

FINAL

ISC/23/ANNEX/12



ANNEX 12

*23rd Meeting of the
International Scientific Committee for Tuna
and Tuna-Like Species in the North Pacific Ocean
Kanazawa, Japan
July 12-17, 2023*

REPORT OF THE BILLFISH WORKING GROUP WORKSHOP

July 2023

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ANNEX 12

REPORT OF THE BILLFISH WORKING GROUP WORKSHOP

*International Scientific Committee for Tuna and Tuna-Like Species
in the North Pacific Ocean (ISC)*

14 - 15 December 2022

Mie University Japan

1. OPENING AND INTRODUCTION**1.1. Welcoming Remarks**

Hiroataka Ijima, the International Scientific Committee for Tuna and Tuna-Like Species in the North Pacific Ocean (ISC) Billfish Working Group (WG) chair, opened the biological study workshop for billfish species. Scientists from Chinese Taipei, Japan, United States of America (USA) participated in the meeting. The participating scientists are listed in Attachment 1.

1.2. Introduction

The WG held a face-to-face two-day meeting. The goals of the meeting were to conduct the biological workshop and share sample treatments such as the Otolith section and fin spine. The WG will also confirm the progress of the International Billfish Biological Sampling (IBBS) program.

1.3. Standard Meeting Protocols

The WG chair introduced protocols. This meeting was This workshop was conducted in face-to-face meetings at the Mie university laboratory, where the Japanese samples are processed. The WG discussed these methodologies and compiled them into a written report later.

2. ADOPTION OF AGENDA AND ASSIGNMENT OF RAPPORTEURS

Prior to the meeting, The WG adopted the draft agenda of the meeting (Attachment 2). The WG chair assigned the numbers for the working papers (WP) (Attachment 3). The rapporteurs were nominated Hiroataka Ijima and Michael Kinney.

3. NUMBERING WORKING PAPERS AND DISTRIBUTION POTENTIAL

The WG agreed to post the finalized working papers on the ISC website and make them publicly available.

4. ONLINE DATABASE

The U.S. presented the database and showed how to import data, what to do with errors, and how to identify errors. It was also reported that the progress sample collection from the WG members.

Japan asked if they could add a few new fleets to the database (Japanese training vessel fleet). Japan was asked to send an email with specific fleet names that Japan wanted to add to the database. Fleet names were shared during the meeting, and the U.S. was able to add the requested new fleets.

Following the fleet additions, Japan was able to successfully upload a small test dataset into the database. Japan agreed to upload the rest of its relevant data following the conclusion of the workshop by the end of February 2023.

Japan asked for specifics (part numbers if possible) for the containers the U.S. uses for shipping gonads, **U.S. members agreed to share this information.**

It was suggested that photos of otoliths, etc., could be shared in the database, but only metadata will be shared now because the purpose is to implement the sampling plan.

It was asked what should be done if both EFL and LJFL are measured for a single specimen. It was responded that EFL should be recorded in the database but that the LJFL measurement could be recorded in the notes section for that specimen.

The WG confirmed that sample collections are doing well. The detail of sampling for each member is as follows.

A. The sampling situation in Japan

It was noted collect fresh gonad samples from fishing vessels is difficult. Therefore, it was reported that Japanese members focus on sampling from research and training vessels.

It was noted that the sampling locations of the research and training vessels are different from the operational locations of the fishing vessels.

B. The sampling situation in Taiwan

Taiwan is collecting samples through port sampling and observers.

It was noted that port sampling is difficult to collect all three samples simultaneously. Thus, the samples are collected mainly by observers.

The WG confirmed that the sample locations are very different from those in Japan and that the collaboration has improved the spatial coverage.

The WG confirmed that samples of the northern and southern hemispheres were collected for billfishes.

It was noted that Taiwanese samples would provide valuable information on the North-South interactions of striped marlin.

The WG also indicated that the Taiwanese samples in the IBBS database would now be confined to the northern hemisphere.

C. The sampling situation in the US

Observers are sampling the U.S. Hawaii longline fleets.

Sampling is going well, there is some effort to have Mexico provide some samples however such work is still ongoing.

Most U.S. samples contain fin spines, otoliths, gonads, and muscle tissue from each individual sampled.

All US-sampled gonads are fixed in formalin in the field, with no freezing.

5. PHYSICAL SAMPLE COLLECTION AND STORAGE

5.1. Otolith Processing Guide

Japanese scientist lectured methodology of otolith processing i) how heads are collected, ii) the Otolith extraction technique, iii) Steps to visualize otoliths and counting rings, and iv) Sample storage and how samples can be shared.

Japan keeps their otoliths in small vials like the U.S. but filled with 70% ethanol. This is to prevent them from drying out and curling up when stored for long periods. It was discussed at the meeting if the 2 years or so that this project will likely run would be long enough to be concurred about curling. The group decided that it is not a major concern for this work as the otoliths will likely be processed sooner rather than later and so long-term storage is not as much of an issue. **The WG agreed that otoliths will not be processed passed cleaning and placing them in vials.**

The WG also discussed that when samples are shared (shipped or personal transport) that the ethanol will need to be replaced by DI water as ethanol creates problems when shipping. It was discussed that some kind of water (DI) would likely be best in order to provide some form of protection when transporting.

Japan makes sure to place their otoliths with the concave part facing upwards in order to avoid trapping an air bubble underneath.

The Japanese samples are very clear and free of bubbles in the epoxy. This is due to an additional step after mixing the epoxy of placing it in a small vacuum chamber that pulls all the air out and so all the bubbles out. This only takes a few minutes but does a very good job of getting bubbles out of the epoxy.

A syringe was filled with epoxy and then a Popsicle stick was used to direct the flow of epoxy into the molds containing the otolith. Japan uses plastic pillboxes to make their molds.

Japan asked the U.S. for the identifying info on the molds and the cutting blades (especially the small ones we use as spacers) so that Japan could perhaps buy some of their own.

Once set in epoxy a dissecting needle was used to score a line in the epoxy mold that could be used to line up the saw blade.

The WG noted that Japan uses only one blade and so has to make two cuts. Japanese scientists were interested in being able to use two blades at the same time like the U.S. is. **The U.S. agreed to share information about this method as they progress and more fully test the method.**

The WG discussed the strategy for daily otolith reading as the process is incredibly slow. The U.S. indicated that daily counting was planned for a subset of otoliths, with the majority being counted using annual rings. The difference is in the processing step, where daily otoliths are sectioned thinner than annual otoliths. Taiwan agreed that this was the approach they intended to use. After some discussion, Japan indicated it would attempt this strategy also.

5.2. Fin Spine Processing Guide

Japanese scientists cut dorsal and fin spines from a single individual into individual fin spines and placed them in a small net bag (almost like a hair net, but in Japan it is used as a disposable drain catch in a sink).

The WG confirmed that the spines are boiled for a few minutes until the flesh can be easily stripped off by hand or using a sponge.

The WG also confirmed that marlin's spines have a small hole in their base that a wire can be passed through to keep them together and in order.

It was indicated that swordfish spines have a tendency to split down the middle as they dry, which makes it difficult to keep them aligned properly when they are cut and mounted on slides

(hard to tell how far apart the two halves are after they split). To counteract this, Japan places a small zip-tie around the spine near the base (about 2 inches from the base). **The WG agreed to use the method of Japanese zip-tie around the spine for the Swordfish.**

Japan noted that spines are boiled a second time to make sure they are as clean as possible before being air dried for 1 or 2 days.

The WG agreed that fin spines would be boiled, cleaned, and dried.

5.3. Gonad Processing Guide

Japan informed that various thawing methods are being investigated to utilize frozen samples, and some results were reported.

The WG recognized that the Taiwanese observer sample is also frozen so that this technique can be used.

Following this information, the **WG agreed to be placed in formalin and kept cold if they had previously been frozen.** Japan is currently working on the effects of keeping frozen gonads in cold formalin to counteract some of the degradations that happen when samples are fully defrosted. More details will come from this work as it progresses.

This thawing method is still being established and will be shared with the WG after the method is officially finalized.

6. OTHER ITEMS

6.1. General Notes

The WG agreed that the three countries would develop a manual for sample processing jointly. Priority will be given to otoliths, dorsal fins, and gonads in that order.

Aging of spines was difficult for all, with several rings not being complete around the entire spine and so being counted as checks. Taiwan is attempting an AI approach to count spine rings, and **it was agreed that members would share spine and otolith photos to help train the AI approach.** It is unclear how many images will be needed to train the AI, but as single images can be altered (rotated, black and white, etc.) a smaller training set can sometimes be augmented to be more helpful.

It was reported that Taiwan had created an AI for Bluefin tuna otoliths, which could be applied to billfishes. It was noted that creating an AI could find differences in readers, and factors regarding annual ring formation can also be ascertained.

Japan also reported building an AI to determine the maturity stage of swordfish gonads.

It was reported that Japanese researchers would attempt to create a Dynamic Energy Budget (DEB) model for billfish based on the information obtained from the sampling program.

6.2. How to Share and Analyze Samples

The WG agreed that sharing the physical samples is the way to go.

All parties discussed sample sharing, and it seems the best way for otoliths and spines is to bring them to meetings. We will discuss this more at the upcoming meeting at the end of Jan., but the next WG meeting (April, 2023) is a likely time to share.

The method of processing and analyzing the samples will be left to the person in charge.

The fin spines will be handed over with the flesh removed, using the same boiling process displayed by Japan in this meeting. (In-person sample exchanges will be at future ISC meetings).

Otoliths will be removed from the head and handed over in dried condition (In person sample exchanges will be at future ISC meetings).

Gonads are to be soaked in formalin and sent by mail. **The U.S. agreed to share methods for this approach as they have been using the same approach for shipping gonad samples from the U.S. territories.**

Physical Samples will be aggregated for each species based on the nation tasked with performing the biological work on that species:

Age and Growth

1. Striped Marlin otoliths and fin spines will be aggregated and stored by Japan.
2. Blue Marlin otoliths and fin spines will be aggregated and stored by Taiwan.
3. Swordfish otoliths and fin spines will be aggregated and stored by the U.S.

Reproduction

4. Striped Marlin gonads will be aggregated and stored by the US.
5. Blue Marlin gonads will be aggregated and stored by Taiwan.
6. Swordfish gonads will be aggregated and stored by Japan.

6.3. Workshop report

The WG agreed a meeting would be held on February 1, 2023, at 9:00 (JST) to report this meeting and prepare the otolith manual.

6.4. Change of the Person in Charge in Japan

It was reported that the person in charge in Japan was changed. Mrs Yuki Ishihara is in charge of the thawing method for swordfish and marlin frozen gonads, and Dr. Hiroshi Ashida is in charge of swordfish L50. These changes will be noted in the working paper table used to denote project leads for the various IBBS projects.

It was proposed that an updated list of contact persons be shared with the WG. **The U.S. agreed to update the above-mentioned table and share it (Attachment 4).**

7. CIRCULATE WORKSHOP REPORT

The WG Chair prepared a draft of the workshop report for the IBBS projects workshop and reviewed it with the WG members via e-mail.

8. ADOPTION

The WG adjourned IBBS projects workshop at 10:26 am on 1st February 2023 (JTS). The WG Chair appreciated the participating scientists' collaboration in the IBBS projects.

APPENDIX 1. LIST OF PARTICIPANTS.

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APPENDIX 2. MEETING AGENDA.

**INTERNATIONAL SCIENTIFIC COMMITTEE FOR TUNA AND
TUNA-LIKE SPECIES IN THE NORTH PACIFIC**

**BILLFISH WORKING GROUP (BILLWG)
INTERSESSIONAL WORKSHOP ANNOUNCEMENT and AGENDA**

- Meeting Style:** Face to face meeting
- Meeting Dates:** December 14-15, 2022 10:00-17:00 (JST)
- Meeting Venue:** Mie University 1577 Kurimamachiya-cho Tsu city, Mie 514-8507 JAPAN
- Meeting Goals:** The ISC BILLWG will conduct the biological workshop and share sample treatments such as the Otolith section.
- Meeting Attendance:** Please respond to Hirotaka Ijima (Email: ijima@affrc.go.jp) if you plan on attending this meeting
- Working Papers:** Submit working papers to Hirotaka Ijima by November 30th.
- BILLWG Contact:** Hirotaka Ijima (Ph.D, ISC BILLWG Chair)
Highly Migratory Resources Division, Fisheries Stock
Assessment Center, Fisheries Resources Institute (FRI), Japan
Fisheries Research and Education Agency. 2-12-4 Fukuura,
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DRAFT AGENDA**December 14th (Wednesday), 10:00 - 17:00 (JST)**

1. Opening of Billfish Working Group (BILLWG) workshop
 - a. Welcoming remarks
 - b. Introductions
 - c. Standard meeting protocols
2. Adoption of agenda and assignment of rapporteurs
3. Numbering working papers and distribution potential
4. Online Database
 - a. Basics of login and navigation
 - b. Data entry
 - c. Batch data entry
 - d. Searching and editing the database
5. Physical Sample Collection and Storage
 - e. Otolith processing guide
 - i. How heads are collected
 - ii. Otolith extraction technique
 - iii. Steps to visualize otoliths and counting rings
 - iv. Sample storage and how samples can be shared
 - f. Fin spine processing guide
 - i. How fin spines are collected, cleaned, and sectioned
 - ii. Steps to visualize fin spine sections and counting rings
 - iii. Sample storage and how samples can be shared
 - g. Gonad Processing guide
 - i. How gonads are collected and stored
 - ii. Example of gonad processing and staging
 - iii. Sample storage and how samples can be shared
 - h. DNA processing guide
 - i. How muscle is collected and stored
 - ii. Sample storage and how samples can be shared

December 15th (Thursday), 10:00 - 17:00 (JST)

5. Physical Sample Collection and Storage
 - e. Otolith processing guide
 - i. How heads are collected
 - ii. Otolith extraction technique
 - iii. Steps to visualize otoliths and counting rings
 - iv. Sample storage and how samples can be shared
 - f. Fin spine processing guide
 - i. How fin spines are collected, cleaned, and sectioned
 - ii. Steps to visualize fin spine sections and counting rings
 - iii. Sample storage and how samples can be shared
 - g. Gonad Processing guide
 - i. How gonads are collected and stored
 - ii. Example of gonad processing and staging
 - iii. Sample storage and how samples can be shared
 - h. DNA processing guide
 - i. How muscle is collected and stored
 - ii. Sample storage and how samples can be shared
6. Circulate workshop report
7. Adoption

APPENDIX 3. THE LIST OF WORKING PAPERS AND PRESENTATIONS.

ISC/22/BILLWG-04/01 Length-Based Proportional Sampling for Life History
Research: Establishing Uniform Sampling for North Pacific
Billfish Species. Michael J. Kinney¹, Yi-Jay Chang, Hirotaka
Ijima, Minoru Kanaiwa⁴, Eva Schemmel¹, Joseph O'Malley

APPENDIX 4.

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 L_{inf}	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 L_{inf}	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)