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Progress report of biological sampling for billfishes collected by Japan in the North Pacific for 2019-2023[1]

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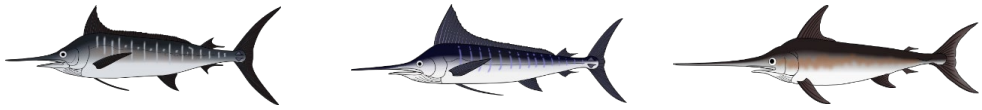

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Abstract

This working paper provides a summary report on the progress of Japanese biological sampling implemented for swordfish, striped marlin, and blue marlin for 2019-2023 under the collaborative study of ISC with US and Taiwan on the growth, the maturity size, and the stock structure in the North Pacific Ocean (NPO). The biological samples include head (otolith), dorsal fins and/or anal fins (spines), gonads (ovary and testis), and tissue samples of muscle (DNA). These samples are collected from one fish with auxiliary information such as body size and capture location using a variety of Japanese fleets such as commercial coastal longline and large-mesh driftnet. The sample sizes of three species are summarized, sampling location are displayed on the maps, and length frequency data are shown. A total number of biological samples (gonads) for swordfish, striped marlin, and blue marlin were 676 (656), 720 (676), and 83 (70), respectively. The summary of Japan's biological sampling of three species for 5 years indicated that there is a limitation of random sampling as well as the achievement of target (sampling protocol) due to biological and physical problems. It is therefore recommended that the working group should review the sampling protocol assigned number of samples by area and species in consideration of current sampling coverage, achievement rates of sampling for each bin compared to their targets, and migration patterns of billfishes especially for the seasonal latitudinal migration of swordfish. It is also recommended that the working group should collect raw gonads fixed by formalin or "Altfix" as much as possible.

Introduction

The ISC BILLFISH Working Group (BILLWG) had commenced a collaborative biological sampling project in 2020 with Japan, the United States of America (US), and Chinese Taipei (i.e., Taiwan). The main objectives are to improve estimates of biological parameters for growth and maturity of striped marlin (*Kajikia Audax*), blue marlin (*Makaira Mazara*) and swordfish (*Xiphias gladius*), and to clarify the stock structures in the NPO, and finally to enhance the accuracy of the stock assessments for these three species. The BILLWG developed a uniform sampling protocol that is available for all the sampling of three species across the NPO (Kinney et al., 2020). The BILLWG determined the proportional length-based otolith sampling designs (Chang et al., 2019) for three species and assigned the tasks to each member countries. The NPO was separated into three sampling regions (i.e., western : west to 160 °E, central: between 160 °E and 160 °W, and eastern: east to 160 °W), and Japan was assigned the western and central regions to collect more than 300 samples per region for each species. A constant length bin size (5 cm) and a different number of samples for each bin were adopted based on the statistical analyses with the length

frequency data of each species, and the different maximum sizes of length bins were given by the species (Kinney et al., 2020). The study on the growth of striped marlin (swordfish) and the maturity size of swordfish (striped marlin) in the NPO were assigned as tasks of Japanese (US) delegation, and both growth and maturity size of blue marlin were assigned as tasks of Taiwanese delegation.

The swordfish is mainly targeted by Japanese coastal longline fishery and Japanese offshore and distant water longline fishery in the northwestern Pacific Ocean. Japanese longliners commonly use a shallow set (3-5 number of hooks between floats) in the night to catch swordfish. This species is also caught by Japanese large-mesh driftnet fishery and Japanese harpoon fishery off the coast of Sanriku (i.e., northeastern of Japan). The operational area of Japanese driftnet fishery is limited to the exclusive economic zone (EEZ) of Japan after the United Nations moratorium on high seas large-scale pelagic driftnet fisheries implemented on 31 December 1992 (Ito et al., 1993). The striped marlin and blue marlin are mainly caught as bycatch by Japanese coastal longline fishery and Japanese offshore and distant water longline fishery in the northwestern Pacific Ocean. These species are occasionally targeted and caught by recreational fishery (i.e., rod and reel) around the coastal waters of Japan. The striped marlin is also caught by Japanese large-mesh driftnet fishery and Japanese harpoon fishery off the coast of Nagasaki-prefecture (i.e., East China sea). The blue marlin is mainly caught in the warmer waters compared to the other two species because blue marlin prefers to the surface warm water ranging from 26 to 30°C (Carlisle et al., 2017).

The main purpose of this working paper is to summarize the progress of biological sampling conducted by Japan for three billfish species from 2019 to 2023 and to discuss the current issues and challenges for the collaborative study.

Materials and Methods

Fisheries Resources Institute in Japan had officially commenced to collect a set of biological samples for the study on growth, maturity size, and stock structure of three billfish species (i.e., blue marlin, striped marlin, and swordfish) since 2019. The biological samples include head, dorsal fins and/or anal fins, gonads, and tissue samples of muscle. The sagittal otoliths were taken from the head and stored dry in vials after cleaning them. Several longer spines were taken from both fins or either fin, and then preserved dry after removing muscle and skin. The weight of gonads (i.e., ovary and testis) were measured and a part of it was preserved in the 10 % formalin or in the freezer. Researchers can use formalin on the research vessels, whereas fishermen usually preserve gonads in the freezer because formalin contains harmful substance. The big issue in the use of

frozen gonad is the destruction of a cell, so that it makes difficult to identify the maturity stage of gonads. Recently, we started to ask fishermen of "Kesennuma" for the use of Altfix which is fixative solutions of tissue without using the formaldehyde.

These samples were collected from one fish with a batch number and auxiliary information about the body size (i.e., eye fork length), capture location, vessel name, and time and date. In addition, the biological samples were collected from multiple Japanese fisheries including commercial coastal longline fishery, commercial offshore and distant-water longline fishery, large-mesh driftnet fishery, harpoon fishery, research vessels (longline fishery), and recreational fishery.

The sample sizes for three species collected by Japan were summarized by year, month, gear, sex, regions, and storage way of gonad, and the sampling locations of gonads for three species were displayed on the maps by year, month, gear, sex, and storage way of gonad. In addition, length frequency of number of samples (gonads) for three species were shown to confirm the achievements of the sampling for each species in each region.

Results

Annual changes in the number of biological samples

The number of biological samples were significantly increased in 2023 for swordfish and striped marlin because Japan intensified the sampling of these species to accelerate the growth and maturity studies (**Table 1**, **Figure 1A**). The sampling locations and sampled species were largely changed by year (**Figure 1**). In 2019, the samples of swordfish were widely collected in the NPO and those of the other two species were collected in the waters around Japan. However, the sampling locations were limited to the western and central areas since 2020 except for a few samples of striped marlin in 2022. The samples of swordfish and striped marlin were mainly collected in the western area and followed by central area. The number of samples for blue marlin was much smaller than those of the other two species for 2019-2023, and the sampling locations were limited to the subtropical areas between 10 and 25 °N and temperate area in the northwestern Pacific.

Monthly changes in the number of biological samples

The number of biological samples were largely changed by month and species (**Table 2**, **Figure A2**). The samples of swordfish and striped marlin were collected throughout the year (**Figure A2**), however, there was no sample of blue marlin in January and March with lower number of samples throughout the year compared to those of the other two species. The samples of striped marlin were mainly collected from January to May and those of swordfish were mainly collected from

April to July. The sampling locations were also largely changed by month and species (**Figure 2**). The samples of swordfish were widely collected in the NPO from April to June, while the sampling locations of the other two species were limited to the western and central NPO throughout the year. In January and March, the sampling of swordfish and striped marlin was conducted only in the western area.

Gear-specific changes in the number of biological samples

The number of biological samples were largely changed by gear and species (**Table 3**, **Figure A3**). The samples of blue marlin were mainly collected from recreational fleet and research longline fishery, while those of swordfish were mainly collected from driftnet fishery, offshore and distant water longline fishery, and research longline fishery. The samples of striped marlin were collected from all types of fisheries; coastal longline, driftnet, harpoon, offshore and distant water longline, recreational, and research longline. Driftnet and research longline fisheries provided many samples of striped marlin and swordfish. In addition, many samples of swordfish were collected from offshore and distant water longline fisheries. The sampling locations of coastal longline, harpoon, recreational, and driftnet fisheries were limited to the waters around Japan, whereas the samples from the other two fisheries (i.e., offshore-and-distant water longline fishery and research longline fishery) were widely collected in the western and central NPO (**Figure 3**).

Area-specific changes in the number of biological samples

The number of biological samples of striped marlin and swordfish were significantly large in the western area compared to the central areas in the NPO (**Table 4**, **Figure A4**).

Sex-specific changes in the number of biological samples

The number of biological samples for blue marlin and striped marlin were almost the same between male and female, but those of swordfish were differed by sex (**Table 5**, **Figure A5**). The biological samples of female were significantly large compared to those of male for swordfish. In addition, the sampling locations of female swordfish were denser in the higher latitude between 35 and 45 °N compared to those of male swordfish (**Figure 4**). We considered that the biased sampling to the female is due to the sex-specific migration patterns of swordfish in the western NPO (Takahashi et al., 2003, Tanaka and Yamaguchi, 2017). The less samples of dorsal fin for female swordfish are attributed to the product form at the Kesennuma fish market because the swordfish are landed without dorsal fin. The sampling locations of striped marlin were almost the same between sexes, whereas those of blue marlin were differed by sex. The samples of male blue marlin were mainly collected in the lower latitude of the central area in the NPO, but those of female blue

marlin were mainly collected in the coastal waters of Japan.

Changes in the number of biological samples by storage way of gonad

Many biological samples of striped marlin and swordfish were preserved by the formalin and less samples were preserved by freeze (**Table 6**, **Figure A6**). Altfix was used to preserve the samples of swordfish and striped marlin because the Fisheries Research Institute in Japan requested the fishermen of "Kesennuma" to collect the gonad samples of these two species and to preserve the gonad using the Altfix since 2023. Therefore, the sampling locations with Altfix were limited to the northwestern Pacific Ocean where is the main operational area of "Kesennuma" fleets (**Figure 5**). The sampling locations for three species were almost similar between formalin and frozen samples, however frozen samples were solely collected in the tropical areas between 5 and 15 °N (**Figure 5**).

Length frequency of number of samples (gonads)

Except for blue marlin, biological samples for the other two species were collected from a wide range of body size in the NPO (**Figure 6**). Observed number of samples in the western area exceeded the target at many bins (**Figure 7**). For striped marlin, the samples of small size less than 120 cm eye-fork-length (EFL) and those of large size more than 200 cm EFL are lacking due to the difficulties in the catch of year-of-young and extra-large size of striped marlin by Japanese fleets. For swordfish, the sampling coverage regarding the body size is much higher compared to the striped marlin and the number of samples reached the target at many bins of middle and large sizes more than 150 cm EFL. On the contrary, the sampling coverage is lower in the central area of NPO (**Figure 8**) due to low fishing effort of Japanese fleets in this region.

Discussions

This working paper provides a summary report on the progress of Japanese biological sampling implemented for three key billfish species: swordfish, striped marlin, and blue marlin in the NPO from 2019 to 2023 under the collaborative study with US and Taiwan. The maps of sampling location indicated that the operational area of Japanese fleets mostly limited to the western area, though the offshore and distant water longline fishery and research vessels collected the samples to some extent in the central area (**Figure 3**). The main reasons for that Japanese offshore and distant water commercial longline fleets typically operate in the western area is the soaring fuel prices. This situation makes a difficulty in achievement of the target number of samples for each bin and species in the central area (**Figure 8**). In addition, even in the western area, we need to make more effort to collet the samples of small size less than 1-meter EFL for striped marlin and

swordfish (**Figure 7**). Since the spawning grounds of three species in the NPO are in the tropical and subtropical waters below 20 °N (Nishikawa, 1958), the year-of-young fishes smaller than 1-meter EFL are mainly collected by longline of research vessels operated in the tropical and subtropical areas (10–30°N) as shown in **figure 3**. Many Japanese commercial offshore and distant water longline fleets were operated in the tropical and subtropical areas to target tropical tunas such as a bigeye tuna using deep-set gear. Since not only the year-of-young fishes of striped marlin and swordfish are usually stayed in the surface water but also those low-value fishes are commonly discarded, it is therefore difficult to collect samples of those small fishes in the tropical and sub-tropical area using those fleets. On the other hand, we can collect some samples of small sized billfishes from Japanese research and training vessels, run by local government, operated in the subtropical waters around Hawaii. However, the fishing effort (i.e., number of hooks) has been continuously declining due to the reduction of total number of fishing vessels (Kai, 2019). This is one of the issues to collect the samples of small size of striped marlin and swordfish in the central area.

Considering the conundrum to collect the samples in the central area, the utilization of samples collected by US and Taiwan in this area is useful. The sampling area of US is however restricted to the water around Hawaii and east of the international date line. By contrast, the sampling area of Taiwan is widely covered between 160 °E and 160 °W, but all sample of gonad are frozen. The frozen samples of billfishes are also collected by Japan (**Table 6**). Then, Japan attempted an examination of histological methods for estimating an accurate mean maturity size of the billfishes from frozen gonad samples (Kurashima et al., 2020). Histological sections of gonads for male and female billfishes (i.e., swordfish and striped marlin) were made using "Paraffin section method" and "Cryofracture technique" for different storage ways of gonad samples (i.e., Raw and Frozen specimen without fixation, respectively, and Row, Frozen, and Defrost specimen fixed by 10% buffered formalin, respectively). The study concluded that frozen specimens may be possible to estimate the maturity stage, whereas defrosted specimens may be difficult to estimate the maturity stage. The development of histological methods is continuously required to effectively use the frozen samples.

The migration patterns of three species in the northwestern Pacific is little known except for swordfish. Past electronic tagging studies such as an archival tag and pop-up satellite tags for swordfish suggested that this species conduct a cyclic seasonal migration between the food rich "Oyashio" cold current area (40–45°N) during summer and the subtropical area (10–20°N) in the winter (Takahashi et al., 2003, Tanaka and Yamaguchi, 2017). However, no common migration patterns were observed for 12 fishes with electronic tags, though 6 fishes showed northward movement for foraging at the end of summer (Tanaka and Yamaguchi, 2017). These results

indicated that the current sampling plan does not consider the horizontal movement patterns of swordfish. It is therefore the WG should consider the latitudinal coverage of samples as well as the longitudinal coverage of samples at least for swordfish.

Recommendations

The WG should review the sampling protocol assigned number of samples by area and species in consideration of current sampling coverage, achievement rates of sampling for each bin and each species compared to their targets, and migration patterns of billfishes especially for the seasonal latitudinal migration of swordfish.

The WG should collect raw gonads fixed by formalin or "Altfix" as much as possible.

Acknowledgements

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252 **Table 1.** Summary of yearly biological samples for three species collected by Japan for 2019-
253 2023.

Species	Year	Gonad	Otolith	Dorsalfin	Analfin	Muscle
Blue marlin	2019	27	35	35	35	0
	2020	0	2	2	0	0
	2021	20	21	21	21	21
	2022	15	17	17	13	17
	2023	8	6	7	7	7
	Total	70	81	82	76	45
Striped marlin	2019	34	27	51	53	5
	2020	10	7	11	11	11
	2021	67	61	59	59	63
	2022	61	69	48	42	70
	2023	504	385	380	375	396
	Total	676	549	549	540	545
Swordfish	2019	101	46	18	44	84
	2020	41	42	41	41	42
	2021	80	80	78	75	81
	2022	39	22	16	15	21
	2023	395	272	76	272	308
	Total	656	462	229	447	536

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Table 2. Summary of monthly biological samples for three species collected by Japan for 2019-2023.

Species	Month	Gonad	Otolith	Dorsalfin	Analfin	Muscle
Blue marlin	1	0	0	0	0	0
	2	3	3	3	3	3
	3	0	0	0	0	0
	4	15	17	17	13	17
	5	8	6	7	7	7
	6	1	1	1	1	1
	7	19	22	22	21	5
	8	10	16	16	14	0
	9	1	2	2	2	0
	10	1	3	3	3	1
	11	7	8	8	8	8
	12	1	1	1	1	1
	Total	70	81	82	76	45
Striped marlin	1	108	83	66	58	77
	2	230	185	168	170	187
	3	71	62	64	64	64
	4	46	44	46	46	45
	5	59	57	64	60	62
	6	30	29	28	29	29
	7	27	8	7	8	8
	8	32	18	30	30	13
	9	28	28	37	37	12
	10	16	14	15	15	19
	11	27	19	22	21	27
	12	2	2	2	2	2
	Total	676	549	549	540	545
Swordfish	1	17	13	2	13	13
	2	7	9	7	8	9
	3	4	4	4	4	4
	4	86	73	58	69	72
	5	107	92	92	87	105
	6	165	105	37	106	122
	7	131	93	6	92	93
	8	11	7	4	10	10
	9	24	17	5	12	23
	10	53	30	12	25	48
	11	33	15	1	17	21
	12	18	4	1	4	16
	Total	656	462	229	447	536

262 **Table 3.** Summary of gear-specific biological samples for three species collected by Japan for
263 2019-2023.

Species	Gear	Gonad	Otolith	Dorsalfin	Analfin	Muscle
Blue marlin	Coastal LL	0	2	2	2	0
	Recreational	30	40	40	37	5
	Research LL	40	39	40	37	40
	Total	70	81	82	76	45
Striped marlin	Coastal LL	44	59	63	63	46
	Driftnet	406	308	273	268	304
	Harpoon	67	52	51	49	60
	Offshore and distant-water LL	15	14	15	15	16
	Recreational	27	9	28	29	0
	Research LL	117	107	119	116	119
	Total	676	549	549	540	545
Swordfish	Coastal LL	4	5	5	5	4
	Driftnet	244	165	5	165	165
	Harpoon	1	1	1	1	1
	Offshore and distant-water LL	168	81	23	79	138
	Research LL	239	210	195	197	228
	Total	656	462	229	447	536

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Table 4. Summary of sex-specific biological samples for three species collected by Japan for 2019-2023.

Species	Sex	Gonad	Otolith	Dorsalfin	Analfin	Muscle
Blue marlin	Male	37	38	39	36	35
	Female	32	38	38	37	10
	Unknown	1	5	5	3	0
	Total	70	81	82	76	45
Striped marlin	Male	300	239	240	238	240
	Female	370	269	266	259	278
	Unknown	6	41	43	43	27
	Total	676	549	549	540	545
Swordfish	Male	125	95	72	88	113
	Female	521	352	145	346	407
	Unknown	10	15	12	13	16
	Total	656	462	229	447	536

Table 5. Summary of area-specific biological samples for three species collected by Japan for 2019-2023. “West” is 160 °E to west, “East” is 160 °W to east, and “Central” is the water between “West” and “East” in the NPO.

Species	Area	Gonad	Otolith	Dorsalfin	Analfin	Muscle
Blue marlin	West	38	49	49	46	12
	Central	32	32	33	30	33
	Total	70	81	82	76	45
Striped marlin	Central	50	44	49	49	50
	East	2	2	2	2	2
	West	624	503	498	489	493
	Total	676	549	549	540	545
Swordfish	Central	137	111	103	104	137
	East	38	1	0	0	38
	West	481	350	126	343	361
	Total	656	462	229	447	536

Table 6. Summary of storage way for gonad of three species collected by Japan for 2019-2023.

Species	Year	Gonad
Blue marlin	Altfix	0
	Formalin	27
	Frozen	16
	Others	27
	Total	70
Striped marlin	Altfix	2
	Formalin	577
	Frozen	68
	Others	29
	Total	676
Swordfish	Altfix	85
	Formalin	384
	Frozen	137
	Others	50
	Total	656

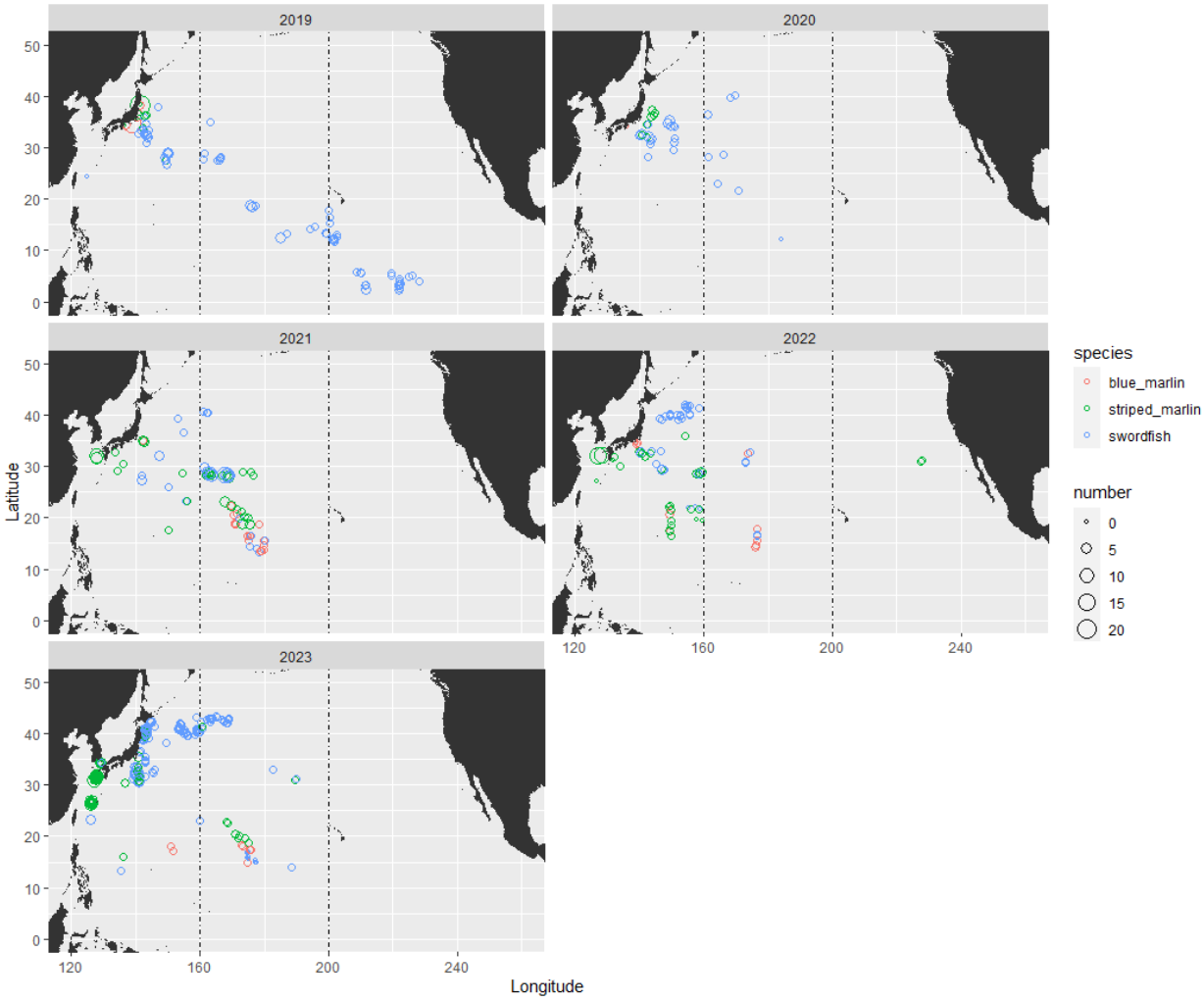
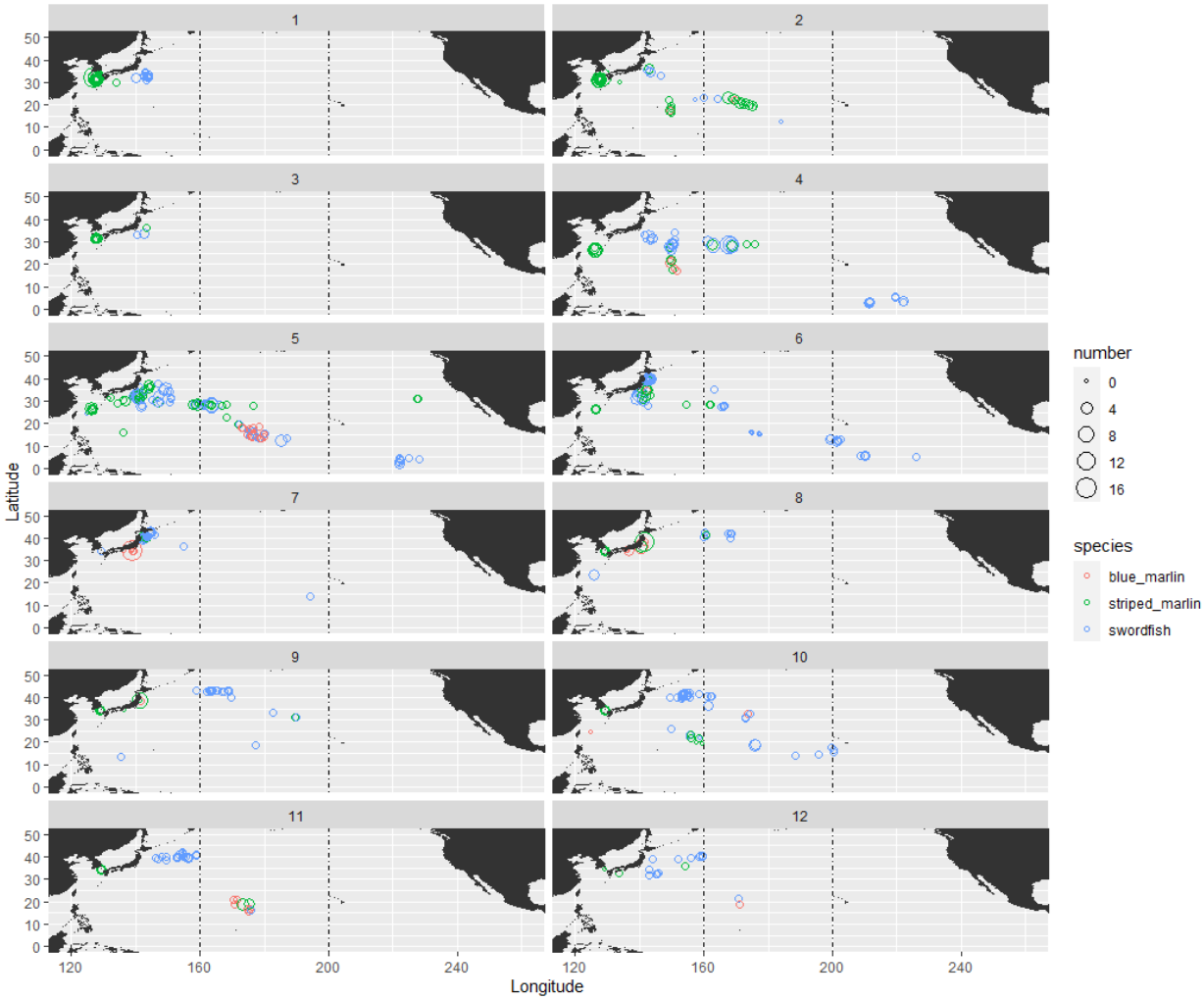


Figure 1. Annual changes in sampling locations of gonads for three species in the NPO from 2019 to 2023. Vertical broken lines denote the delimitation of three regions.



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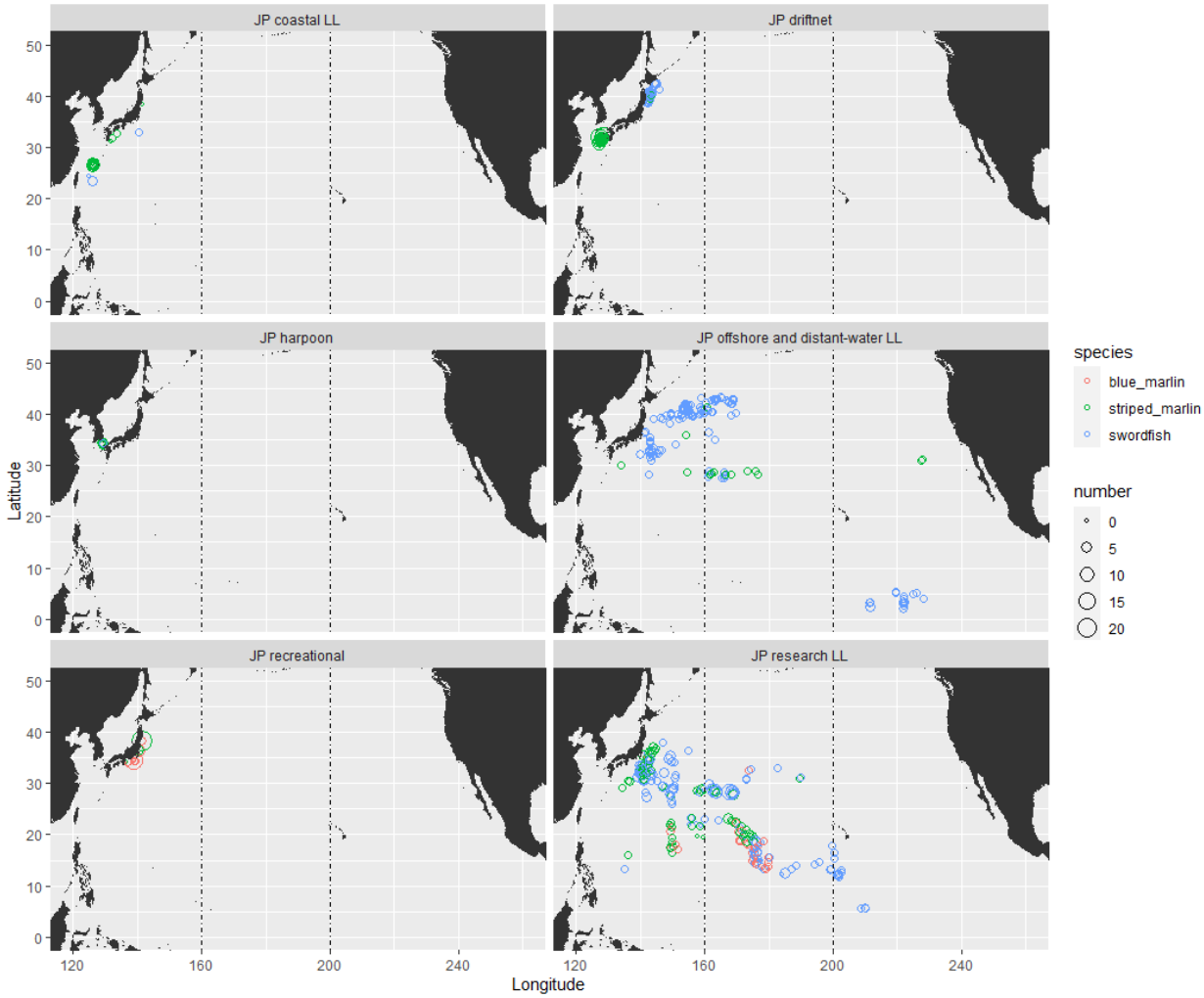
284 **Figure 2**. Monthly changes in sampling locations of gonads for three species in the NPO from
285 2019 to 2023. Vertical broken lines denote the delimitation of three regions.



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287 **Figure 3**. Gear specific sampling locations of gonads for three species in the NPO from 2019 to
288 2023. Vertical broken lines denote the delimitation of three regions.

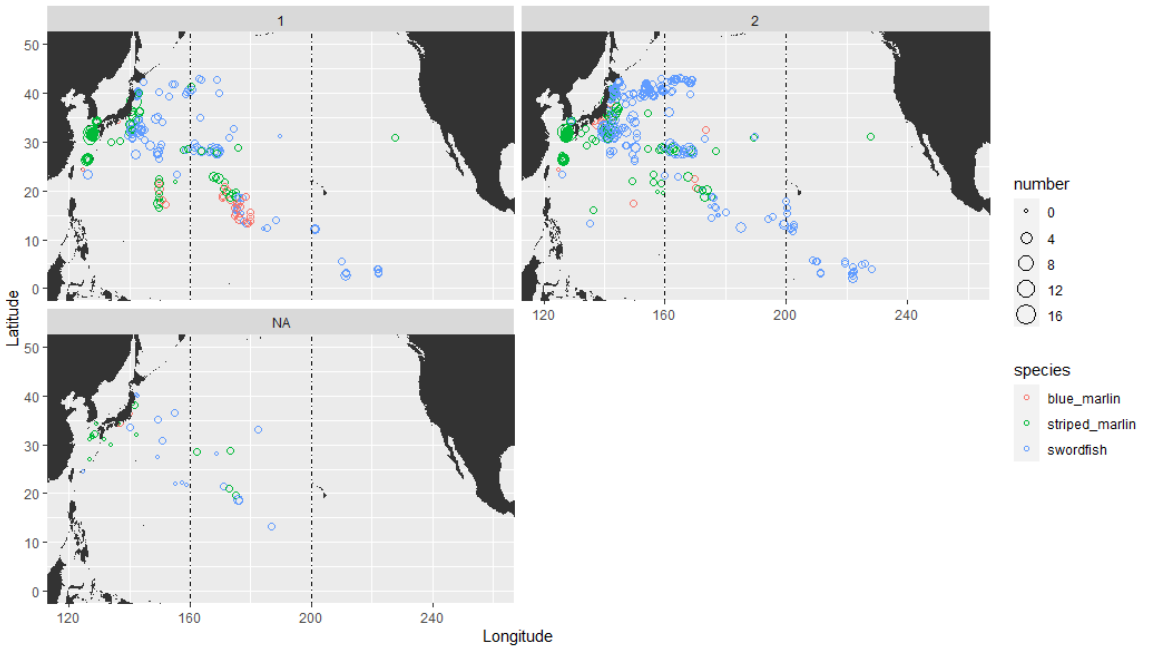


Figure 4. Sex specific sampling locations of gonads for three species in the NPO from 2019 to 2023. "1", "2", and "NA" denote male, female, and unknown, respectively. Vertical broken lines denote the delimitation of three regions.

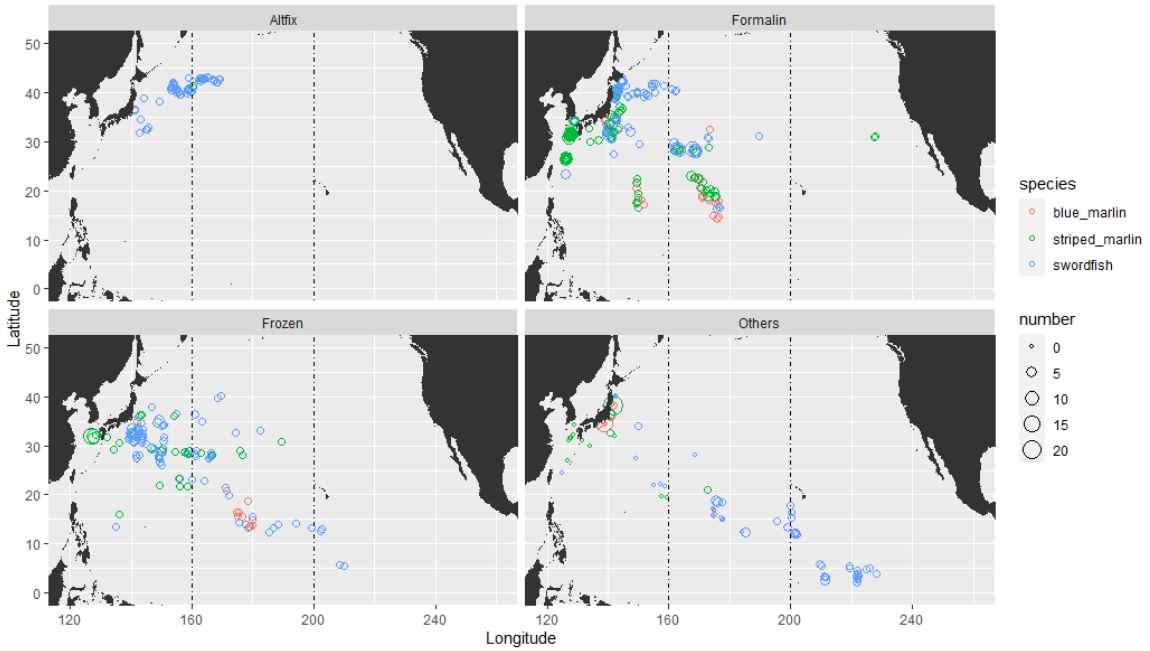
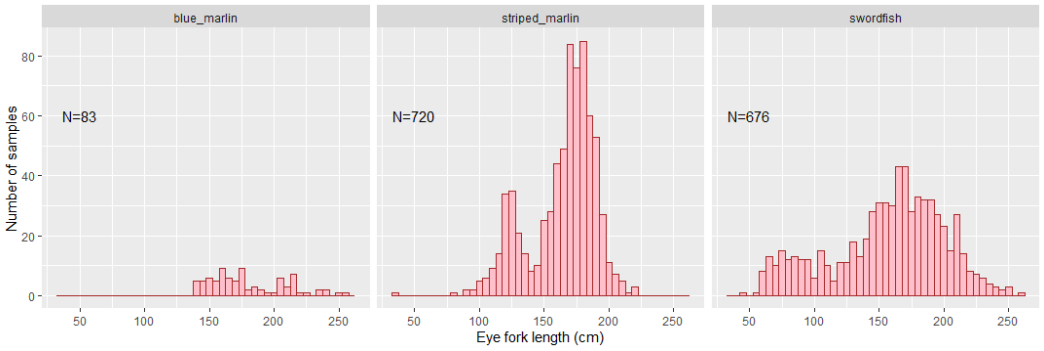


Figure 5. Sampling locations of gonads preserved by different storage ways for three species in the NPO from 2019 to 2023. Vertical broken lines denote the delimitation of three regions.



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298 **Figure 6**. Length frequency of observed number of biological samples for three species collected
299 by Japan in the north Pacific between 2019 and 2023.
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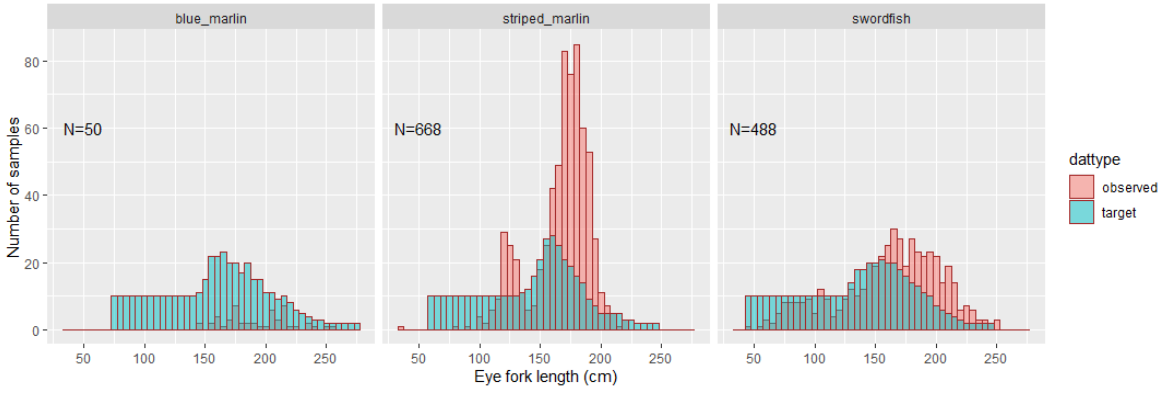

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302 **Figure 7**. Length frequency of observed and target number of biological samples for three
303 species collected by Japan in the western north Pacific between 2019 and 2023.
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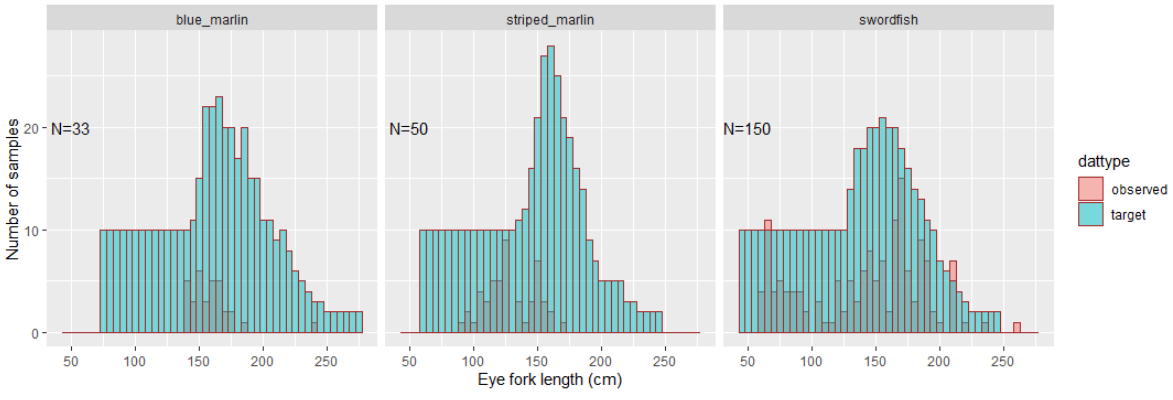

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306 **Figure 8**. Length frequency of observed and target number of biological samples for three
307 species collected by Japan in the central north Pacific between 2019 and 2023.
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Appendix

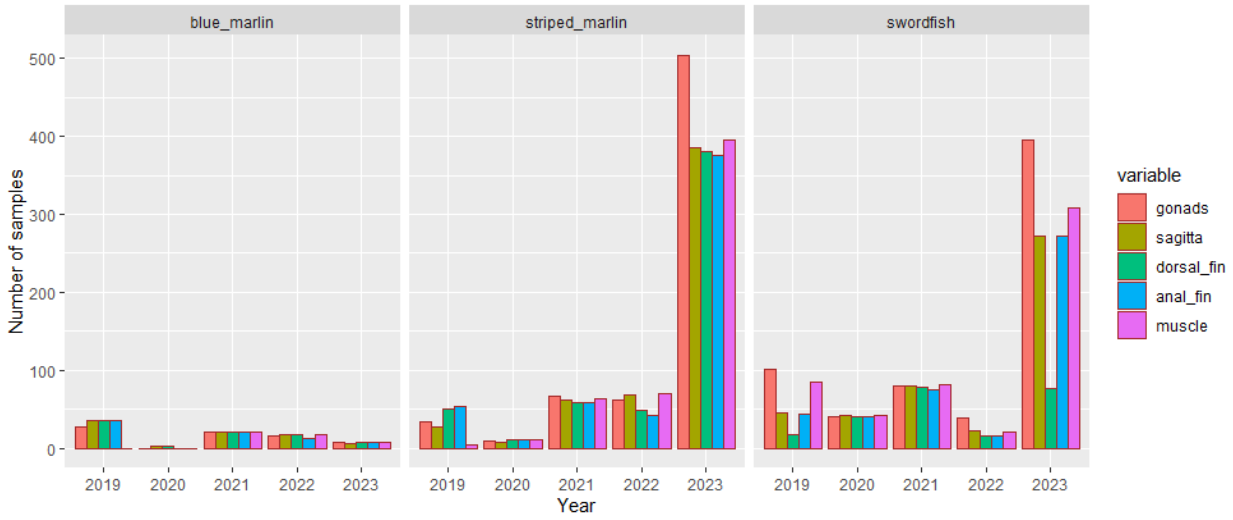


Figure A1. Annual changes in number of different types of biological samples for three species. collected by Japan in the north Pacific between 2019 and 2023.

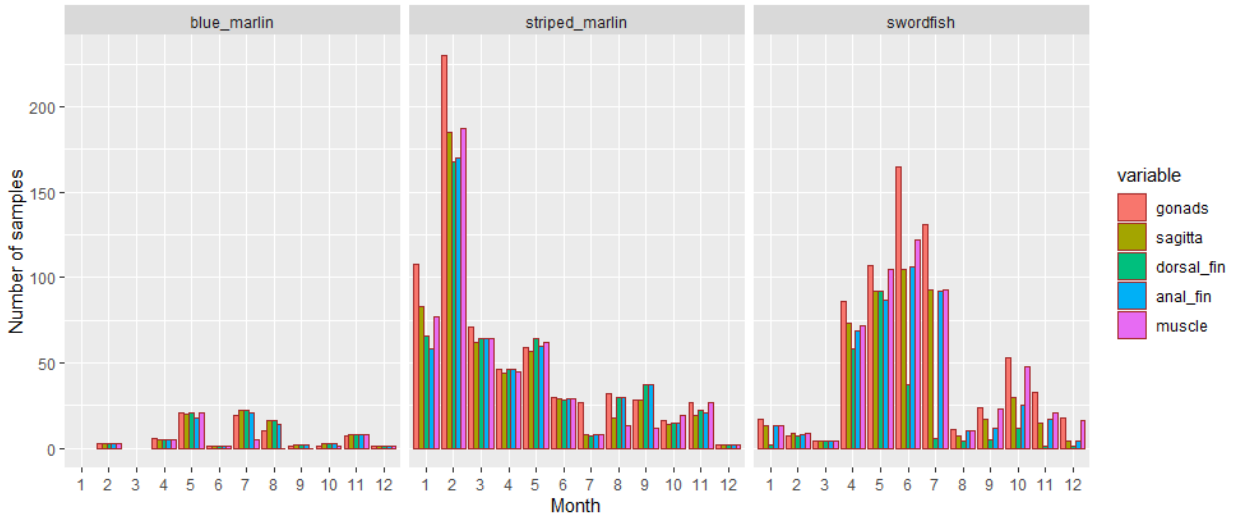
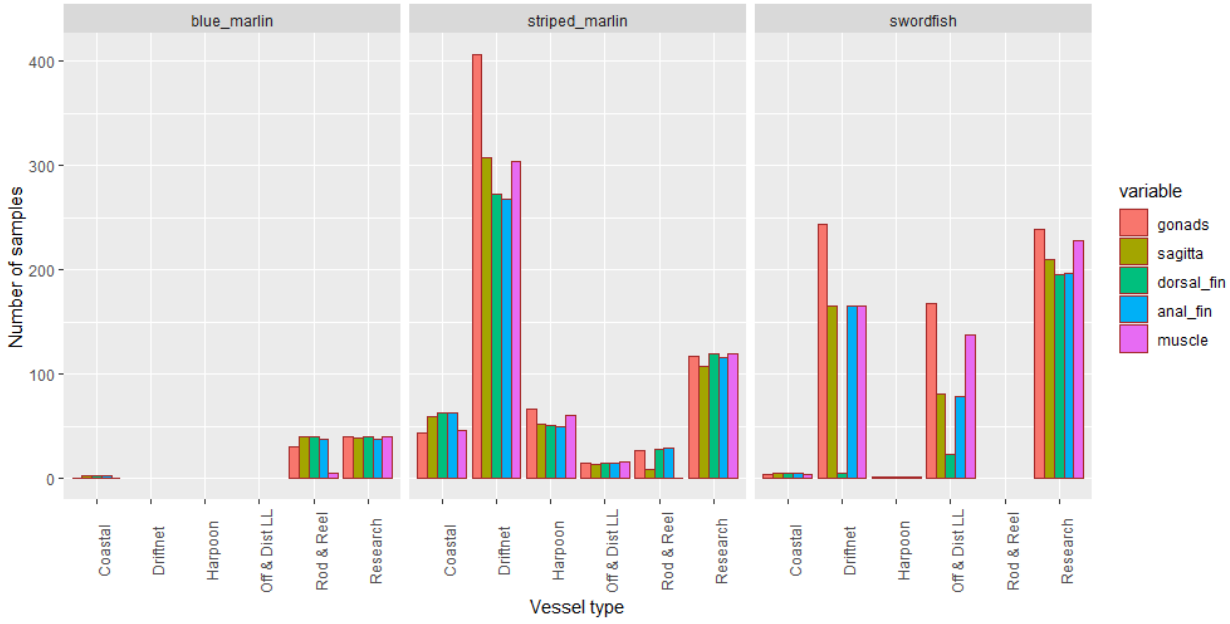


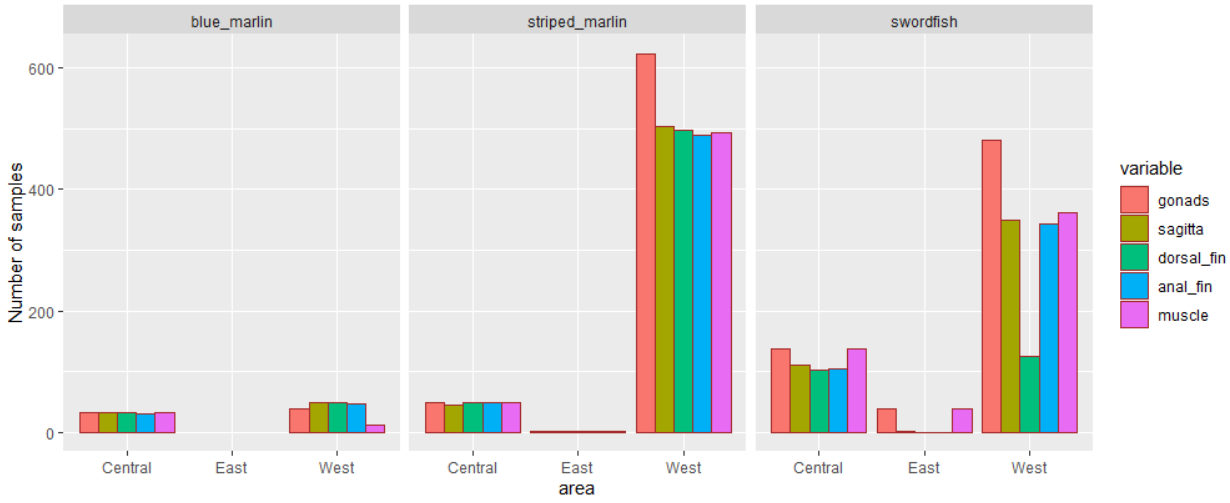
Figure A2. Monthly changes in number of different types of biological samples for three species collected by Japan in the north Pacific between 2019 and 2023.



319

320 **Figure A3**. Gear-specific number of different types of biological samples for three species
321 collected by Japan in the north Pacific between 2019 and 2023.

322



323

324 **Figure A4**. Area-specific number of different types of biological samples for three species
325 collected by Japan in the north Pacific between 2019 and 2023.

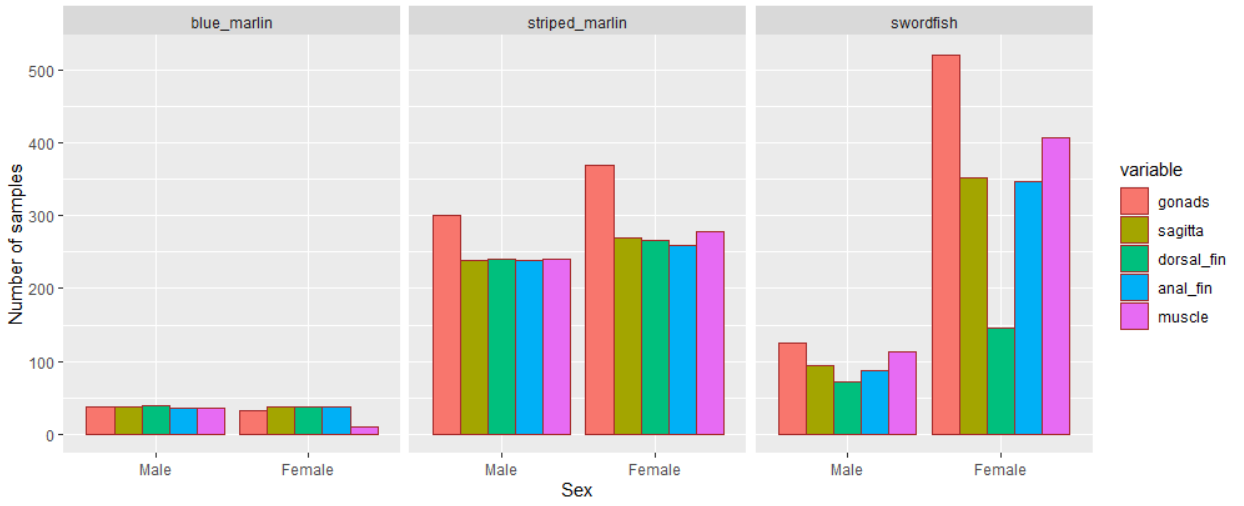


Figure A5. Sex-specific number of different types of biological samples for three species collected by Japan in the north Pacific between 2019 and 2023.

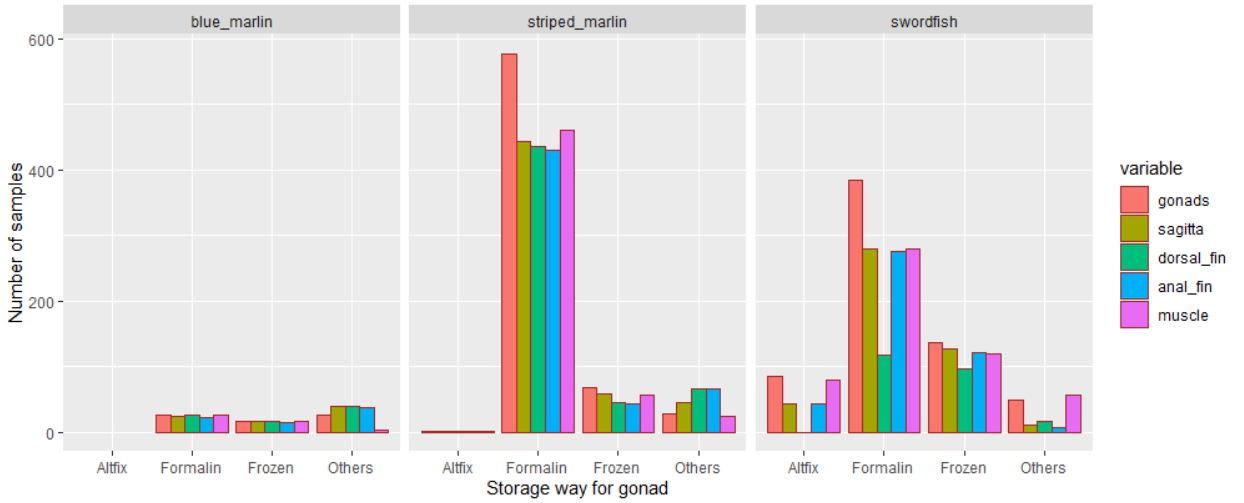


Figure A6. Number of different types of biological samples by storage way of gonad for three species collected by Japan in the north Pacific between 2019 and 2023.