SCIENTIFIC COMMITTEE
NINETEENTH REGULAR SESSION

Koror, Palau
16 – 24 August 2023

Length-Based Proportional Sampling for Life History Research: Establishing Uniform Sampling for North Pacific Billfish Species

WCPFC-SC19-2023/SA-IP-11

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Abstract

For highly migratory species such as tuna and billfish, basic biological processes like growth and reproduction are notoriously difficult to estimate accurately. The ISC Billfish Working Group (BILLWG) indicated that improved estimates of these basic biological processes were key components in efforts to reduce model uncertainty in the assessment of billfish in the North Pacific. Here, the United States, as a member of the BILLWG, outlines a collaborative biological sampling effort with Japan and Taiwan in order to improve estimates of growth, maturity, and stock structure by developing a uniform sampling protocol that can be used across the North Pacific. Our design sets out guidelines for a length-based proportional sampling approach that can be used to sample pelagic species of interest in the North Pacific. We also evaluate the impact of using length frequency data from past years to inform current proportional sampling efforts.

1.0 Introduction

The prevalence of marine species life history studies in the scientific literature belies the difficulty of obtaining representative data, and thus, accurate estimates of fundamental life history parameters. For wide ranging highly migratory pelagic species such as tuna and billfish these problems are compounded, making the accurate estimation of basic biological processes such as growth and reproduction notoriously challenging. A myriad of factors contribute to this, but often, an ineffective sampling design, or a single study's inability to access samples from across the species range are chief among them. Sampling limitations among highly migratory species commonly result in regionally specific studies, sometimes with conflicting biological parameter estimates. Issues can arise when stock assessments, with their associated assumptions of stock structure, are forced to rely upon regionally specific life history studies that do not match the stocks assumed range.

1.1 A representative case of sampling related issues for pelagic species

In the case of *Kajikia audax* (striped marlin) in the Western and Central North Pacific (WCNPO), various studies have independently produced estimates for the species basic biological parameters, such as length at 50% maturity (L_{50}) and asymptotic size (L_∞). Samples collected from the Taiwan Tungkang, Singkang, and Nanfangao fish markets from 2004–2010 resulted in an estimated L_{50} of 181 cm eye-to-fork length (EFL) (Chang *et al.* 2018), which was used in the most recent assessment of striped marlin. Alternatively, samples collected from the Hawaiian longline fishery from 2008–2011 indicated a much lower L_{50} of 161 cm EFL (Humphreys & Brodziak 2019). Asymptotic size also varied among studies, indicating a larger L_∞ from waters off Taiwan [228.7 cm EFL converted from lower jaw fork length of 263.44 cm (Sun et al. 2011)] from 2004–2010, compared to waters off Hawaii [181.7 cm EFL (Fitchett 2019)] from 2003–2010. These disparate estimates from individual studies were investigated with various sensitivity analyses during the striped marlin assessment (ISC 2019); however, in the end a single parameter had to be selected for the base case model. From a biological perspective, it is difficult to determine if these differences reflect true natural spatial variability in life history attributes or simply different sampling designs or analytical methods. It must also be noted that this example is not an isolated case, similar issues persist for other pelagic species in the North Pacific, such as blue marlin and shortfin mako sharks. In order to make meaningful progress on these issues a more rigorously designed, representative sampling approach is needed.

1.2 Comprehensive sampling approach

Despite many recent advances in the study of life history, including methodological standardization on otolith processing and ageing, and validation of growth increments (Campana 2001; Kopf *et al.* 2011),there remains a lack of standardization in sample selection and sampling design. This is especially true for many pelagic species in the Pacific, as is clear from the example above. Improved datasets, and thus, improvements in sampling design, are needed in order to reduce bias in parameter estimates and make meaningful advances in life history studies of highly migratory pelagic species in the Pacific.

Luckily, several recent papers have focused on the issues of age and growth estimation in pelagic fish and their relationship to sampling design (Chang *et al.* 2019; Goodyear 2019). While various approaches are possible for collecting biological samples for growth and age estimation, random and size stratified sampling have proven the most reliable for reducing bias and producing representative samples. However, studies indicate that random sampling of fishery catch is not cost or time efficient due to the large sample sizes needed to collect suitable numbers of the least abundant size classes, something which typically leads to further oversampling of the more common age classes (Brouwer & Griffiths 2005; Chih 2009b). In some cases, the random sampling approach is altered and specific individuals are then targeted to supplement the least abundant classes (i.e. typically the lower and upper tails of the size distribution), leading to an amplification of biases (Chang *et al.* 2019; Goodyear 2019). Both Chang et al. (2019) and Goodyear (2019) used simulations to show how a disproportionate selection of samples from the upper end of the size distribution can lead to an inflated asymptotic length (L_∞). Furthermore, the ability to impose a truly random sampling design on fishery collected samples is often not feasible due to extraneous factors such as size-selective gear, shifting fisheries practices, and fish movements and aggregations (Lee *et al.* 2017).

Given the issues associated with collecting samples using a true random sampling design, size stratified sampling is the preferred option. Size stratified sampling sets predetermined size bins with a fixed number of total samples. Generally, two approaches can be implemented when conducting size stratified sampling. The first is to set a predetermined number of samples for each size bin (e.g. 10 fish/2 cm length bin across the entire size distribution), this approach is commonly referred to as Fixed Otolith Sampling (Chang *et al.* 2019). This methodology, while increasing efficiency compared to random sampling, is also known to be susceptible to bias in estimating mean length-at-age, tending to overestimate the asymptotic length for long-lived fish (Goodyear 1995; Chih 2009a; Coggins *et al.* 2013). The second approach is a proportional sampling design, termed Proportional Otolith Sampling (POS) (Chang *et al.* 2019). This approach entails determining a total number of samples, then allocating them across size bins based on the population's proportional length frequency. While POS has been identified as the preferred option based on simulations (Chang *et al.* 2019; Goodyear 2019) it comes with a caveat of its own, as it requires knowledge of the length frequency of a population prior to sampling. One sampling approach we have not mentioned here, but which appears in the sampling literature, is age-based proportional sampling (or age-stratified sampling), a design that Goodyear (2019), using simulations, indicated as being the least bias of the sampling approaches investigated. The reason for this omission here is due to its logistical improbability, with even Goodyear indicating "…ages would seldom be available to select observations for inclusion in stratified designs in actual studies".

In this document we look to 1) explore the effect of utilizing past length data on the catch of *K. audax*, *Makaira nigricans* (blue marlin), and *Xiphias gladius* (swordfish) in the North Pacific to inform current POS sampling efforts, potentially allowing us to avoid one of the major caveats of POS as stated above; 2) outline a sampling designs for highly migratory species which are of interest to the ISC (International Scientific Committee for Tuna and Tuna-like Species in the North Pacific Ocran) BillfishWG and the Pacific Islands Fisheries Science Center, specifically, pelagic billfish species (*K. audax*, *M. nigricans,* and *X. gladius*) in the North Pacific; and 3) detail sample and data collection, storage, and distribution logistics in order to provide a guide for use in a collaborative billfish sampling effort. Ultimately, these efforts will result in a standardized collection of representative samples which will facilitate the reduction of bias in life history parameters used to inform stock assessments.

2.0 Methods

With several studies indicating the effectiveness of POS for studies on the age and growth of pelagic fish (Chang *et al.* 2019; Goodyear 2019) our primary interest here is to evaluate the impact of utilizing length frequency data from past years to inform current POS sampling efforts. After which, our focus will be the creation of a practical framework to guide the collection and distribution of biological samples to improve our estimates of the age and growth of billfish in the North Pacific.

2.1 Effect of Lagged Length Frequencies Data

To test the potential effect of using length frequency data from past years to inform current POS sampling efforts, length data for *K. audax*, *M. nigricans,* and *X. gladius* were collected from three major fishing nations in the North Pacific (Japan, Taiwan, and the United States) over a five year period (2014-2018). The recommended assessment schedule for billfish, such as *Kajikia audax,* in the North Pacific is every 5 years (ISC 2019), a period over which the ISC, and thus the fisheries management councils, feel the stock can reliably be managed based on the stock status established in the previous assessment. For the same reason, we decided to examine the catch over the last 5 years in order to test the sensitivity of basing our proportional sampling on past length frequency data. Due to potential differences in life history across the North Pacific, samples were assigned to three gross regions (eastern, central, and western) defined as generally equal areas divided by lines of longitude (160° E and W), thus allowing comparison both temporally and spatially by insuring that samples be collected from across the species range.

A Kruskal-Wallis rank sum test was used to test for differences across regions and years, and a pairwise Wilcoxon test was used to investigate which comparisons were statistically significant.

2.2 Approaches for total sample size, and sample binning

Several studies investigating sampling design for life history analysis indicate that 200 to 500 samples is a suitable total sample size, however, this number can be highly dependent on the maximum size of the fish, with larger fish requiring additional sampling (Kritzer *et al.* 2001; Brouwer & Griffiths 2005; Chang *et al.* 2019).

In terms of length bins for larger pelagic fish, such as billfish, Coggins *et al.* (2013) suggests computing $L_\infty/30$ as an approach to determine the number of size bins. Obvious aliasing, or alternating peaks and valleys in length frequency plots indicating sharp differences in neighboring length categories, can also be used to guide the selection of length bins based on data quality. We used a combination of these two approaches, selecting the smallest suggested length bin size between the two approaches.

Giving these general guidelines, we established appropriate total sample and length bin sizes independently for each of our species of interest, with considerations of tractable sample sizes guiding our final decisions.

3.0 Results

3.1 Results of using lagged length frequencies

With over 25,000 individual *K. audax* length measurements from 2014-2018 split into three regions of the North Pacific, results from the Kruskal-Wallis rank sum test indicate that there were no significant differences in length frequencies between areas (eastern, central, western) or years ($\chi^2 = 7.1964$, df = 14, p-value = 0.9269), with adjusted pairwise Wilcoxon test p-values indicating that no single comparison was statistically significant either. Results from *X. gladius* (over 200,000 individuals, $\chi^2 = 23.591$, df = 14, p-value = 0.0513), and *M. nigricans* (over 50,000 individuals, $\chi^2 = 12.497$, df = 14, p-value = 0.5664) also indicated no significant differences in length frequencies by area or year, with adjusted pairwise Wilcoxon test p-values indicating the same for individual years and areas.

3.2 Appropriate total sample size and bin size

The scientific literature, as well as a recent simulation approach investigating the effect of various total sample sizes, indicated that overall sample size had little to do with the accuracy of estimated life history parameters (Kritzer *et al.* 2001; Brouwer & Griffiths 2005; Chang *et al.* 2019; Schemmel *et al.* 2022). With the most resent approach (which focused on bottomfish) estimating growth parameters of simulated *Pristipomoides auricilla* and *Etelis coruscans* populations indicating little to no variation with sample sizes ranging from 50 to 1000 (Schemmel *et al.* 2022). However, as expressed in the literature, precision in growth parameter estimates was highest between 200-400 samples, after which, increases in sample size resulted in limited precision gains (Schemmel *et al.* 2022).

For each billfish species, seven length bins sizes were investigated (2, 3, 5, 7, 8, 10, and 15 cm) to allow for comparison of length frequency data with different binning structures. For each species, moderate to severe aliasing in length frequency plots was apparent for data binned into 2, and 3 cm bins (Figure 1, using *M. nigricans* as an example). Obvious aliasing was reduced in 5 cm bins for all species indicating 5 cm as an appropriate bin size for each species (5 cm is also the same length bin structure used in the latest assessment for each species) (ISC 2016, 2018, 2019). Investigations into length bin size, using the Coggins *et al.* (2013) $L_\infty/30$ calculation, indicated for striped marlin, 214 cm EFL (Sun *et al.* 2011; ISC 2019), blue marlin (226 male, 304 female), and swordfish (206 male, 226 female), no less than 5cm for each. Given this, a 5 cm bin structure was adopted for our sampling purposes as it was the smallest suggested length bin size between the two approaches.

4.0 Discussion

Results from the Kruskal-Wallis rank sum test indicated no significant difference in billfish length frequency data between years and areas. With the concern of knowing a populations length frequency prior to sampling elevated, the scientific literature on sampling design indicates that POS is the preferred method for highly migratory pelagic species (Chang *et al.* 2019; Goodyear 2019). Our base POS sampling design therefore combined all areas and the last five years of length frequency information independently for each species across the North Pacific (Figure 2). Despite length frequency consistency across regions, our derived POS design was mirrored for each of the three regions (eastern, central, and western), thus maintain sampling consistency and allowing regions to be looked at independently, or combined in future age, growth, and reproduction work. Importantly, the gross spatial structure presented in this sampling approach is designed to ensure that samples are distributed across the north Pacific as much as possible. However, set specific, or trip specific location information for each sample will be required for an analysis of spatial variability.

Results from Schemmel *et al.* (2022) indicate that total sample sizes greater than 200 produced reliable estimates of L_∞ and K. However, sample sizes should be increased to be able to test for spatial and sex-specific differences in growth, and account for species with increased variability in age at length. With the literature indicating 200–500 total samples (Kritzer *et al.* 2001; Brouwer & Griffiths 2005; Chang *et al.* 2019) we elected to use a sample size of 300 for each of our defined North Pacific regions (eastern, central, and western), resulting in 900 samples across the Pacific. This should allow enough samples within each region to estimate growth independently of the other regions, making comparisons of spatially explicit growth parameters estimates possible, while also keeping the total sample size tractable.

To improve consistency in sampling practices across labs, details are provided in the attached appendix (Appendix I and Appendix I Sampling Infographic) on how to collect, store, and share samples. Additionally, once samples are collected a single central database which all countries can access and input data will be made available. This central database is an important component of this research as it allows participating countries to track total sample collations and avoid redundant sampling, while also providing a common labeling structure to facilitate the sharing of samples between labs. A central database will also be useful for keeping track of what samples were shipped and when. Details of this centralized database are included in this work (Appendix II).

To encourage sample independence, general guidelines regarding a limit on the number of samples per length bin and per trip are necessary. We propose that, within any single length bin, no more than two samples be collected from a single set. Beyond that, for any given trip no more than 10 samples total should be collected, in order to insure sample independence, and also to reduce sampling burdens for any one boat on any one trip. While 300 samples per region is a clean and simple approach, there are of course caveats that need to be considered which will modify total sample sizes. For instance in order to examine sexually explicit growth a general rule will be applied to ensure that any sampling bin with one sample allotted to it via the proportional sampling approach (length bins in the tails of the distribution) will instead have two samples allotted in order to allow for one female and one male to be collected. Furthermore, care will need to be taken by those collecting samples to try and ensure that length bins are filled with both male and female samples, however, this practice will be dependent on the sample source used by each researcher. Therefore, we leave this statement as guidance which each researcher should seek to fulfill as they see fit.

In order to better assess size at maturity (L_{50}), additional samples of smaller individuals are needed to insure enough samples of immature animals. In general, when looking to improve estimates for L_{50}, L_∞ can be used along with the life history invariant of 0.66 (Jensen 1996) to identify an appropriate upper length bin bound for these supplemental samples. This is because individuals > 0.66 x L_∞ are predominantly mature and therefore, contribute limited information to the maturity ogive estimation. As these samples are meant specifically for the estimation of size at maturity, and not meant for use in determining length at age, identifying a lower bound for these supplemental samples is of less importance and so will be left open, thus allowing for the collection of all small individuals up to the total supplemental sample size of 10 per length bin in order to improve our ability to accurately define the maturity ogive for our species of interest. For striped marlin this means collecting additional samples below 140 cm EFL (currently estimated L_{50} used in assessment, 161 cm), for blue marlin below 200 cm EFL (currently estimated L_{50} used in assessment, 179.76 cm), and for swordfish below 150 cm EFL (currently estimated L_{50} used in assessment, 143.6 cm). This supplemental sampling aims to allow all size bins below 0.66 x L_∞ to have a maximum of 10 individuals from each region for maturity estimation, while keeping the POS derived sample sizes intact for length at age estimation (Table 1). Based on current maturity ogives for these species (Kapur et al. 2017) this supplemental sampling, along with the larger POS sampling approach will allow for the collection of an adequate number of samples from across the needed size range in order to estimate key life history parameters like L_{50}.

These additional samples will only be used for estimating maturity and will not be included in the aged samples so as to avoid known issues related to adding supplemental age samples to either the upper or lower ends of a species length distribution (Goodyear 2019). Despite this, efforts should still be taken to collect ageing structures (fin rays and otoliths) from these supplementally sampled individuals as such samples may be valuable for future research, while neglecting to collect such structures would be a waste of sampling effort.

Samples will be collected from Japan (commercial, and training vessel longlines, and driftnet fishery), Taiwan (deep-water longline), and the US (Hawaii deep and shallow set longline). Members of the scientific community from each of these nations have agreed to analyze specific life history parameters using this collaborative dataset (Table 2). This sampling design should allow us to accurately estimate age, growth, and maturity for *K. audax* , *M. nigricans,* and *X. gladius* across the North Pacific. Additionally, the mirrored sampling across gross regions of the Pacific will allow us to examine spatial variability without concerns of disparate sampling or analysis confounding our results.

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Tables

Table 1: Proportional length based otolith sampling designs for *K. audax*, *M. nigricans,* and *X. gladius* (S_marlin, B_marlin, and Swordfish, respectively). Bin_width_cm indicates the size of the length bin used in this sampling approach. N_inds specifics the number of individuals in the fisheries catch data for a particular length bin, while Perc indicates the percentage of the fisheries catch in each length bin. N_samp indicates the number of samples (out of the total ~300) that should be sampled from each length bin, and sup_mat_samps indicates the number of supplemental maturity samples that should have gonads collected from each size bin. For each species this sampling strategy will be applied to each region of the North Pacific (eastern, central, and western), resulting in ~900 total samples for each species.

length_bins	species	bin_width_cm	n_inds	perc	n_samp	sup_mat_samps
45	S_marlin	5	1	3.78E-05	0	10
60	S_marlin	5	1	3.78E-05	0	10
65	S_marlin	5	1	3.78E-05	0	10
70	S_marlin	5	1	3.78E-05	0	10
75	S_marlin	5	1	3.78E-05	0	10
80	S_marlin	5	7	0.00026438	0	10
85	S_marlin	5	10	0.000377686	0	10
90	S_marlin	5	26	0.000981984	0	10
95	S_marlin	5	96	0.003625788	2	8
100	S_marlin	5	299	0.01129282	3	7
105	S_marlin	5	521	0.019677456	6	4
110	S_marlin	5	664	0.02507837	8	2
115	S_marlin	5	640	0.024171923	7	3
120	S_marlin	5	738	0.027873248	8	2
125	S_marlin	5	785	0.029648374	9	1
130	S_marlin	5	953	0.035993504	11	0
135	S_marlin	5	1083	0.040903426	12	0
140	S_marlin	5	1417	0.053518148	16	0
145	S_marlin	5	1895	0.071571553	21	0
150	S_marlin	5	2393	0.09038033	27	0
155	S_marlin	5	2430	0.091777769	28	0
160	S_marlin	5	2217	0.083733051	25	0
165	S_marlin	5	1873	0.070740643	21	0
170	S_marlin	5	1649	0.06228047	19	0
175	S_marlin	5	1370	0.051743022	16	0
180	S_marlin	5	1238	0.046757563	14	0
185	S_marlin	5	798	0.030139366	9	0
190	S_marlin	5	609	0.023001095	7	0
195	S_marlin	5	434	0.016391585	5	0
200	S_marlin	5	476	0.017977868	5	0
205	S_marlin	5	438	0.01654266	5	0

210	S_marlin	5	448	0.016920346	5	0
215	S_marlin	5	257	0.009706538	3	0
220	S_marlin	5	223	0.008422404	3	0
225	S_marlin	5	139	0.005249839	2	0
230	S_marlin	5	123	0.004645541	2	0
235	S_marlin	5	109	0.004116781	2	0
240	S_marlin	5	55	0.002077275	2	0
245	S_marlin	5	23	0.000868678	0	0
250	S_marlin	5	11	0.000415455	0	0
255	S_marlin	5	9	0.000339918	0	0
260	S_marlin	5	9	0.000339918	0	0
265	S_marlin	5	4	0.000151075	0	0
270	S_marlin	5	1	3.78E-05	0	0
275	S_marlin	5	1	3.78E-05	0	0
280	S_marlin	5	1	3.78E-05	0	0

length_bins	species	bin_width_cm	n_inds	perc	n_samp	sup_mat_samps
45	B_marlin	5	2	3.84E-05	0	10
70	B_marlin	5	1	1.92E-05	0	10
80	B_marlin	5	1	1.92E-05	0	10
85	B_marlin	5	5	9.59E-05	0	10
90	B_marlin	5	7	0.000134251	0	10
95	B_marlin	5	12	0.000230145	0	10
100	B_marlin	5	25	0.000479469	0	10
105	B_marlin	5	60	0.001150726	0	10
110	B_marlin	5	362	0.006942713	2	8
115	B_marlin	5	374	0.007172858	2	8
120	B_marlin	5	494	0.00947431	3	7
125	B_marlin	5	544	0.010433248	3	7
130	B_marlin	5	883	0.01693485	5	5
135	B_marlin	5	1113	0.021345966	6	4
140	B_marlin	5	1923	0.036880766	11	0
145	B_marlin	5	2603	0.049922326	15	0
150	B_marlin	5	3780	0.072495733	22	0
155	B_marlin	5	3845	0.073742352	22	0
160	B_marlin	5	3973	0.076197234	23	0
165	B_marlin	5	3432	0.065821522	20	0
170	B_marlin	5	3413	0.065457126	20	0
175	B_marlin	5	2955	0.056673251	17	0
180	B_marlin	5	3396	0.065131087	20	0
185	B_marlin	5	2652	0.050862085	15	0
190	B_marlin	5	2664	0.051092231	15	0

195	B_marlin	5	1862	0.035710861	11	0
200	B_marlin	5	1874	0.035941006	11	0
205	B_marlin	5	1614	0.030954527	9	0
210	B_marlin	5	1735	0.033275158	10	0
215	B_marlin	5	1372	0.026313266	8	0
220	B_marlin	5	1108	0.021250072	6	0
225	B_marlin	5	813	0.015592336	5	0
230	B_marlin	5	740	0.014192286	4	0
235	B_marlin	5	558	0.010701751	3	0
240	B_marlin	5	450	0.008630444	3	0
245	B_marlin	5	417	0.007997545	2	0
250	B_marlin	5	350	0.006712568	2	0
255	B_marlin	5	170	0.00326039	2	0
260	B_marlin	5	149	0.002857636	2	0
265	B_marlin	5	98	0.001879519	2	0
270	B_marlin	5	87	0.001668553	2	0
275	B_marlin	5	46	0.000882223	0	0
280	B_marlin	5	53	0.001016475	0	0
285	B_marlin	5	31	0.000594542	0	0
290	B_marlin	5	22	0.000421933	0	0
295	B_marlin	5	12	0.000230145	0	0
300	B_marlin	5	19	0.000364397	0	0
305	B_marlin	5	14	0.000268503	0	0
310	B_marlin	5	8	0.00015343	0	0
315	B_marlin	5	8	0.00015343	0	0
320	B_marlin	5	6	0.000115073	0	0
325	B_marlin	5	1	1.92E-05	0	0
330	B_marlin	5	1	1.92E-05	0	0
350	B_marlin	5	1	1.92E-05	0	0
360	B_marlin	5	2	3.84E-05	0	0

length_bins	species	bin_width_cm	n_inds	perc	n_samp	sup_mat_samps
40	Swordfish	5	8	3.53E-05	0	10
45	Swordfish	5	16	7.07E-05	0	10
50	Swordfish	5	36	0.000158976	0	10
55	Swordfish	5	54	0.000238464	0	10
60	Swordfish	5	176	0.000777217	0	10
65	Swordfish	5	252	0.001112833	0	10
70	Swordfish	5	428	0.00189005	2	8
75	Swordfish	5	515	0.002274243	2	8
80	Swordfish	5	566	0.002499459	2	8
85	Swordfish	5	550	0.002428803	2	8

90	Swordfish	5	782	0.003453316	2	8
95	Swordfish	5	1614	0.007127433	2	8
100	Swordfish	5	2248	0.00992718	3	7
105	Swordfish	5	2805	0.012386895	4	6
110	Swordfish	5	3601	0.015902035	5	5
115	Swordfish	5	4582	0.020234137	6	4
120	Swordfish	5	7532	0.033261352	10	0
125	Swordfish	5	10575	0.046699257	14	0
130	Swordfish	5	13706	0.06052577	18	0
135	Swordfish	5	13874	0.061267659	18	0
140	Swordfish	5	15026	0.066354897	20	0
145	Swordfish	5	14752	0.065144911	20	0
150	Swordfish	5	15684	0.069260628	21	0
155	Swordfish	5	15450	0.068227283	20	0
160	Swordfish	5	14994	0.066213585	20	0
165	Swordfish	5	13354	0.058971336	18	0
170	Swordfish	5	12101	0.053438081	16	0
175	Swordfish	5	10813	0.047750266	14	0
180	Swordfish	5	9504	0.041969715	13	0
185	Swordfish	5	8438	0.037262253	11	0
190	Swordfish	5	7272	0.032113191	10	0
195	Swordfish	5	5156	0.022768924	7	0
200	Swordfish	5	4447	0.019637976	6	0
205	Swordfish	5	3851	0.017006037	5	0
210	Swordfish	5	3210	0.014175377	4	0
215	Swordfish	5	2363	0.010435021	3	0
220	Swordfish	5	1859	0.008209354	2	0
225	Swordfish	5	1231	0.005436103	2	0
230	Swordfish	5	941	0.004155461	2	0
235	Swordfish	5	582	0.002570115	2	0
240	Swordfish	5	442	0.001951874	2	0
245	Swordfish	5	289	0.001276226	0	0
250	Swordfish	5	232	0.001024513	0	0
255	Swordfish	5	136	0.000600577	0	0
260	Swordfish	5	110	0.000485761	0	0
265	Swordfish	5	70	0.00030912	0	0
270	Swordfish	5	55	0.00024288	0	0
275	Swordfish	5	38	0.000167808	0	0
280	Swordfish	5	27	0.000119232	0	0
285	Swordfish	5	21	9.27E-05	0	0
290	Swordfish	5	24	0.000105984	0	0
295	Swordfish	5	8	3.53E-05	0	0
300	Swordfish	5	15	6.62E-05	0	0

305	Swordfish	5	9	3.97E-05	0	0
310	Swordfish	5	3	1.32E-05	0	0
315	Swordfish	5	6	2.65E-05	0	0
320	Swordfish	5	2	8.83E-06	0	0
330	Swordfish	5	2	8.83E-06	0	0
345	Swordfish	5	2	8.83E-06	0	0
350	Swordfish	5	2	8.83E-06	0	0
360	Swordfish	5	1	4.42E-06	0	0
390	Swordfish	5	1	4.42E-06	0	0
400	Swordfish	5	1	4.42E-06	0	0
440	Swordfish	5	1	4.42E-06	0	0

Table 2: List of projects for *K. audax*, *M. nigricans,* and *X. gladius* (S_marlin, B_marlin, and Swordfish (a,b,c), respectively) to be undertaken using samples collected using this protocol. (JP-Japan, TW-Taiwan, US-United States). Priority was specified by the ISC billfish working group based on the projects perceived benefit to improvements to current stock assessment efforts.

a)

No	Species	Category	Project description	Participants	Priority	Lead scientist
1	All Billfish	Data and sample collection	Collaborative biological sampling of highly migratory species	JP, TW, US	High	Michael Kinney (US)
2	Striped Marlin	Biological parameter	Update estimation of growth curve and key parameters like Linf	JP, TW, US	High	Minoru Kanaiwa (JP)
3	Striped Marlin	Biological parameter	Update estimation of size at 50% maturity	JP, TW, US	High	Michael Kinney (US), Mrs Yuki Ishihara is working on frozen tissues
4	Striped Marlin	Biological parameter	Update of estimation of weight-length relationship. Update of conversion factor. Length conversion factors.	JP, TW, US	Low	Yi-Jay Chang (TW)
5	Striped Marlin	Stock structure	Collection, and storage of genetic information in line with biological sampling protocol	JP, TW, US	Low	Michael Kinney (US)

7	Striped Marlin	Biological parameter	Genetic marker for sex identification	JP, TW, US	Mid	On hold as genetic storage is settled

b)

No	Species	Category	Project description	Participants	Priority	Lead scientist
1	All Billfish	Data and sample collection	Collaborative biological sampling of highly migratory species	JP, TW, US	High	Michael Kinney (US)
2	Blue Marlin	Biological parameter	Update estimation of growth curve and key parameters like Linf	JP, TW, US	Low	Yi-Jay Chang (TW)

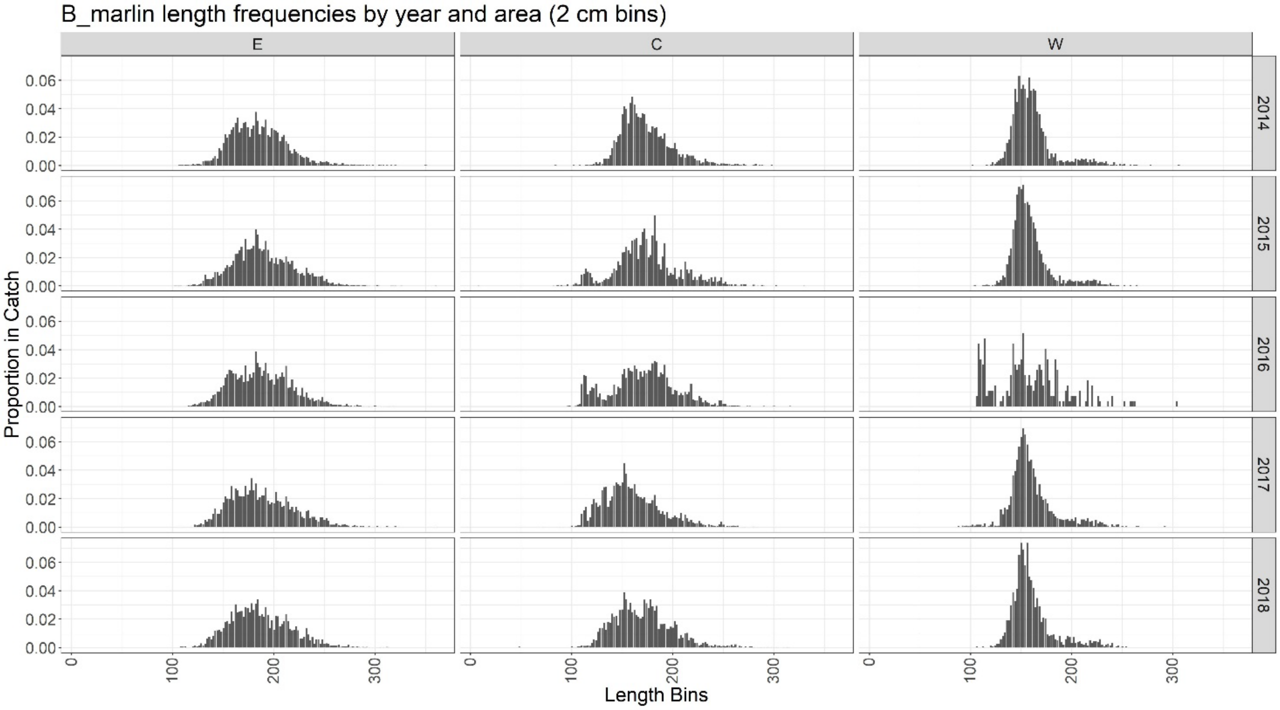
c)

No	Species	Category	Project description	Participants	Priority	Lead scientist
1	All Billfish	Data and sample collection	Collaborative biological sampling of highly migratory species	JP, TW, US	High	Michael Kinney (US)
2	Swordfish	Biological parameter	Update estimation of growth curve and key parameters like Linf	JP, TW, US	High	Michael Kinney (US); Minoru Kanaiwa (JP) point of contact for JP

3	Swordfish	Biological parameter	Update estimation of size at 50% maturity	JP, TW, US	Low	Dr. Hiroshi Ashida (JP), Mrs Yuki Ishihara is working on frozen tissues
4	Swordfish	Stock structure	Collection, and storage of genetic information in line with biological sampling protocol	JP, TW, US	Low	Michael Kinney (US)

Figures

a)


B_marlin length frequencies by year and area (2 cm bins)
E
C
W
2014
2015
2016
2017
2018
0.06
0.04
0.02
0.00
Proportion in Catch
0
100
200
300
Length Bins

b)

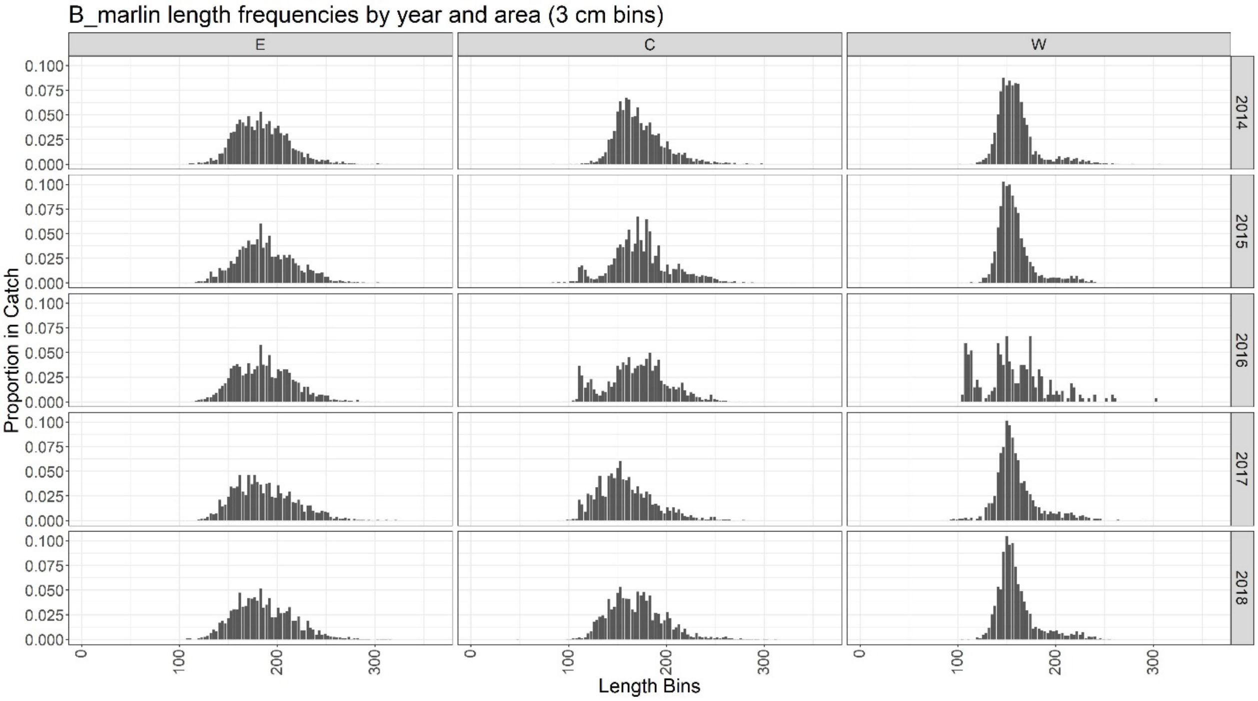
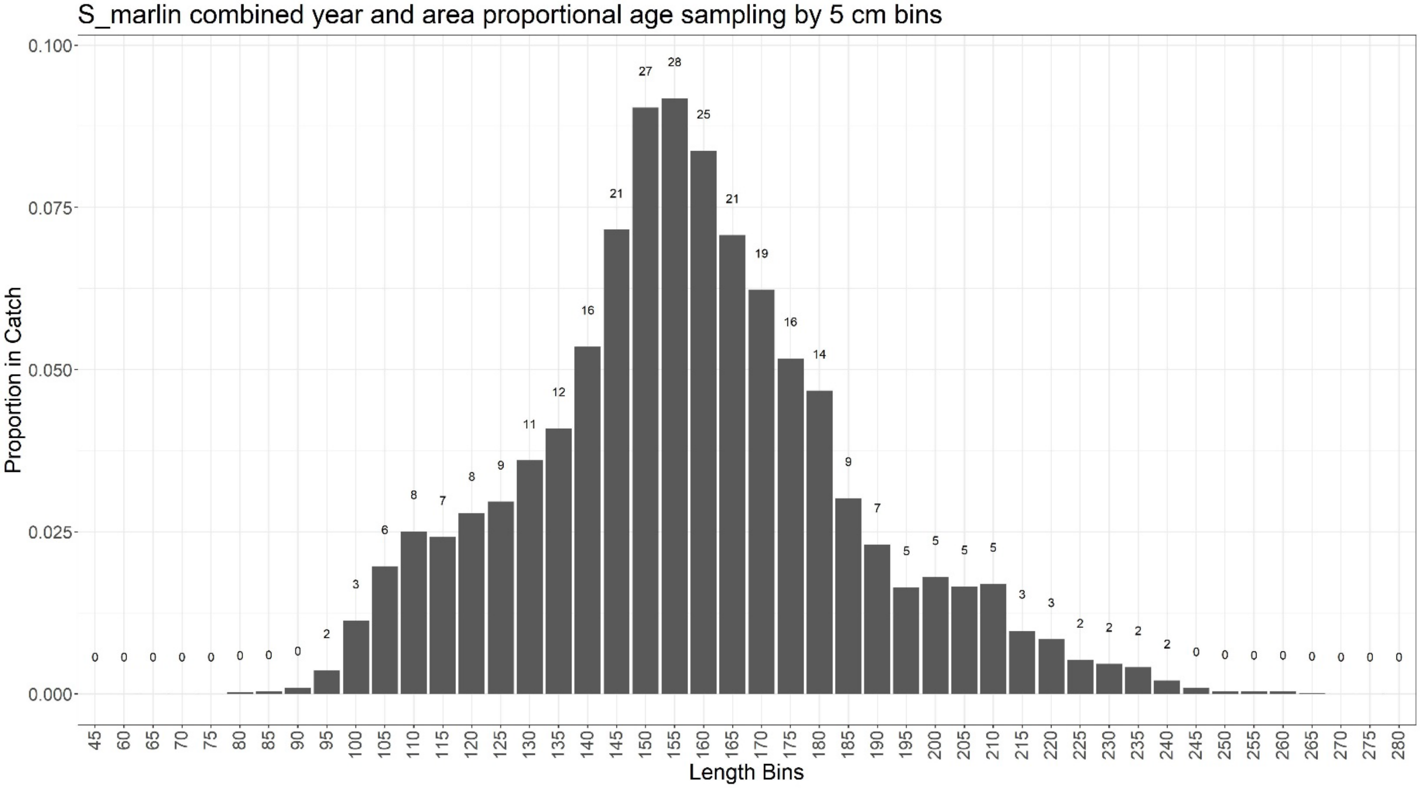
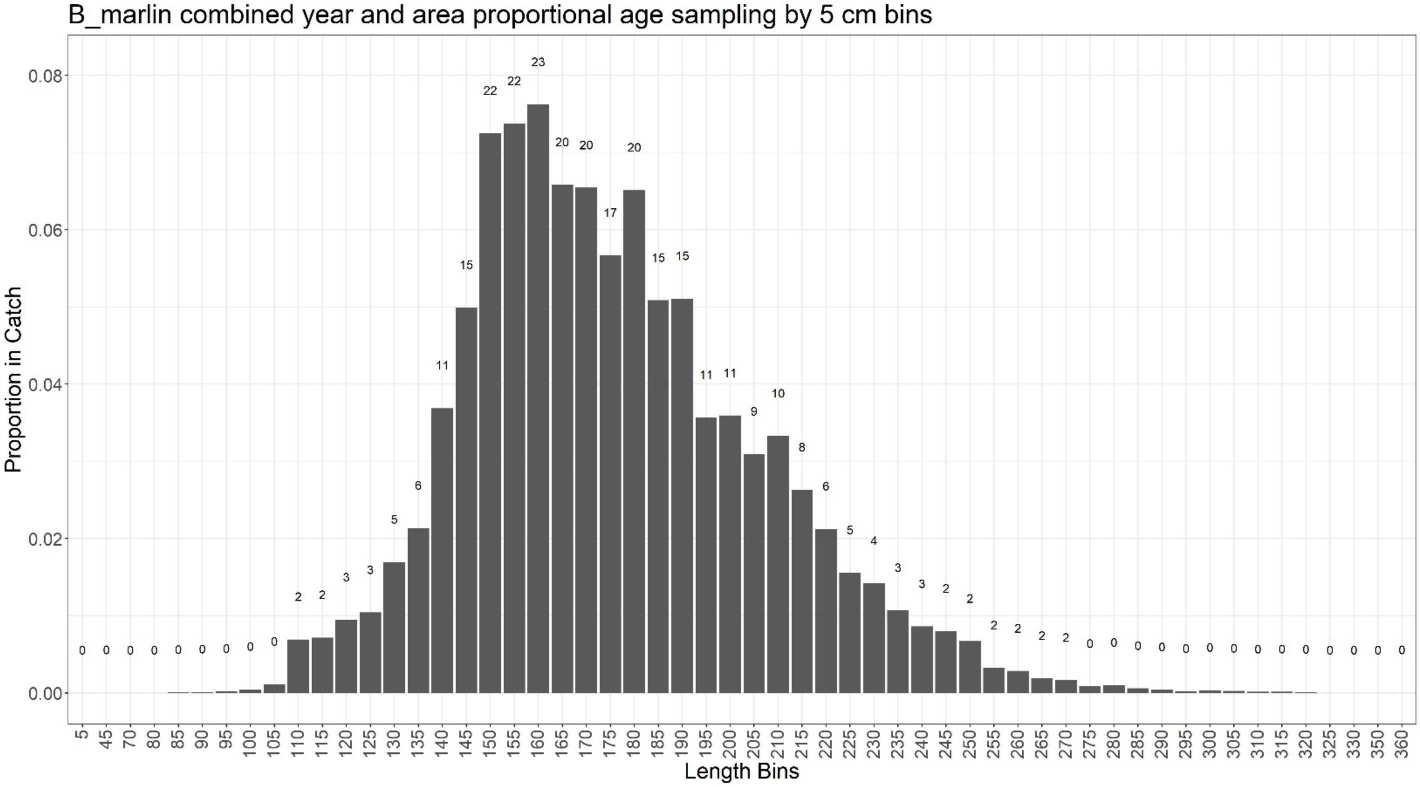


Figure 1: Length frequency plots for *K. audax* (B_marlin), in the North Pacific, a) 2 cm size bins and b) 3 cm size bins. Length information sourced from longline fisheries of Japan, Taiwan, and the US (Hawaii) used in the last assessment (2016) of blue marlin in the North Pacific. Aliasing appears as spikes in the length frequency plots indicating unrealistic discrepancies in the numbers of animals in adjacent length bins, suggesting a mismatch in plotting resolution compared to data recoding resolution.

a)


S_marlin combined year and area proportional age sampling by 5 cm bins
Proportion in Catch
0.100
0.075
0.050
0.025
0.000
Length Bins
45 60 65 70 75 80 85 90 95 100 105 110 115 120 125 130 135 140 145 150 155 160 165 170 175 180 185 190 195 200 205 210 215 220 225 230 235 240 245 250 255 260 265 270 275 280
0 0 0 0 0 0 0 0 2 3 6 8 7 8 9 11 12 16 21 27 28 25 21 19 16 14 9 7 5 5 5 5 3 3 2 2 2 2 0 0 0 0 0 0 0 0

b)


B_marlin combined year and area proportional age sampling by 5 cm bins
Proportion in Catch
0.08
0.06
0.04
0.02
0.00
Length Bins
5 45 70 75 80 85 90 95 100 105 110 115 120 125 130 135 140 145 150 155 160 165 170 175 180 185 190 195 200 205 210 215 220 225 230 235 240 245 250 255 260 265 270 275 280 285 290 295 300 305 310 315 320 325 330 350 360
0 0 0 0 0 0 0 0 0 0 2 2 3 3 5 6 11 15 22 22 23 20 20 17 20 15 15 11 11 9 10 8 6 5 4 3 3 2 2 2 2 2 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0

c)

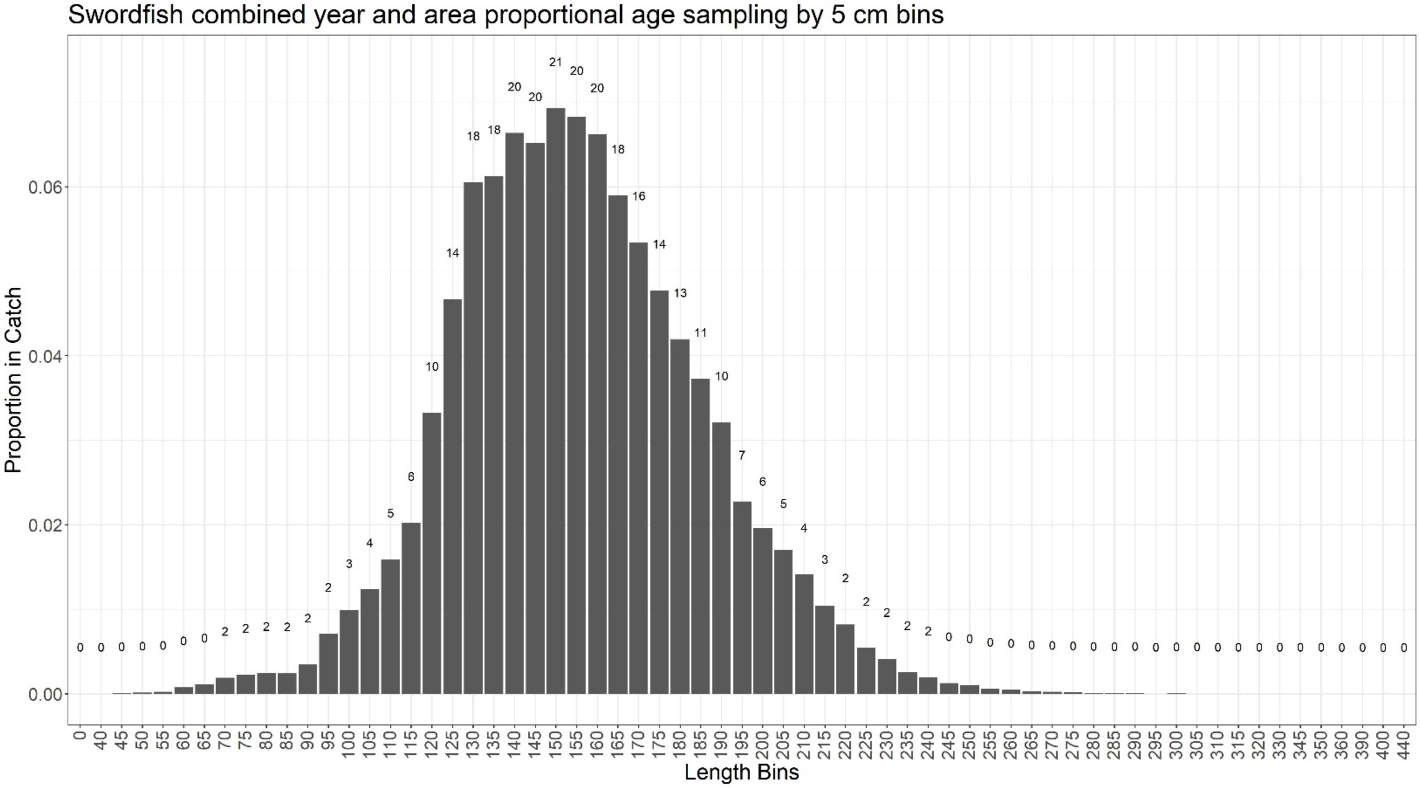

Swordfish combined year and area proportional age sampling by 5 cm bins

Figure 2: Proportional length based otolith sampling designs for *K. audax*, *M. nigricans,* and *X. gladius* (S_marlin, B_marlin, and Swordfish, [a,b,c], respectively). Bars indicate the proportion of the combined catch across each region over the last five years (2014-2018) in each 5 cm length bin. Numbers above the bars indicate sample sizes for each length bin (out of 300 total) in a given region. The same sampling strategy will be applied to each region of the North Pacific (eastern, central, and western), resulting in ~900 total samples for each species (not including supplemental samples for maturity estimation).

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.

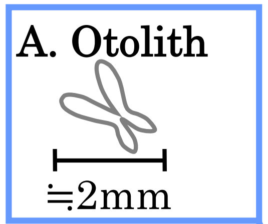
Appx. I Table 1: Life history studies on billfish species of interest. 1A = first anal fin, 1D = first dorsal fin, -num = additional fin spine number, L = longest spine. Fin spines are counted from the head of the fish to the tail, meaning that 1D, or the first dorsal fin spine, is the dorsal fin spine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

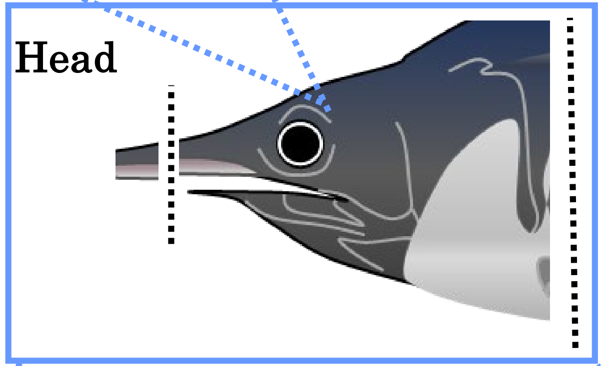
Sampling Infographic

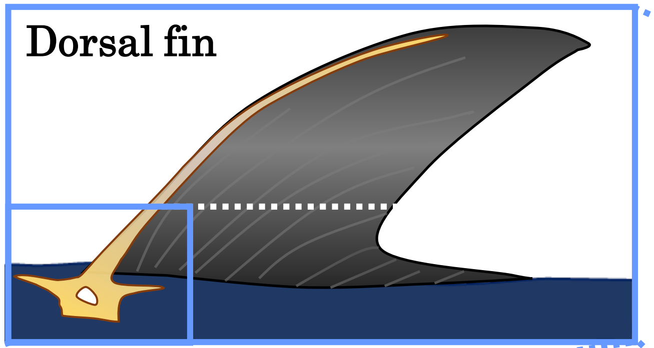
Sampling infographic originally created by:
Miyuki Kanaiwa (Mie University, Graduate School of Bioresources)

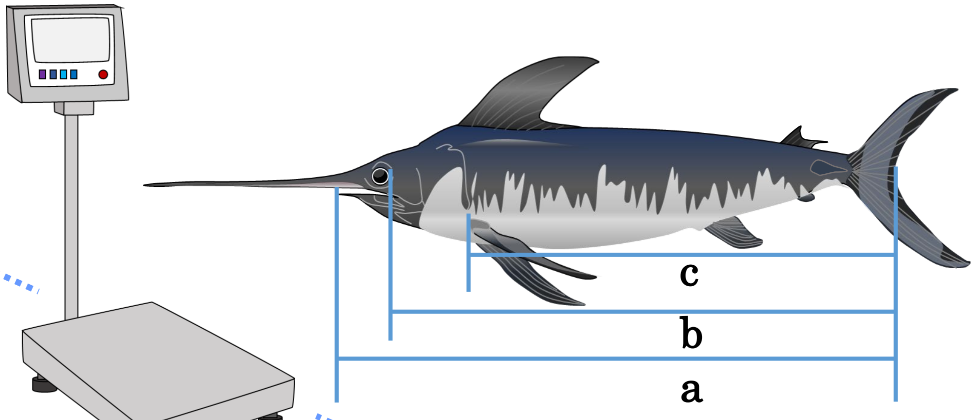
A. Otolith



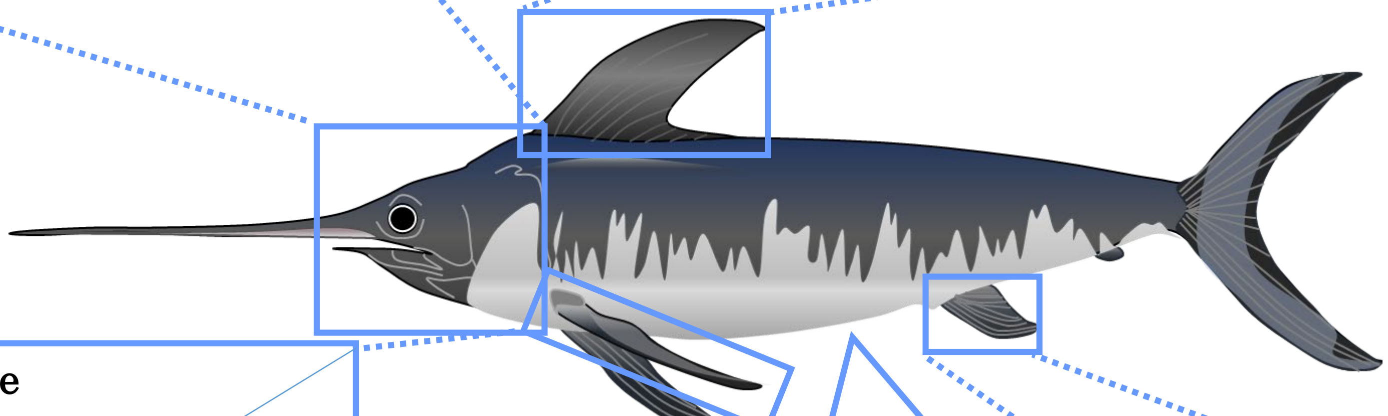
Take the Sagittal Otoliths from both sides of the head from each whole otic capsule. Otoliths should be cleaned and stored dry in labeled vials or coin envelopes.



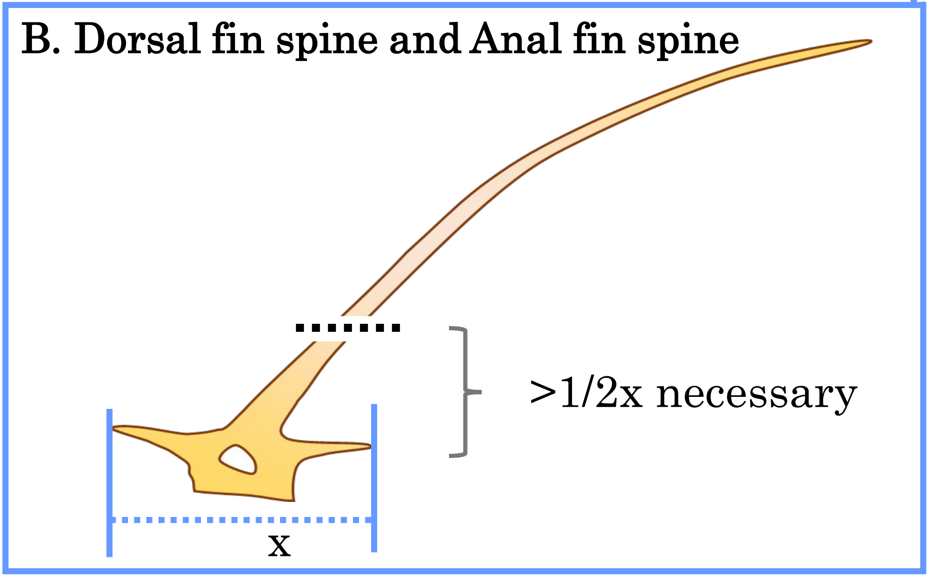




Measure the a:LJFL, b:EFL and c:PFFL of the billfish and record the whole or dressed weight. In the case of dressed weight, record its condition in detail (ex. bodyweight without the bill, caudal fin, gills and viscera).

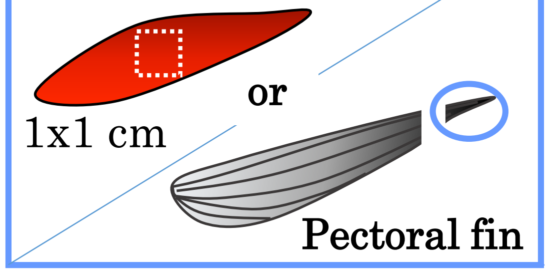


B. Dorsal fin spine and Anal fin spine



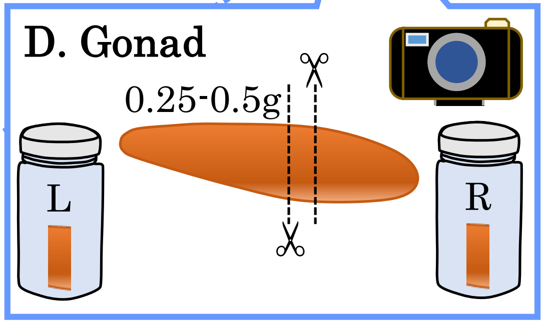
Sample a few spines, including the longest spines of the dorsal and anal fins. Measure the widest point at the base of the spine, section the spine at ½ of this measurement (dotted line).

C. Muscle

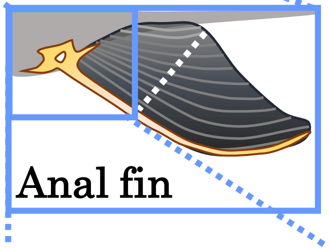


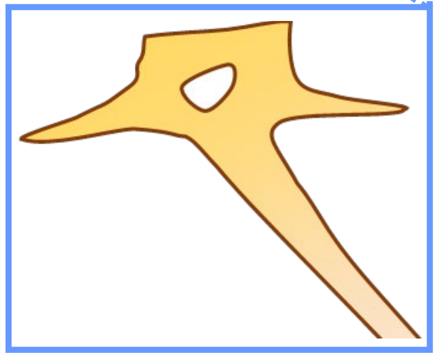
Fin clips should be taken from the tip or trailing edge of pectoral fin. Muscle tissue should be taken using a biopsy punch (3mm preferred) or a clean area cut out with a clean knife (1x1 cm).
Fin/muscle tissue should be fixed with ethanol (>80% but <95% conc), use enough ethanol to allow samples to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

Original concept that follows differs slightly from what has been implemented for this project:

- 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