eastern North Pacific region. Similar lengths at 50% maturity where determined for female swordfish by Wang et al. (2003) for Taiwan (150 cm EFL or 168

cm LJFL at 50% maturity) and by DeMartini et al. (2000) for Hawaii (144 cm EFL or 162 cm LJFL at 50% maturity). Male length at 50% maturity for Hawaii swordfish is 102 cm EFL; no values for males are available for Taiwan. These two studies developed estimates of length at 50% maturity based on the examination of histological sections and reproductive developmental criteria to distinguish between mature and immature states. In the eastern Pacific, female reproductive activity was investigated using a combination of histology and a gonad index based on the ratio of the natural log (*ln*) of total ovary weight to *ln* EFL; females with gonad indices ≥ 1.375 were considered reproductively mature (Hinton et al. 1996). The senior author can confirm whether an equivalent estimate of female length at 50% maturity can be derived from this study.
For striped marlin, length at 50% female maturity is reported to be 143 cm EFL (168 cm LJFL) in the study by Klett-Traulsen and Rodriguez (1996). Size at 50% male maturity was not reported. An additional reproductive maturity study in this region is reported in Gonzalez-Armas et al. (2006) but length at 50% maturity was not determined. Information on size at 50% maturity for the western and central regions of the North Pacific is not available.

For blue marlin, Tamaki Shimose (National Research Institute of Far Seas Fisheries, Shimizu, Japan) is preparing for publication the results of a length at 50% maturity study on blue marlin sampled off Yonaguni Island, Japan. Female length at 50% maturity is tentatively reported to be 178 cm EFL or 206 cm LJFL; no estimate was provided for males. For the central and eastern North Pacific, no estimates of length at 50% maturity are available for either sex.

POTENTIAL COLLABORATORS

Potential collaborators in these studies will likely be the same participants previously identified as collaborators in future age & growth studies.

RESEARCH TASKS

The proposed length at 50% maturity research will require successful completion of a series of coordinated research tasks similar to that proposed for the age & growth studies. These tasks include sampling, data recording, gonad extraction and preservation, sample preparation, histological-based protocols for classifying reproductive stages and maturity, and establishing reference standards.

Field Sampling

Length-Stratified Sampling

The strategy of utilizing length-stratified sampling for collecting hardparts is also optimal for collecting gonads across as wide a range of sizes as are available in each fishery. Gonads could be concurrently sampled with hardparts by utilizing the same sample collection size of 5-6 gonads (2-3 female/2-3 male) per 5 cm length interval. However, if spawning information is known, and particularly if spawning is known to be seasonal in the region sampled, the number of gonad samples per length interval should at least double (≥10 female/≥10 male per 5 cm length interval).

If the fishery or fisheries being sampled extend over a wide latitudinal area subject to differences in sea surface temperature, it will be necessary to ensure that sample location is known and that sample collection extends across these areas.

Species Identification
Same as the "Species Identification" section under Objective 1.

Sample Labels
Same as the "Sample Labels" section under Objective 1.

Data and Sample Storage
Data requirements will be similar to those outlined in "Data and Sample Storage" under Objective 1.

For sample storage, a 1 to 2 cm slice of gonad tissue through the middle of either gonad lobe should be retained for histology. The sample should also include the outer gonad wall. Samples should be preserved in a neutral buffered solution of 4% formaldehyde (10% Formalin) to properly fix gonad tissues prior to histological preparation. If this is not possible at the time of collection, the sample should be stored in an air tight bag and stored on ice or frozen. Samples originally stored on ice (and particularly frozen) and then preserved later in formaldehyde yield suboptimal histology sections but can potentially still be used to distinguish between mature and immature stages.

Gonad Processing and Preparation
Gonad samples need to be preserved in neutral buffered 4% formaldehyde (Hunter 1985) for approximately 3 months prior to removal for histology preparation. It is important that at least two-thirds of the volume is occupied by the fixative solution in order to ensure that the gonad sample is properly fixed prior to histological processing.
Since histology requires specialized skills, equipment, laboratory set-up, and the use of noxious chemicals, it is assumed that the histological preparations will be outsourced at cost. After placing a subsample of each gonad specimen in a histological cassette, the preparation of the gonad histology slides follows a fairly standard protocol used in most hospital laboratories in the preparation of biopsy slides. This involves embedding the fixed gonad sample in paraffin; the production of multiple thin-sections 5-7 micron thick, sections stained in hematoxylin and counter-stained in eosin, and stained sections mounted on individual microscope slides overlain with clear mounting sealant and coverslip.

Classification of Reproductive Stage and Maturity
Field Criteria
Field criteria for establishing sex of billfish can be based on a combination of shape, texture, and color. An example of such visual criteria was developed by ICCAT and appears in there field manual (ICCAT 2007); this information is reproduced in Table 1. A needed supplement to this table would include photo examples for each stage and sex. Perhaps the development of such a visual guide could be collectively developed for this objective.

Histological Criteria for Classifying Developmental Stage

Fish gonad histology has been impeded by the lack of a common developmental classification that can be used for most teleosts to identify and delineate typical developmental stages of the ovary and testis. Many published studies have developed there own slightly different set of histological criteria rather than adopt those from previous studies on similar species. The development and adoption of standard criteria prior to this work could be an early goal among collaborators in this research. A good starting point would be to review the series of gonadal developmental stages of Murphy and Taylor (1990) as modified by DeMartini et al. (2000). These criteria were used in both the DeMartini et al. (2000) and Wang et al. (2003) studies of length at 50% maturity in swordfish.

Maturity Criteria

The accurate classification of maturity, especially for females, will determine whether a particular histological slide is interpreted as mature or immature. Development of standard histology criteria should include a common interpretation of mature and immature. The ability to interpret mature from immature based on histology becomes less certain when examining gonads sampled outside the spawning season. Evidence of past spawning is transient and cellular features from past spawning in billfish are probably no longer visible a few days to one week past the cessation of spawning. This is why its important to intensify gonad sampling during the time of year when spawning is known or suspected to occur or peak. Based on typical developmental criteria for histology, a previously mature "resting" female that hasn't spawned recently and a virgin immature female would both be classified as immature. Inclusion of such mature "resting"samples (classified as immature) might bias results toward higher length at maturity values. Collaborative work between CSIRO Australia and PIFSC Hawaii has investigated this in swordfish. Two histological features involving 1) the pattern of primary oocytes clustered along lamellae and, 2) the occurrence of vascularized (blood vessels) muscle walls hold promise as features which may be used to distinguish reproductively inactive (but previously mature) fish from virgin fish. Development of such criteria would increase the accuracy of maturity classifications. Further research on this could also be collaboratively undertaken if other researchers agree to its importance.

Digital Reference Standards

See this same section under Objective 1. Development of a digital library of referenced histology standards is similarly important as it would be for interpreting fin ray sections.

PROJECTED COST

Projected costs assume that gonads are obtained free-of-charge. Projected costs also assume that laboratories conducting this work will not have to invest in new microscopes, and other expensive equipment costs. It is assumed that histological work will be out-sourced. Project cost does not include labor.

Consumable supplies: $5,000 USD
[Microscope slides, specimen jars, formaldehyde, histology cassettes, etc.]

Histology ($10 USD per slide): $10,000 USD
[1,000 gonads examined for histology]

Project Cost per species per lab: $15,000 USD

ESTIMATED TIMELINE

The first three tasks will overlap to varying degrees with each other.

Sample Collection (continuous): 1.5-2 years
Sample Preparation: 1 year
Sample Interpretation and Analysis: 1.0 years
Development of Report: 0.5-1 year
Time Interval between start of Sample Collection to Development of Report: 3 years

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Table 1. Reproduced from Table 4.8.1 of the ICCAT filed Manual. Maturity stages for visual examination of large pelagic gonads.

Gonad Stage	VISUAL CRITERIA Males	VISUAL CRITERIA Females
I	Gonads small ribbon-like; not possible to determine sex by gross examination	Gonads small ribbon-like, not possible to determine sex by gross examination
1	**Immature:** testes extremely thin, flattened and ribbon-like, but sex determinable by gross examination	**Immature:** gonad elongated, slender, but sex determinable by gross examination
2	Enlarged testes, triangular in cross section, no milt in central canal	**Early maturing:** gonads enlarged but individual ova not visible to the naked eye
3	**Maturing:** milt flows freely if testes pinched or pressed	**Late maturing:** gonads enlarged, individual ova visible to the naked eye
4	**Ripe:** testes large, milt flows freely from testes	**Ripe:** ovary greatly enlarged, ova translucent, easily dislodged from follicles or loose in lumen of ovary
5	**Spent:** testes flabby, bloodshot, surface dull red, little or no milt in central canal	**Spawned:** includes recently spawned and post-spawning fish, mature ova remnants in various stages of resorption, and mature ova remnants about 1.0 mm in diameter