Appendix I: Sample collection, storage, shipment

As a general rule for all sampling, otoliths, fin spines, gonads, and DNA are (whenever possible) to be collected for all samples. If all four of these samples cannot be collected from the same individual then it should be considered whether that individual should not be replaced by a different individual in the collaborative sampling effort (exceptions can be made for rare samples that are unlikely to be replaced with samples containing all desired tissues). As this project aims to reduce overall sample sizes (as compared to random sampling) every effort must be made to maximize the utility of each and every sample.

Age samples (otoliths and fin spines)

- Sample collection:
    - Two fin spines should be collected from each specimen. The selection of fin spines for each species should be based the literature in order to keep consistency with other related age and growth studies for billfish (Appx. I Table 1).
        - Swordfish (*Xiphias gladius*) – The first and second anal fins should be collected.
        - Striped marlin (*Kajikia audax*) – The first dorsal as well as the longest dorsal fin spine (if the longest dorsal is the first, then the first and second dorsal fin spines should be collected).
        - Blue marlin (*Makaira nigricans*) – The first and second anal fins should be collected.
    - Both sagittal otoliths should be sampled whenever possible
- Sample storage:
    - Fin spines should be dried, cleaned, and sectioned (following the infographic rules), then stored in labeled coin envelopes for shipment.
    - Otoliths should be cleaned and stored in labeled coin envelopes or vials for shipment.
- Sample shipment:
    - As otoliths are fragile, care must to taken to use packing materials (bubble wrap works well) around samples to protect them during shipment.
- Treatment of received samples:
    - No additional treatment is need for dried ageing samples upon arrival.

Reproductive samples (gonads)

- Sample collection:
    - Sample ~0.25-0.5 grams of gonad tissue from the medium position and store in a labeled perforated tissue cassette. Place cassettes in a 10% buffered formalin solution. Make sure to not fill the formalin container more than 2/3 of the way full with cassettes, allowing for enough formalin to fix the tissues.
    - If a female with hydrated oocytes (female that looks like spawning is eminent) is encountered, take an additional gonad sample (~1 gram) from the medium position of the gonad. Weigh and record the mass of the gonad sample and mark for fecundity analysis.
    - If vials are labeled using pens, cover written label with clear tape to help labels from rubbing off.

- Sample storage:
    - In order to ship formalin samples first remove the excess formalin from the histology tissues and fecundity sample vials being very careful not to lose any loose oocytes or eggs, then place in a sealed plastic bag with a dry paper towel.
    - Place the sample plastic bags in a larger plastic bag with enough absorbent material to soak up any free liquid that might spill.
    - Place the large plastic bag(s) in a strong outer package with cushioning material, such that there is no more than 1L of liquid in this outer package. The US uses metal cans.
- Sample shipment:
    - Ensure samples are labeled as "Scientific research specimens, not restricted, Special Provision A180 applies."
- Treatment of received samples:
    - Once samples arrive, remove packing materials and transfer them back into a 10% formalin solution.

Genetic Samples (Fin Clips/muscle tissue)

- Sample collection:
    - Muscle samples collected with biopsy punches should be taken from an internal location so as to avoid cross contamination with other fish. Use a clean knife to cut into the body of the fish and then use the biopsy to extract an internal sample. Avoid sampling red muscle tissue.
    - If biopsy punches cannot be used, use a clean knife to cut into a clean section of the body and remove and 1x1 cm cube of tissue (trying to avoid areas that may have been contaminated by coming into contact with the boat deck, fishing gear, or other fish).
- Sample storage:
    - Samples should be stored in labeled plastic sample vials filled with ethanol (>80% but <95% conc).
    - Externally threaded cryogenic vials work well as their seal prevents excessive ethanol evaporation, however, parafilm can be used to create an effect seal on most any vial.
    - Ensure labeling of vials is not impacted by ethanol (vails labeled with pen can easily have the label erased if excess ethanol contacts the ink). If vials are labeled using pens, cover written label with clear tape to help labels from rubbing off.
- Sample shipment:
    - Ethanol can sometimes be difficult to ship and inquiries will need to be made by participating countries about specifics of shipping samples stored in ethanol. We are looking into this and more detail will be added.
- Treatment of received samples:
    - Ensure samples are still in a sufficient amount of ethanol following shipment and add more if needed.

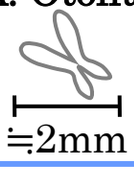
Appx. I Table 1: Life history studies on billfish species of interest. 1A = first anal fin, 1D = first dorsal fin, -num = additional fin spine number, L = longest spine. Fin spines are counted from the head of the fish to the tail, meaning that 1D, or the first dorsal fin spine, is the dorsal fin spine closest to the head of the fish.

Reference	Species	Scientific name	Hard-part	Region	Sample size	Sampling period
Ehrhardt (1992)	Swordfish	*Xiphias gladius*	1A	Northwestern Atlantic	425	Mar-Dec, 1978-1980
Garcia et al. (2017)	Swordfish	*Xiphias gladius*	1A-2	Equatorial and tropical waters of the south-east Atlantic	502	Mar, Apr, Sep, 2006; Jul, Aug, Oct, 2007; Jul-Oct, 2009
Chong and Aguayo (2009)	Swordfish	*Xiphias gladius*	1A-2	Southeastern Pacific off the Chilean coast	1012	Dec/1994-Sep/1996
Valeiras et al. (2008)	Swordfish	*Xiphias gladius*	1A-2	North Pacific	406	2005-2006
DeMartini et al. (2007)	Swordfish	*Xiphias gladius*	1A-2	Waters off Hawaii	1292	March/1994-Jun/1997
Tserpes and Tsimenides (1995)	Swordfish	*Xiphias gladius*	1A-2	Eastern Mediterranean	1100	Feb-Oct, 1987-1992
Shimose and Yokawa (2019)	Striped marlin	*Kajikia audax*	1D-L	Eastern North Pacific	175	Sep-Nov/2004
Kopf et al. (2011)	Striped marlin	*Kajikia audax*	1D-4 or 1D-5 or 6 or 1A-3	Southwest Pacific Ocean	425	Jan/2006-Jan/2009
Melo-Barrera et al. (2003)	Striped marlin	*Kajikia audax*	1D-4	Cabo San Lucas, Baja California Sur, Mexico	399	1988-1993
Hoolihan et al. (2019)	Blue marlin	*Makaira nigricans*	1A-2	Central western Atlantic Ocean	1703	2003-2008
Shimose et al. (2015)	Blue marlin	*Makaira nigricans*	1D-L (5 or 6)	Western North Pacific	571	Feb/2003-Feb/2006

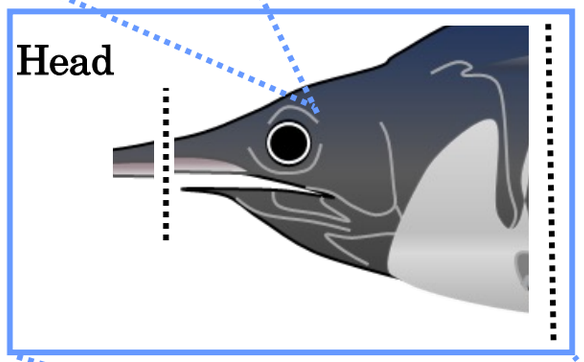
Sampling Infographic

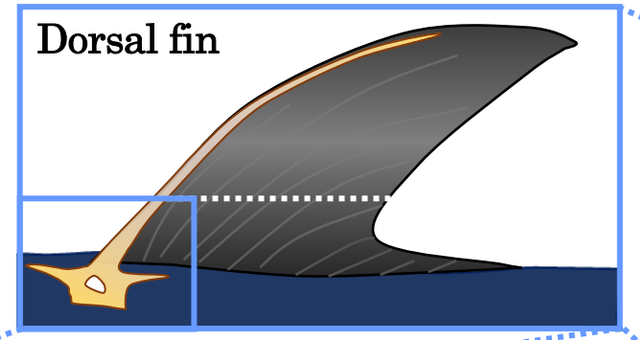
Sampling infographic originally created by:
Miyuki Kanaiwa (Mie University, Graduate School of Bioresources)

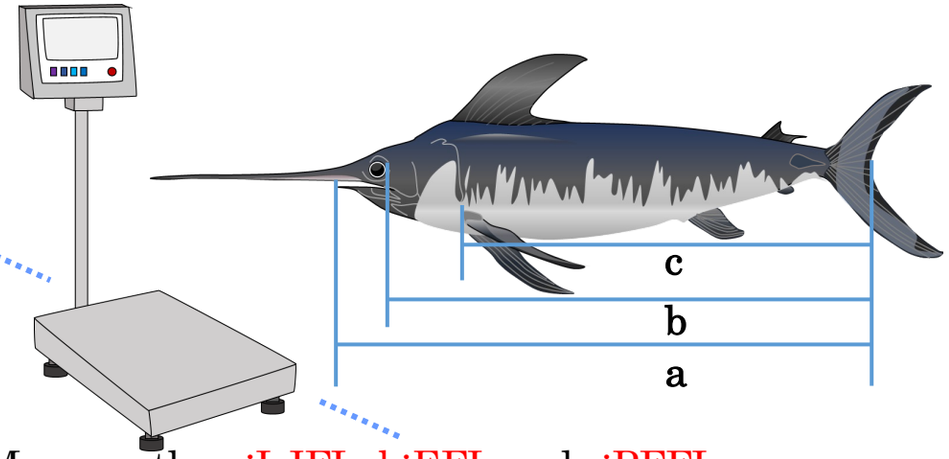
A. Otolith



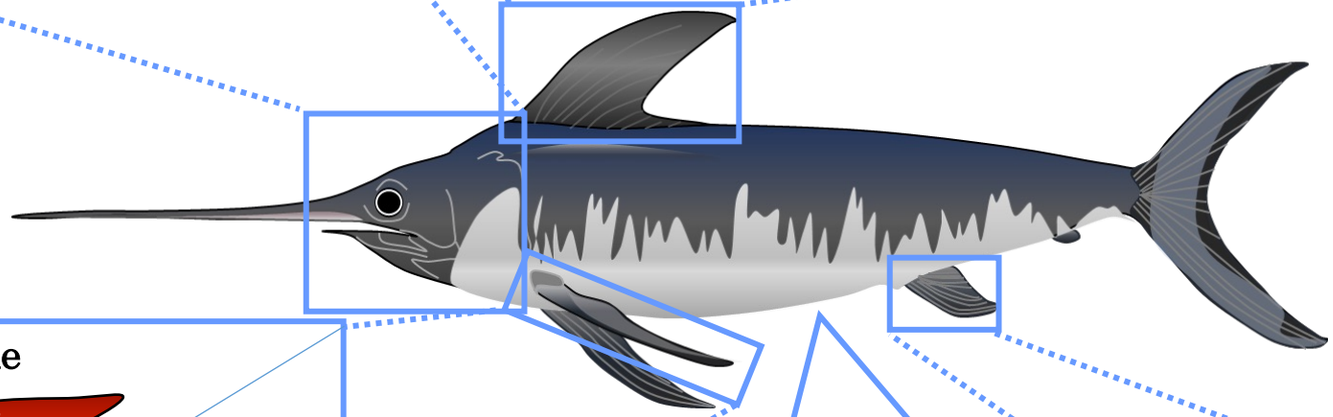
Take the Sagittal Otoliths from both sides of the head from each whole otic capsule. Otoliths should be cleaned and stored dry in labeled vials or coin envelopes.



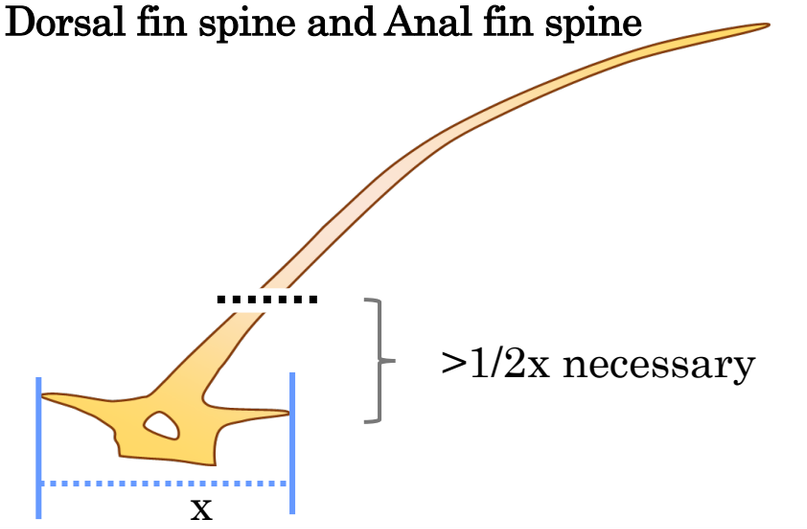




Measure the a:LJFL, b:EFL and c:PFFL of the billfish and record the whole or dressed weight. In the case of dressed weight, record its condition in detail (ex. bodyweight without the bill, caudal fin, gills and viscera).

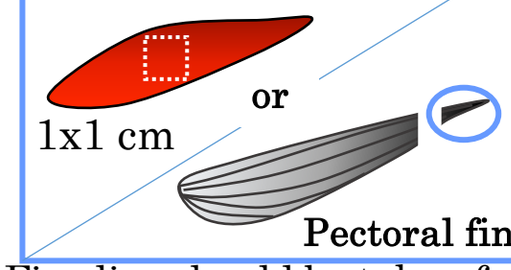


B. Dorsal fin spine and Anal fin spine



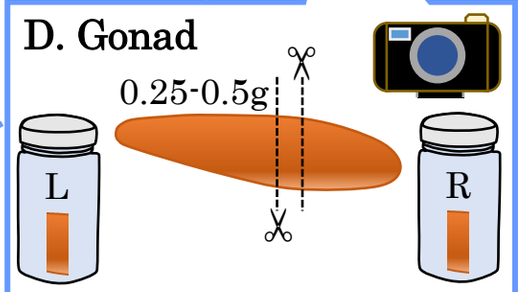
Sample a few spines, including the longest spines of the dorsal and anal fins. Measure the widest point at the base of the spine, section the spine at ½ of this measurement (dotted line).

C. Muscle

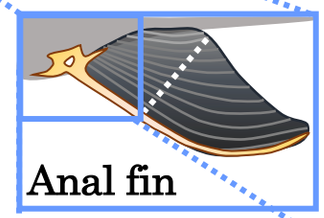


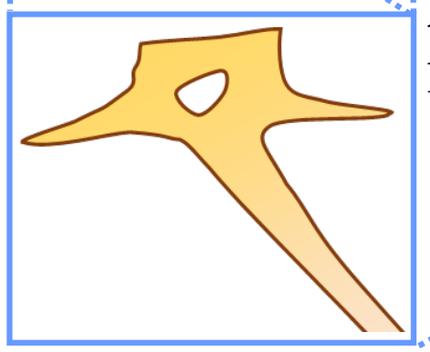
Fin clips should be taken from the tip or trailing edge of pectoral fin. Muscle tissue should be taken using a biopsy punch (3mm preferred) or a clean area cut out with a clean knife (1x1 cm).
Fin/muscle tissue should be fixed with ethanol (>80% but <95% conc), use enough ethanol to allow samples to be fully submerged.

D. Gonad



Weight at least one lobe of the gonad and take photos of the overview and cross-section. Sample ~0.25-0.5g of gonad from medium position and store in perforated tissue cassette buffered with 10% formalin solution.





Appendix II - Centralized database

Work has yet to begin on the database but an outline for its creation is as follows:

- A central source for data maintained and operated by the US (NOAA) that offers write only access to participating members of the international biosampling project.
- The database maintains a list of unique sampling numbers that are allotted to researches for use in the field (researchers can print out sample number lists to use in the field that are then tracked in the database so as to avoid reusing any sample numbers).
- Each unique sample number ends with an identifier to indicate what kind of sample it is:
    - S – fin spine
    - O – otolith
    - G – gonad
    - D – genetic sample (DNA)
- Additional data fields within the database
    - Year
    - Month
    - Day
    - Longitude
    - Latitude
    - Location type (Set level or Grid centroid)
    - Block Width_Longitude (Degrees)
    - Block Width_Latitude (Degrees)
    - Fishery
    - Trip ID/Boat ID
    - Set ID
    - Sample ID (database sample ID omits the above specified suffix)
    - Checkbox for “All samples collected” or “All but genetic samples” (This allows the database to track that all the above suffixed samples exist or not for each sample number)
    - Measured length
    - Measured weight
    - Measurement unit of length
    - Measurement unit of weight
    - Length type
    - Weight type
    - Sex