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Updated sex composition data for biological samples of North Pacific albacore tuna from Japan

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Abstract

This document provides updated sex composition data for North Pacific Albacore tuna (*Thunnus alalunga*) biological samples collected by Japan for upcoming 2026 stock assessment. Samples were collected from commercial, research and training vessels, covering the western central Pacific Ocean. Sex determination for the total of 3,352 individuals utilized conventional macroscopic and histological observation, supplemented by a highly accurate gene-based quantitative PCR (qPCR) method for small or immature gonads. The analysis yielded an overall female proportion of 0.32 (1,024 females / 2,177 males), consistent with recent findings from the previous report. Significant sexual dimorphism was evident: mean fork length (FL) was 99.6 cm for males and 89.7 cm for females, and individuals larger than 100 cm FL were predominantly male (female contribution less than 20 %). Geographically, males were dominant south of 25N. These comprehensive sex data, stratified by size and area, are expected to contribute to enhancing the precision and biological realism of the North Pacific Albacore stock assessment.

Introduction

The ISC Albacore Working Group has considered the necessity of incorporating sex-specific data into the stock assessment model for North Pacific Albacore (ISC, 2025). This is crucial because albacore exhibits sexual dimorphism in growth and potentially in migration and distribution patterns. This document provides the updated sex composition data collected from North Pacific Albacore by Japan. The data, including sex ratios stratified by length, area, and season, are essential for addressing the biological assumptions and improving the accuracy and robustness of the stock assessment.

Materials and Methods

Sample collection

Gonad and muscle samples for the sex determination of the North Pacific albacore have been collected from fish caught by Japanese commercial (C/V), research (R/V) and training vessels (T/V). The geographic area of the collected samples was mainly in the western central Pacific Ocean, covering the areas defined in the previous stock assessment, except for area 5. Samples by T/V were from the central Pacific area (around 170-180E) where the C/V fleet has not recently operated. The R/V collected samples from eastern and southern areas where the C/V is not operated in recent years. Therefore, the number of samples were concentrated in the southern area and around Japan. Figure 3 shows the spatial distribution of the mean fork length (FL) of fish that were sampled. Large fish were mainly collected from the southern area (<30N, Areas 2 and 4), while smaller fish were mainly collected from the northern area (>30N, Area 3). The fish from Area 4 were generally larger, though the number of samples from this area was relatively low. The annual number of samples analyzed by vessel type is shown in Figure 1, and the spatial distribution of sampling areas is shown in Figure 2.

Sex Determination Methods

Conventional and Histological Observation

For adult albacore with larger gonads, sex was visually determined, where ovaries (female) were identified by a granular surface and well-developed blood vessels, whereas testes (male) were characterized as smooth and whitish. For smaller gonads from immature albacore, sectioning allowed identification of an ovarian cavity (female) or a thin sperm duct (male). Immature gonads that remained undetermined were sexed using histological sectioning and microscopic observation of gonad tissues.

Gene-based sex determination (qPCR)

When conventional observation was not possible, particularly for extremely small gonads, a newly applied gene-based quantitative PCR (qPCR) method was employed. Although the sex determination marker for Pacific bluefin tuna (Suda *et al*., 2019) has been applied to albacore (Chiba *et al*., 2021), we confirmed the gene-based method by comparing the results of this PCR analysis using muscle DNA with those of conventional sex determination (accuracy: 100%, n = 28, unpublished data). Consequently, DNA-based sex determination was performed on individuals from which only muscle samples were available. Albacore DNA was extracted from muscle samples preserved in ethanol.

The cording sites of two genes, *geneA* and *geneB*, are constructed at 927 bp with 92% identity in these sequences. The *geneA* is male specific gene while the *geneB* is commonly coded in both sexes. Primer sets were designed at the same location of both genes for amplifying 123 bp (unpublished data). In this region, two TaqMan probes (FAM and Yakima-yellow) were designed to be distinct between both genes. PCR amplification of target genes was performed in a 20 µL reaction mixture containing 1 µL of DNA, 1× TaqPath™ ProAmp™ Master Mix (Thermo Fisher Scientific), and 0.5 µM of each primer and of each probe. Quantitative PCR (qPCR) was conducted using a QuantStudio 3 system (Thermo Fisher Scientific) with the following cycling conditions: an initial denaturation step at 95 ℃ for 20 s, followed by 40 cycles of 95 ℃ for 3 s, and 60 ℃ for 20 s. This analysis determines as male individual by detection of *geneA* fluorescence signal, and *geneB* fluorescence signal indicates as positive control.

Results and Discussions

Gonads were visually identified in n = 3,251 individuals, comprising 987 females and 2,113 males. The gene-based method enabled sex determination for 101 individuals that were indeterminate macroscopically. Among these, 64 were identified as males (*geneA* detected) and 37 as females (only *geneB* detected). Table 1 summarizes the sexed individuals by each method. Aggregating the results, the total sample size was n = 3,352, consisting of 1,024 females and 2,177 males. The overall female proportion (sex ratio) was calculated as females / (females + males) = 0.32, which aligns closely with the results obtained in the U.S. (Craig *et al.*, 2025). Significant sexual size dimorphism was noted that the mean fork length was 89.7 cm in females

and 99.6 cm in males.

Sex rations by area and season

Area-specific patterns in sex ratio (female proportion) exhibited substantial variation, as detailed in Table 2. Area 3 displayed an almost equal sex ratio (female proportion = 0.46), whereas Area 4 demonstrated a strong male bias (female proportion = 0.18). Seasonally, females were slightly more prevalent during the second and third quarters (Q2 = 0.40, Q3 = 0.38), as also summarized in Table 2.

Size-specific and Spatial sex composition

Figure 5 shows the pronounced size-specific dimorphism, as individuals greater than 100 cm FL were predominantly male, with female contribution to these size classes being less than 20%. This observation is consistent with previously reported findings on albacore growth (Chen *et al.*, 2010). Figure 6 further stratifies this pattern by the stock assessment area, showing that while size-specific dimorphism is universal, larger females (>100 cm) were particularly collected in Areas 2 and 4. In terms of geographic distribution, males were dominant in the southern areas, south of 25N, while females were relatively more abundant in the northern areas. Histological observations suggested that one of the estimated spawning areas could be located near 20N, 150E (Ashida *et al.*, 2020), where males outnumbered females (female proportion = 0.3). Conversely, Japanese coastal waters were primarily dominated by immature fish. The collection of spawning/spawning capable fish was concentrated during quarter 2 and 3 (Tsuda *et al.*, 2025). These comprehensive sex data, stratified by size and area, are expected to contribute to enhancing the precision and biological realism of the North Pacific albacore stock assessment.

References

Ashida, H., Gosho, T., Watanabe, K., Okazaki. M., Tanabe. T, Uosaki, K. (2020). Reproductive traits and seasonal variations in the spawning activity of female albacore, *Thunnus alalunga*, in the subtropical western North Pacific Ocean. *J. Sea Res.* 160: 101902.

Chen, KS., Crone, PR. and Hsu, CC. (2020). Reproductive biology of albacore *Thunnus alalunga*. *J. Fish. Biol.* 77: 119-136.

Chiba, SN., Ohashi, S., Tanaka, F., Suda, A., and others, Effectiveness and potential application of sex-identification DNA markers in tunas. (2021). *Mar. Ecol. Prog. Ser.* 659:175-184.

Craig, M., Andrew, M., Tsuda, Y., Chen, C.-Y., O'Malley, J., and Hyde, J. R., Estimating sex ratio in North Pacific Albacore (*Thunnus alalunga*) Using Genetic Methods. (2025). ISC/25/ALBWG-01/08.

Suda, A., Nishiki, I., Iwasaki, Y., Matsuura, A., Akita, T., Suzuki, N., Fujiwara, A., (2019). Improvement of the Pacific bluefin tuna (*Thunnus orientalis*) reference genome and development of male-specific DNA markers. *Sci. Rep.* 9, 14450.

Tsuda, Y., Yokoyama, N., Matsubara N., Ijima H., Aoki, Y. (2025). Review of the biological data

for the North pacific albacore tuna from Japan. ISC/25/ALBWG-01/12.
ISC (2025) Report of the Albacore Working Group Workshop. ISC/25/ANNEX/04.

Table 1. Number of North Pacific albacore individuals successfully sex-identified by each determination method.

Methods	Total	Female	Male	unknown
Conventional observation methods	3,251	987	2,113	151
Gene-based methods	101	37	64	0

Table 2. Sex ratio (female proportion) of North Pacific albacore stratified by the stock assessment area and quarter. Sex ratio is shown as: females / (females + males). The number of samples for female and male are in each parenthesis.

	Quarter 1 Sex ratio (females, males)	**Quarter 2** Sex ratio (females, males)	**Quarter 3** Sex ratio (females, males)	**Quarter 4** Sex ratio (females, males)	**All Quarter** Sex ratio (females, males)
Area 1	**0.36** (35, 61)	**0.42** (25, 34)	-	**0** (0, 3)	**0.38** (60, 98)
Area 2	**0.47** (136, 153)	**0.40** (136, 201)	**0.36** (152, 269)	**0.28** (146, 379)	**0.36** (570, 1,002)
Area 3	**0.48** (133, 143)	**0.39** (47, 73)	**0.48** (29, 32)	**0.40** (2, 3)	**0.46** (211, 251)
Area 4	**0.18** (183, 826)	-	-	-	**0.18** (183, 826)
All Area	**0.29** (487, 1,183)	**0.40** (208, 308)	**0.38** (181, 301)	**0.28** (148, 385)	**0.32** (1,024, 2,177)

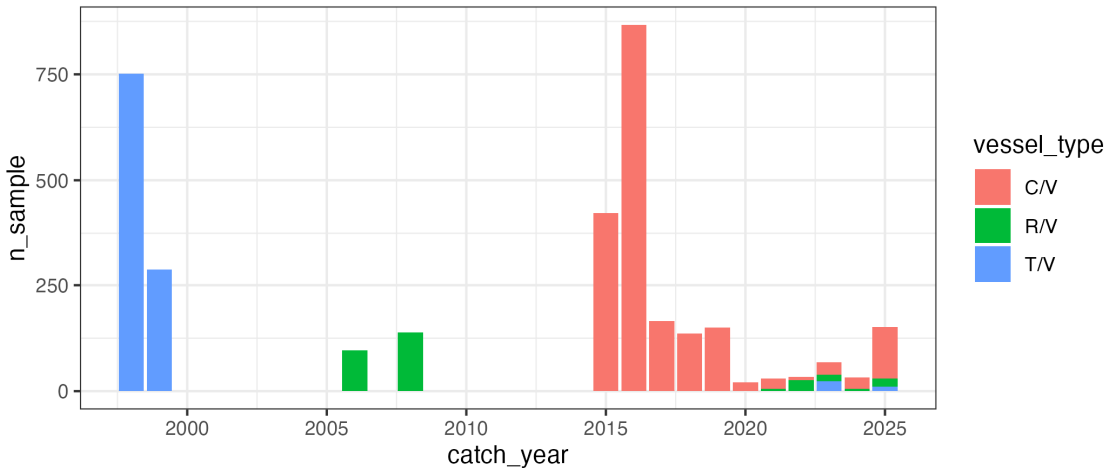

Figure 1. Annual number of North Pacific albacore samples analyzed for sex determination by vessel type (Commercial vessels (C/V), Research vessels (R/V), and Training vessels (T/V))

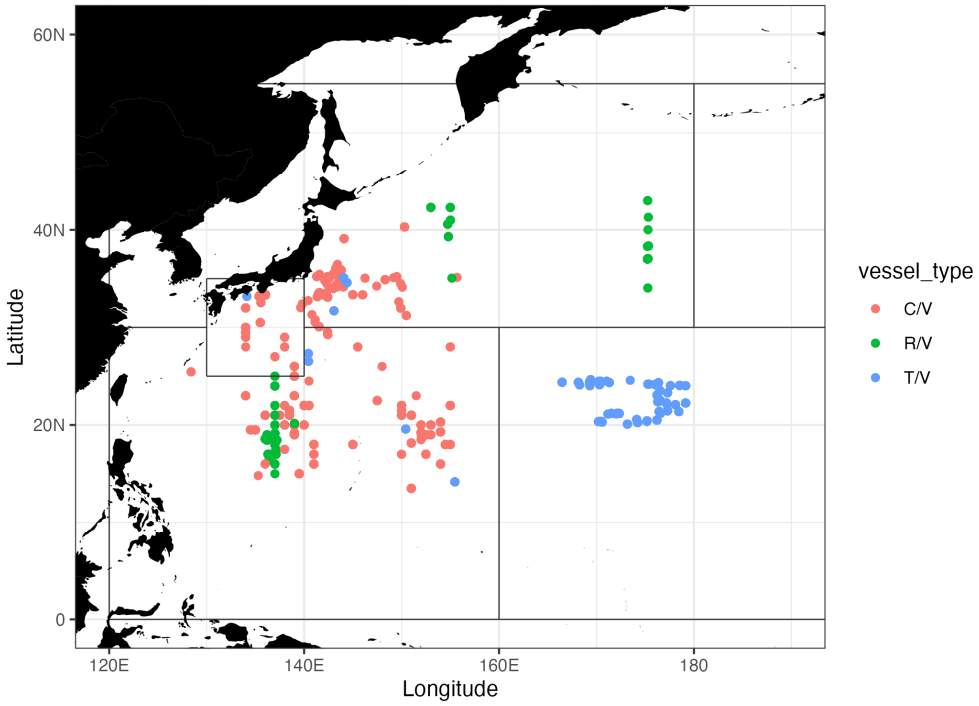

Figure 2. Spatial distribution of sampling locations by vessel type in the Noth Pacific Ocean.

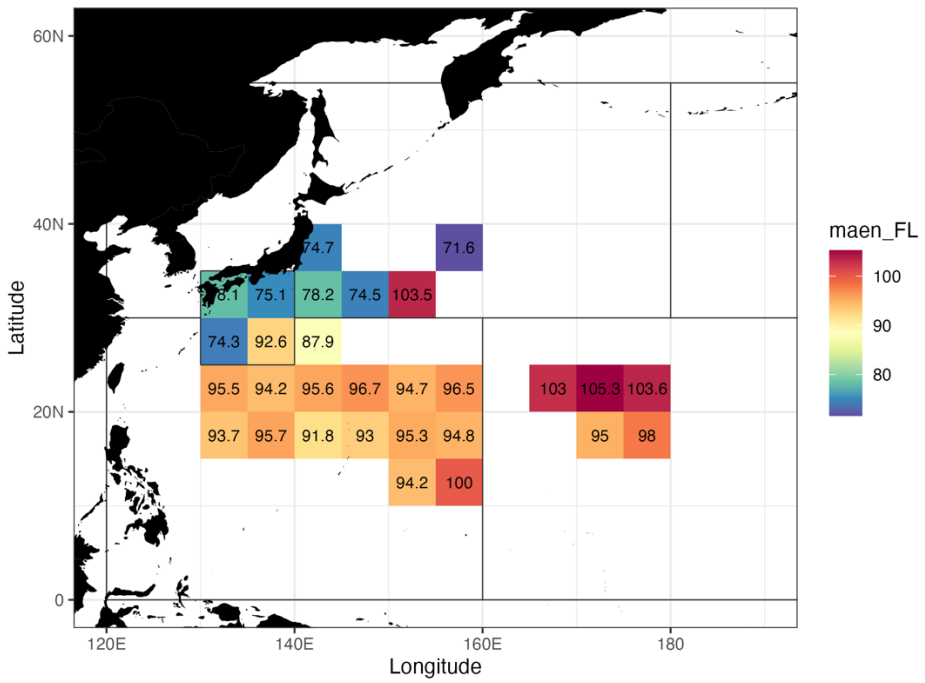

Figure 3. Mean fork length (FL, cm) of sampled North Pacific albacore stratified by 5x5 latitude-longitude grid. The number in the cell indicated the mean FL.

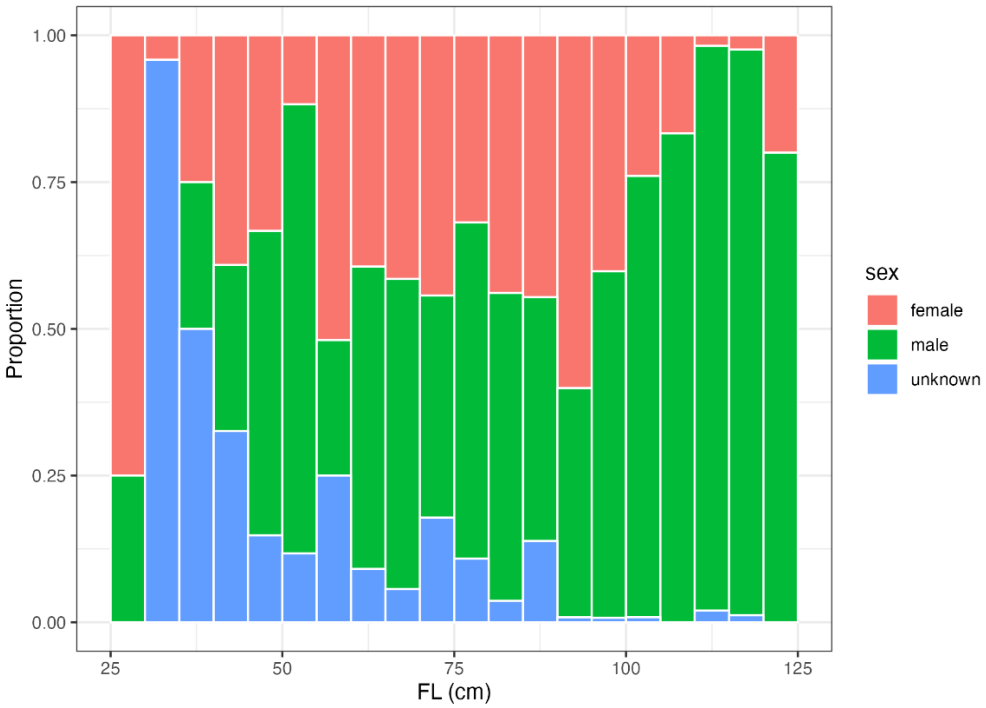

Figure 5. Proportion of female, male, and sex-unknown North Pacific albacore by fork length (FL, cm) class.

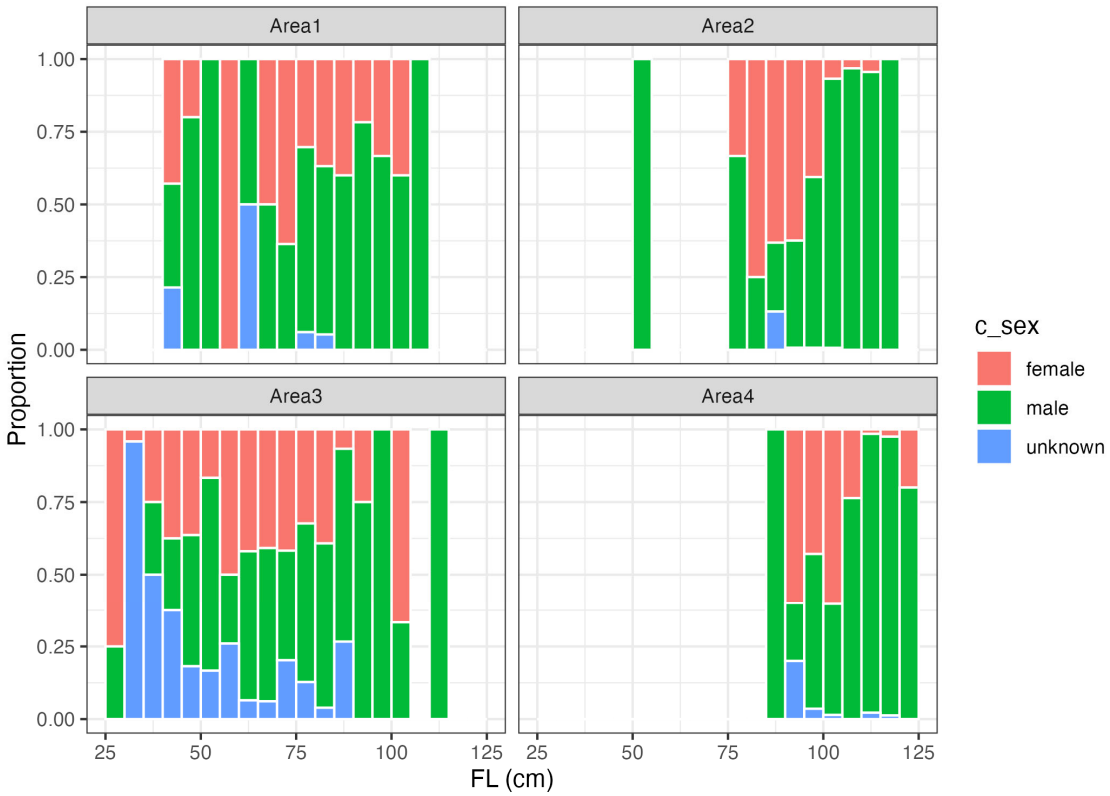

Figure 6. Proportion of female, male, and sex-unknown North Pacific albacore by fork length (FL, cm) class, stratified by the stock assessment areas.