Gonadal information and histological analysis of southern bluefin tuna collected by Taiwanese scientific observer program with updated data

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ABSTRACT

Gonadal samples of SBT were collected by Taiwanese scientific observer project operating in the Indian Ocean. Over 1,600 samples were collected from 2010 to 2025, including 781 females and 871 males. The fork lengths of both sexes primarily ranged from 90 to 150 cm. The variation of the monthly gonado-somatic index (GSI) showed that highest peaked in April and gradually declined thereafter, with elevated values observed from May to July in female. In males, GSI peaked in March and then decreased steadily. Overall, the monthly GSI patterns for both sexes were consistent with updated data.

According to the histological observations, we process the SBT samples for both sexes. There were 1085 histological SBT samples collected from 2010 to 2024 including 584 females and 501 males were classified by sexual maturity stage. The majority of these samples were diagnosed as immature status for both sexes, while approximately 14.9% in females and 8.6% in males were identified as mature. And most of mature individuals were reproductively inactive. Also, most of the mature females were diagnosed to be in the regressed or regenerating stage (Stage 7) during the period from April to August.

1. INTRODUCTION

Multiple biological indices of southern bluefin tuna (SBT, *Thunnus maccoyii*) had been investigated in the waters off Australia such as age-at-first-maturity, gonad index, ovary size-frequency and fecundity (Thorogood, 1986). Also, several studies related to the reproductive biology of SBT were conducted in the waters of Australia and Indian

Ocean. The collection of ovaries from the spawning ground and the main feeding ground were obtained for understanding the spawning dynamics of SBT in the Indian Ocean (Farley and Davis, 1998). Furthermore, the maturity of SBT had been investigated using histological observations of the gonad samples in the southwest Indian Ocean (Chen et al., 2013). However, there is no consensus standard for identifying the maturity stages in SBT. Developing the guidelines of the diagnosis maturity stages will be helpful for further investigating of the reproductive indices of SBT with broadly coverage of sample distribution.

Therefore, developing an independently estimated maturity criteria for SBT was supported by the ESC, and also listed as a high priority in the Scientific Research Plan (SRP) since 2015 (Farley et al., 2013a, 2014). In order to enhance the integrity of the reproductive studies and ensure the continuity of the project, we conducted the biological sample collection of SBT as an essential objective in the Taiwanese scientific observer program annually. Here, we presented the results of monthly GSI variation, histological analyses and the maturity status of SBT collected by Taiwanese scientific observer program.

2. MATERIALS AND METHODS

GSI calculation

The SBT gonad samples were collected from the operational fleets in the Indian Ocean by Taiwanese scientific observer program. The biological information, such as the fork length, body weight, sex, sampling date and location, was recorded for each specimen.

Here, for the calculation of gonado-somatic index (GSI), we adopted a length-based formula for calculating the GSI (Chen et al., 2013):

$$GSI = \frac{GW}{L^3} \times 10^4$$

where GSI is the gonado-somatic index, GW is the weight (g) of gonad and L is the fork length (cm) of each specimen.

Histology Section and Maturity status

For the histological experiments, the gonad samples were preserved in 10% buffered formalin for fixing tissue as first step. Further histological processes were conducted and stained using Harris' hematoxylin and eosin to prepare histological slides for investigating the reproductive characters. We performed histological classification to identify the maturity stages of the gonad samples for both sexes.

The criteria for diagnosing the gonadal developmental stages of SBT require further development; therefore, we adopted the classification criteria proposed by Farley et al.

(2013b), originally developed for albacore in the southern Pacific Ocean. This system was applied to categorize the developmental stages of SBT gonads into seven stages:
1. Immature; 2. Developing; 3. Spawning capable; 4. Spawning;
5. Regressing – potentially reproductive; 6. Regressed; 7. Regenerating
Individuals are identified sexually mature while these specimens most advanced oocytes reach stage 3 or higher. Within these mature status, stages 3 and 4 indicate reproductively active status, while stages 1–2 and 5–7 denote a reproductively inactive state. Detailed histological criteria for each developmental stage are summarized by Farley et al. in Table 1. (2013b).

3. RESULTS AND DISCUSSION

From 2010 to 2025, we collected 1,652 gonad samples of southern bluefin tuna (SBT) between March and September annually. The collection comprised 781 females and 871 males, primarily measuring between 90 and 150 cm in fork length in both sexes. The range of fork length of female and male samples were ranged around 65~180 cm and 60~195 cm, respectively. The sampling area were distributed around 30°E-110°E in longitude and 29°S-42°S in latitude in the south Indian Ocean (Fig. 1 and Fig.2).

First, for the GSI calculation part, both male and female specimens showed larger gonad weights as fork length increased, with the wider diversity occurring in individuals larger than 150 cm. (Fig. 3). The relationship between fork length and the gonadosomatic index (GSI) for both sexes was observed similarly that the GSI values generally increasing as fork length increased. However, some individuals exhibited relatively low GSI values that did not follow this trend. And most of the GSI values in males were lower than 0.5 (Fig. 4). These deviations may be associated with the reproductive maturity status of the samples and warrant further investigation.

In order to investigate the monthly average variation trend of GSI and gonadal maturity status, we calculated the mean GSI of each month for both sexes. In females, the highest of mean GSI values peaked in April and remained elevated through July, followed by a declining trend that reached the lowest levels in September. And in males, the maximum value was revealed in March, and then gradually declined, reaching a minimum in September based on updated data through 2025 (Fig. 5). These monthly average variation trends of GSI for both sexes were consistent with those trends observed in previous years. However, due to the temporal coverage of sample collection limited to March through September, as a result of the seasonal operation of the Taiwanese SBT longline fishery in the Indian Ocean, the annual GSI cycle could not be fully characterized (Fig. 5).

Second, for the histological section and maturity status part, some samples were

excluded due to inadequate preservation, primarily resulting from the difficulties associated with processing frozen specimens. Here, we had processed 1,085 gonad samples including 584 females and 501 males which were collected between 2010 and 2024 for histological diagnoses (Fig. 6). Sexual maturity stages of the samples were examined based on the developmental stage criteria outlined by Farley et al. (2013b). Histological diagnosis revealed that the majority of individuals for both sexes were identified as being in the immature stage (Stage1) and the developing stage (Stage2). Approximately 12.0% of the samples of females and males were classified as mature but in reproductively inactive phases, specifically the regressed or regenerating stages. Only 1.6% of the samples were at active phases (Histological slides of females and males were shown in Fig. 7 and Fig. 8).

Furthermore, the proportion of maturity stages was analyzed across 5 cm fork length intervals for both sexes (females in Fig. 9 and males in Fig.10). The majority of female gonadal samples based on data updated through 2024 were classified as immature. The smallest fork lengths recorded for mature female and male were 97 and 93 cm, respectively. With these specimens were identified as stage 3 for both sexes. Although both gonad weight (Fig. 11) and GSI (Fig. 12) exhibited a gradual, overlapping increase with fork length, we could not establish a distinct threshold to reliably distinguish mature individuals from immature ones. Considerable overlap was observed in fork length, gonad weight, and GSI values between immature and mature specimens. However, individuals with fork lengths less than approximately 90 cm were exclusively immature, with no overlap detected in this size class.

We also demonstrated the monthly distribution of gonadal maturity developmental stages from March to September and found that most mature females and males were in a reproductively inactive status (Fig. 13), and the immature individuals were distributed throughout the entire SBT fishing season from March to September. For the mature females were primarily diagnosed as being in regressed (Stage 6) or regenerating (Stage 7) phases, predominantly from April to August. For the mature males, the majority of individuals were classified as being in inactive phases (stage 5 and stage 7) during March to August. These findings suggest that mature SBT may migrate into the Taiwanese longline fishing grounds in the Indian Ocean after completing reproductive activity elsewhere.

To improve the spatial and temporal coverage outside the Taiwanese SBT longline fishery fishing season from March to September, we collaborated with other researchers from CSIRO and provided the information to the SRP of CCSBT. It will be helpful to improve histological criteria for gonadal maturity stage and the annual reproductive cycle, and also advance the study of SBT reproductive biology.

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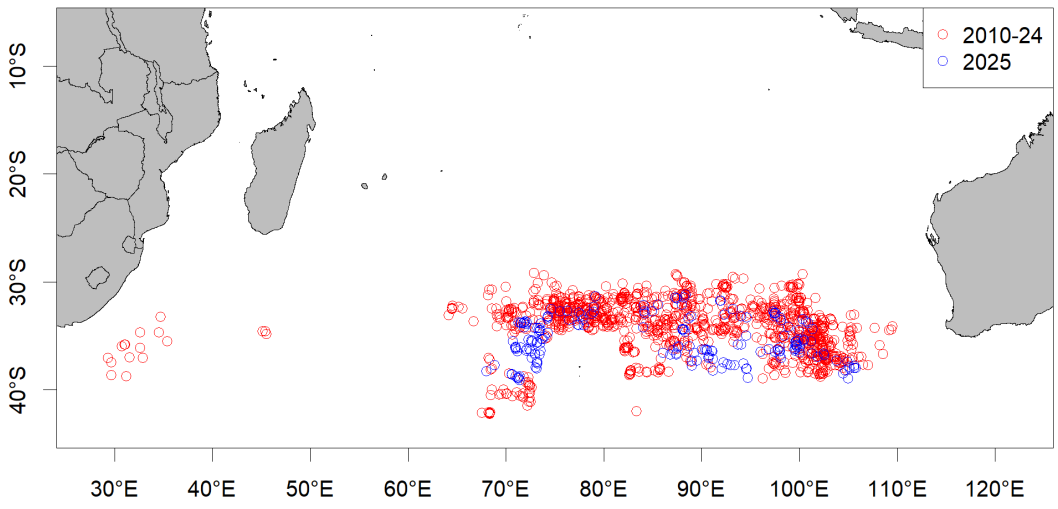


Fig. 1. Sampling locations of southern bluefin tuna collected by Taiwanese scientific observer program from 2010 to 2025.

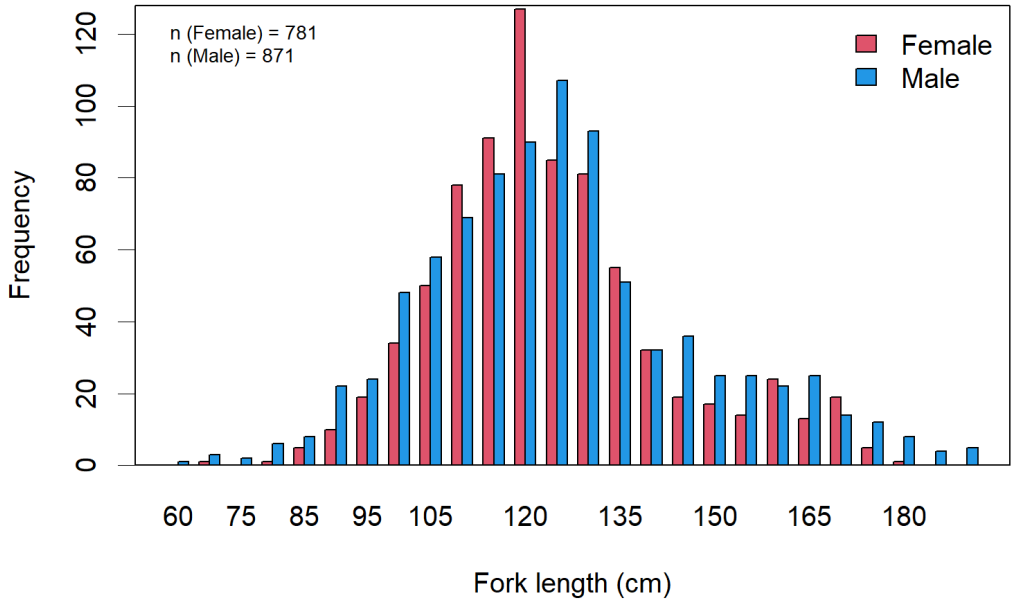


Fig. 2. Length frequency distributions for gonad samples of southern bluefin tuna collected by Taiwanese scientific observer program from 2010 to 2025.

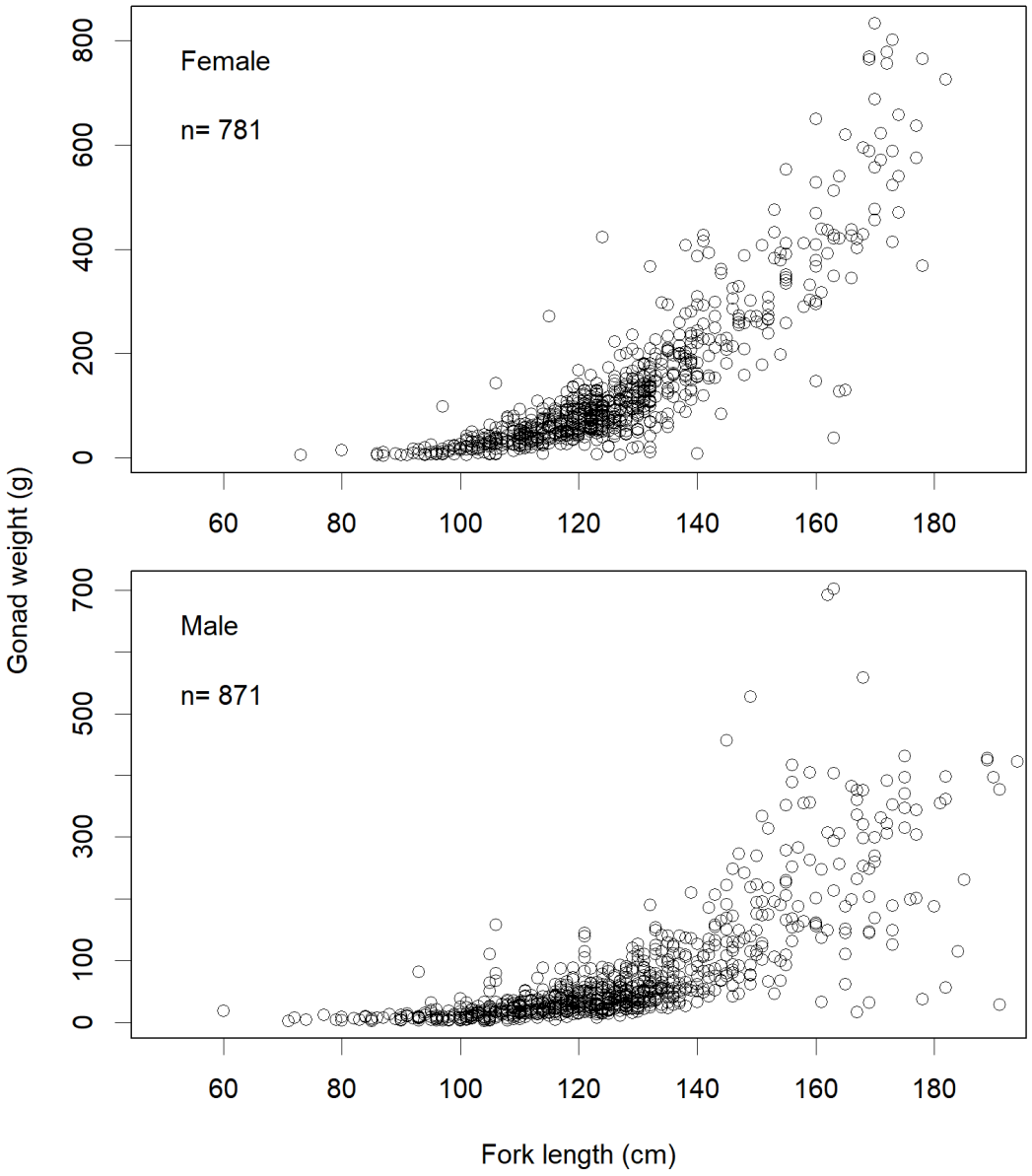


Fig. 3. Relationship between fork length and gonad weight of samples of southern bluefin tuna collected by Taiwanese scientific observer program from 2010 to 2025.

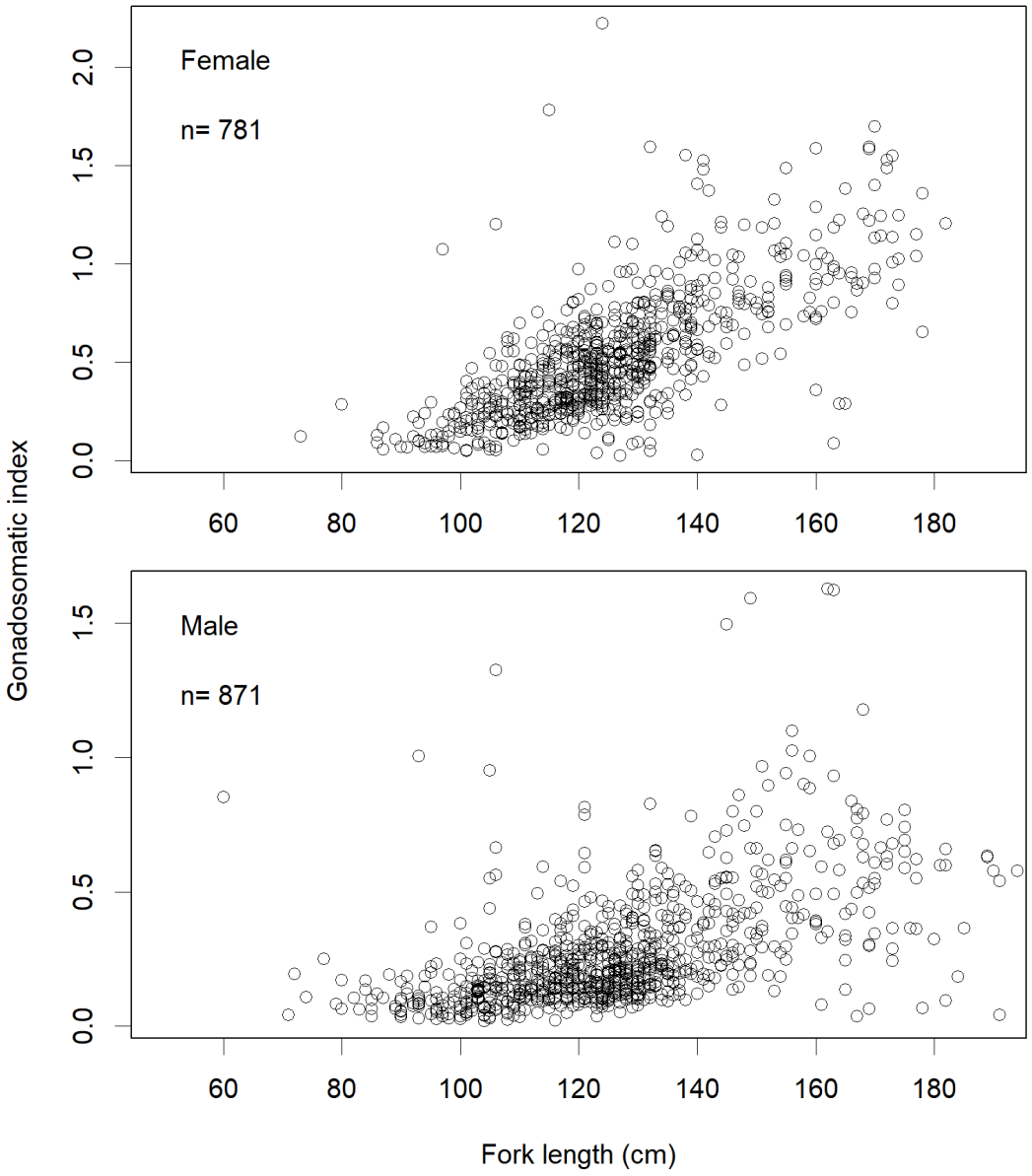


Fig. 4. Relationship between fork length and gonadosomatic index (GSI) of samples of southern bluefin tuna collected by Taiwanese scientific observer program from 2010 to 2025.

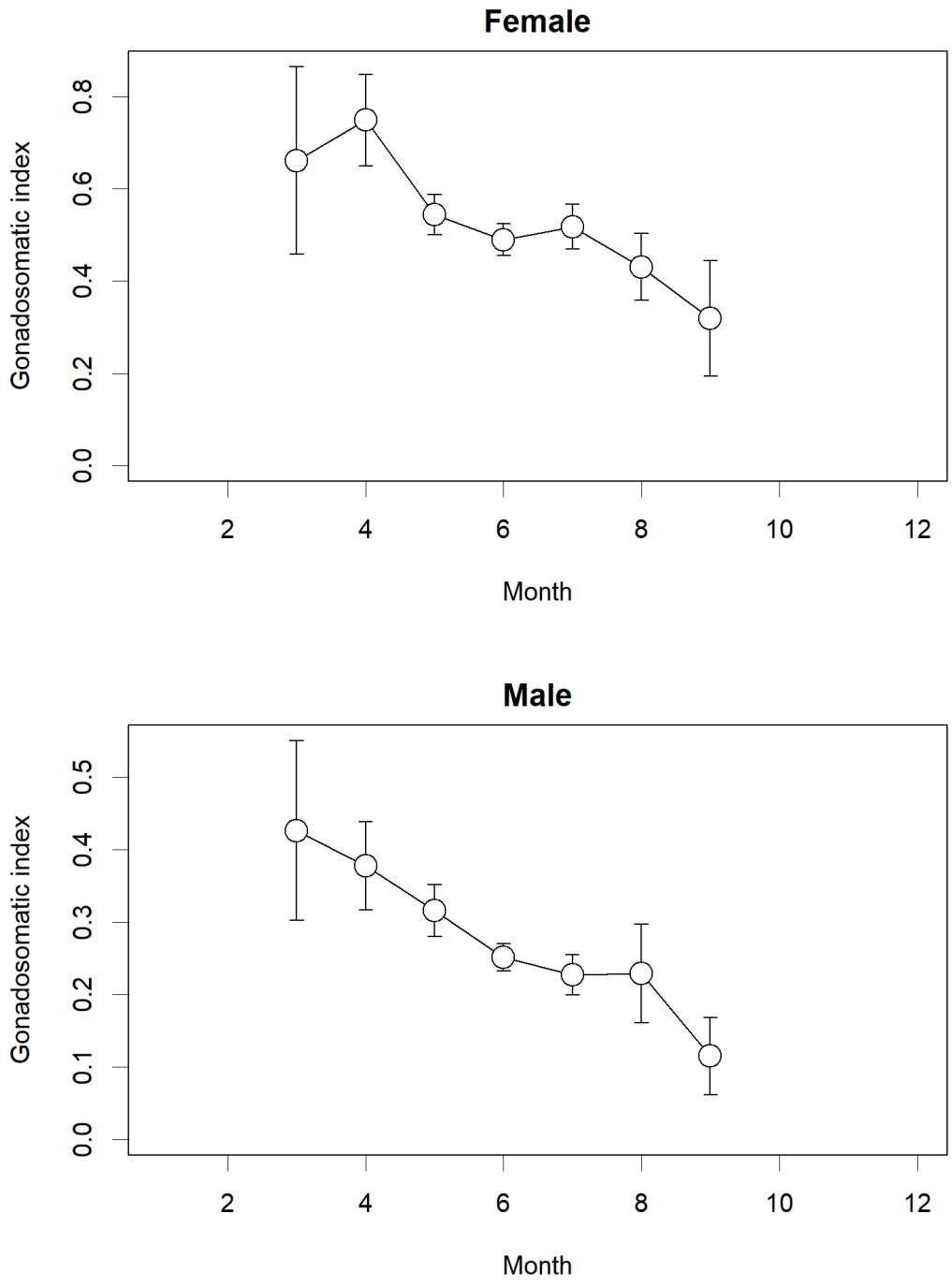
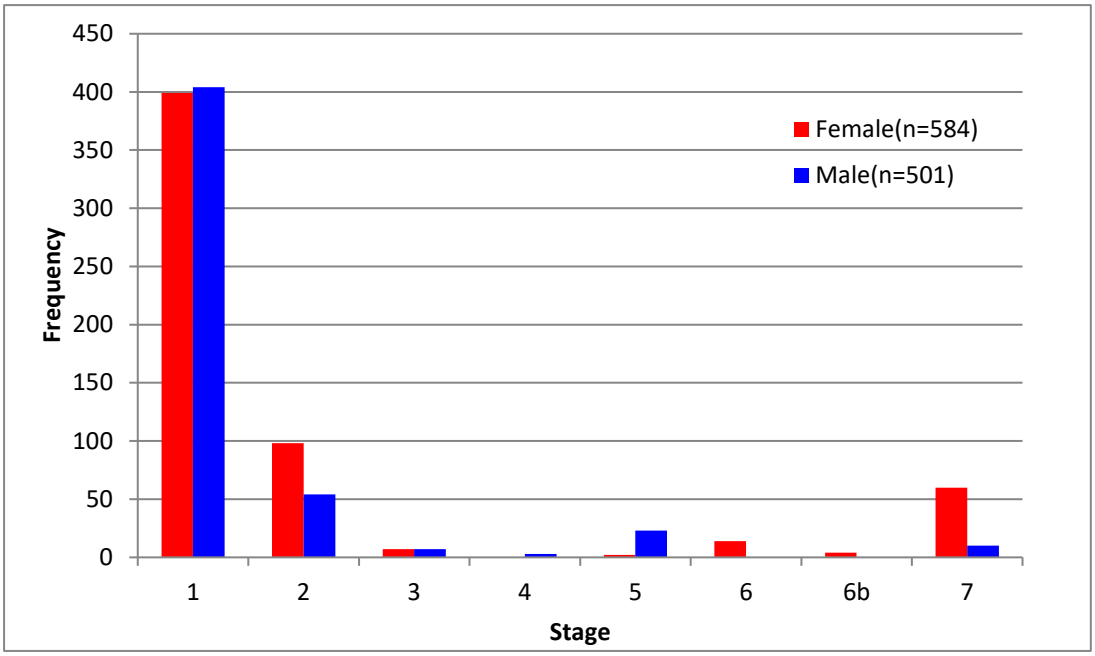


Fig. 5. Monthly trends of gonadosomatic index (GSI) for gonad samples of SBT collected by Taiwanese scientific observer program. Vertical bars represent the 95% confidence interval for means during 2010-2025.

(a)



(b)

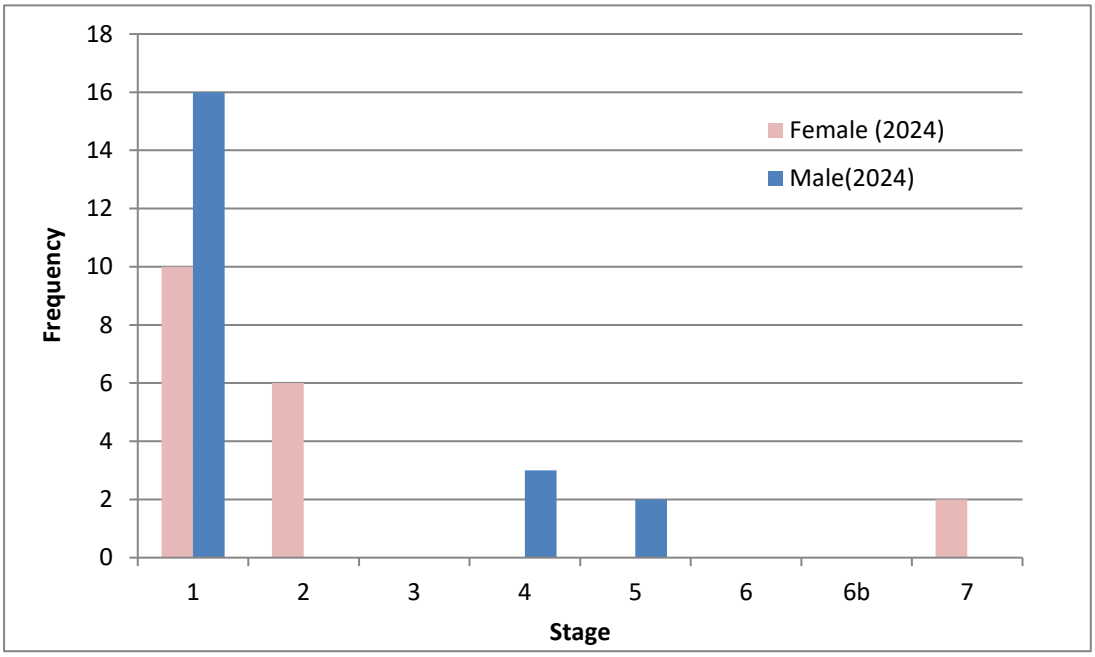
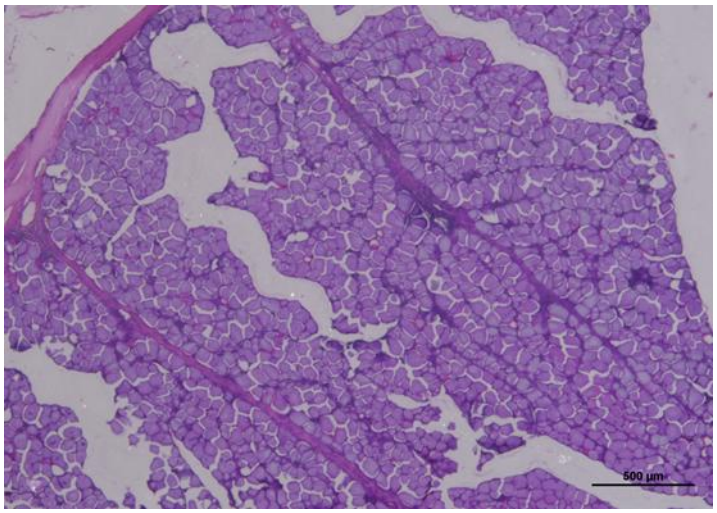
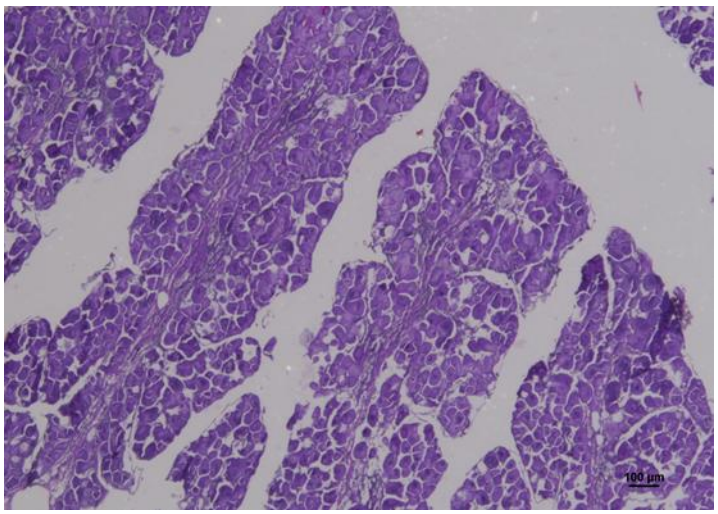


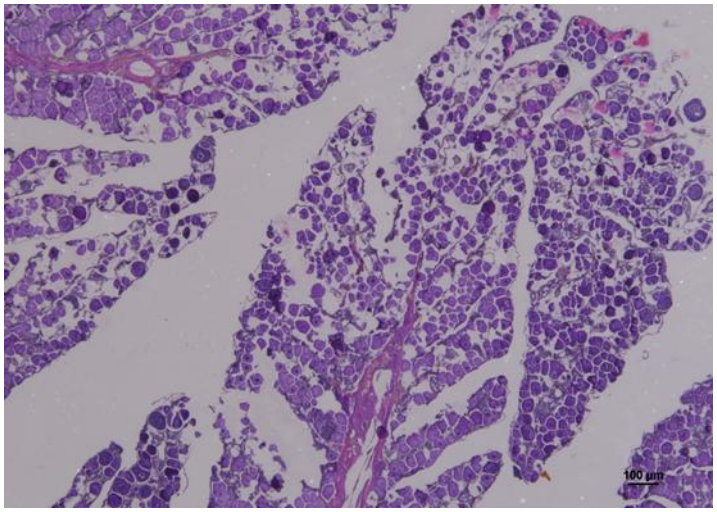
Fig. 6. Number of SBT samples by maturity stages for gonad samples of females and males collected by the Taiwanese scientific observer program. (a) The observed period was from 2010 to 2024. (b) The histological observation in 2024.



FL: 131cm, GW: 93g (Immature status, Stage 1)

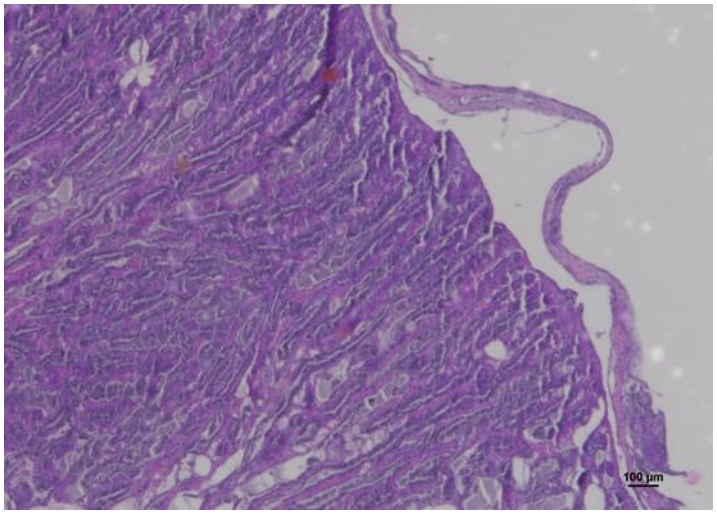


FL: 114cm, GW: 25 g (Immature status, Stage 2)

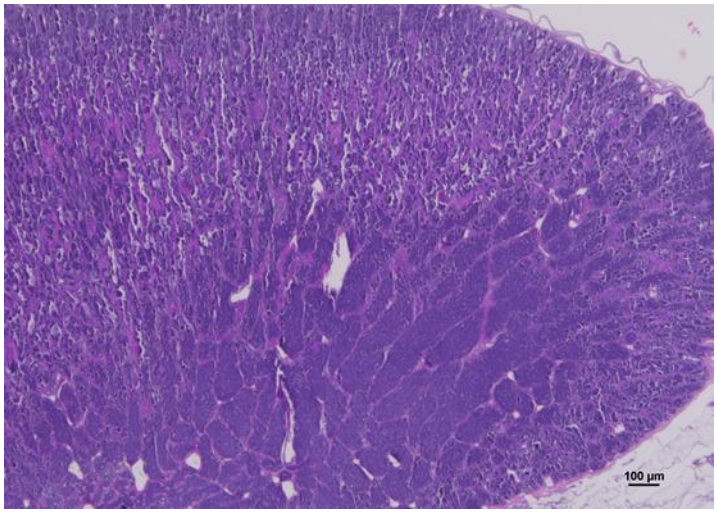


FL: 172cm, GW: 778 g (Mature status, Stage 7)

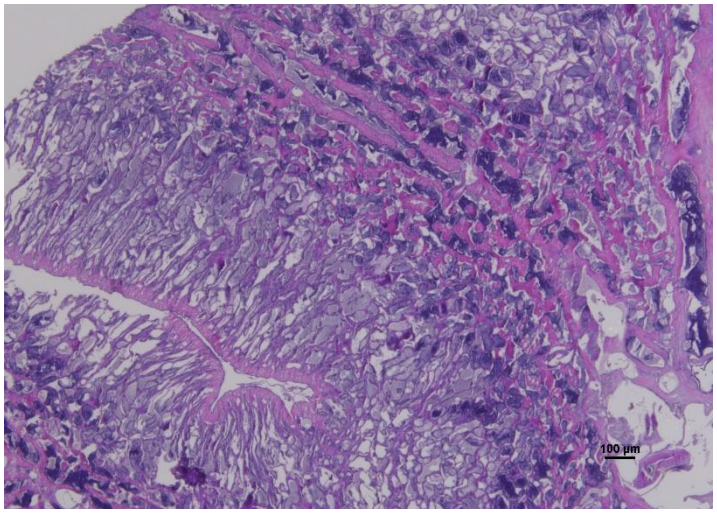
Fig. 7. Histological sections and measurements of ovaries of female SBT collected by Taiwanese scientific observer program in 2024.



FL: 118cm, GW: 17 g (Immature status, Stage 1)



FL: 158cm, GW: 355g (Mature status, Stage 4)



FL: 160cm, GW: 66 g (Mature status, Stage 5)

Fig. 8. Histological sections and measurements of testes of male SBT collected by Taiwanese scientific observer program in 2024.

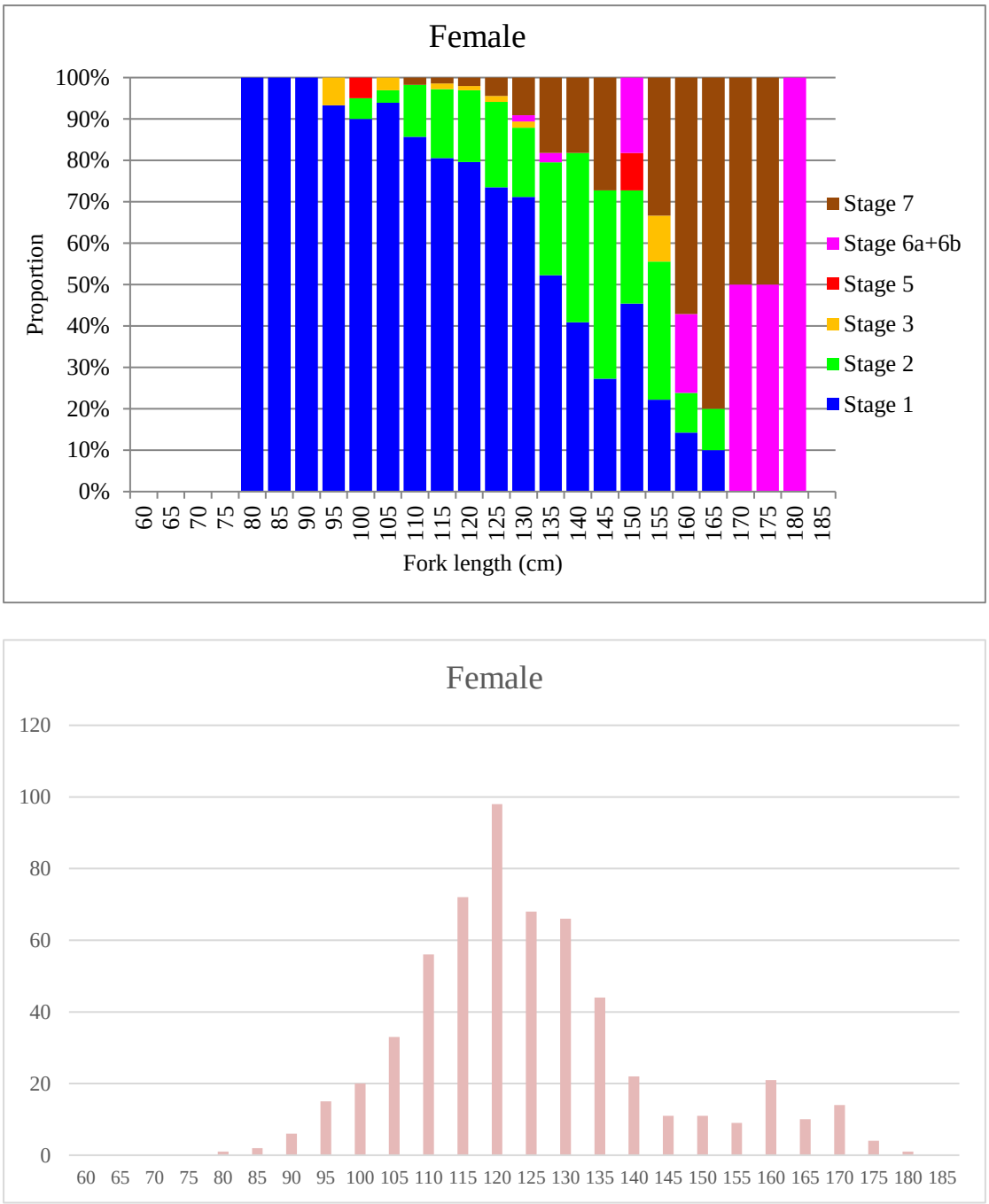
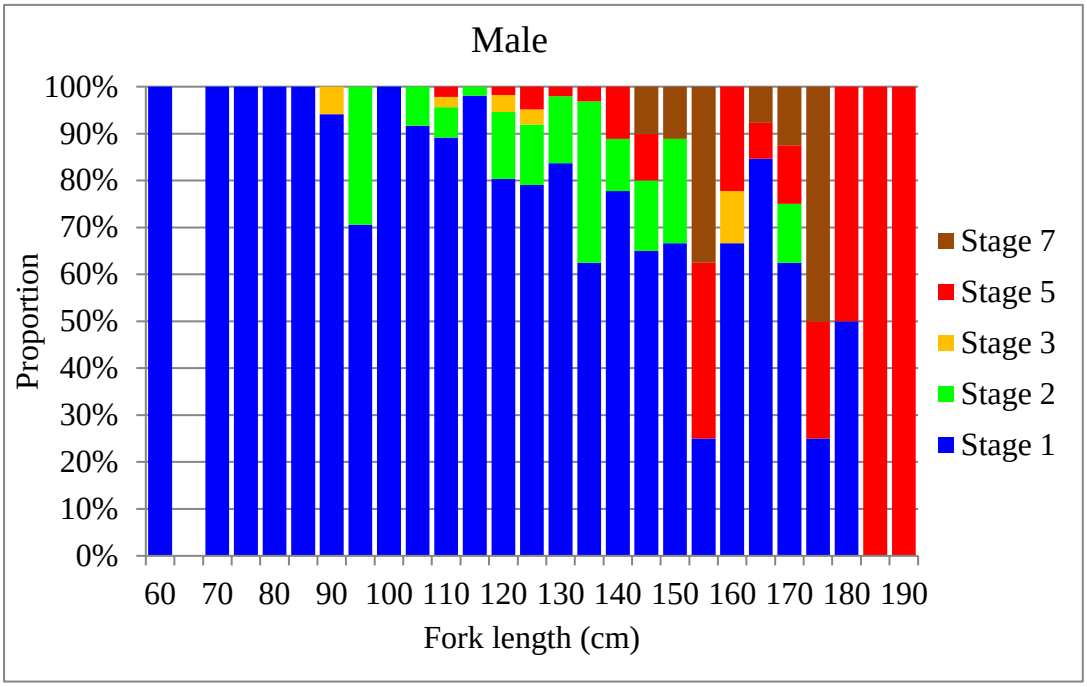


Fig. 91. Proportion of maturity stages by fork lengths with 5 cm intervals for gonad samples of SBT females collected by Taiwanese scientific observer program during the period of 2010 to 2024.



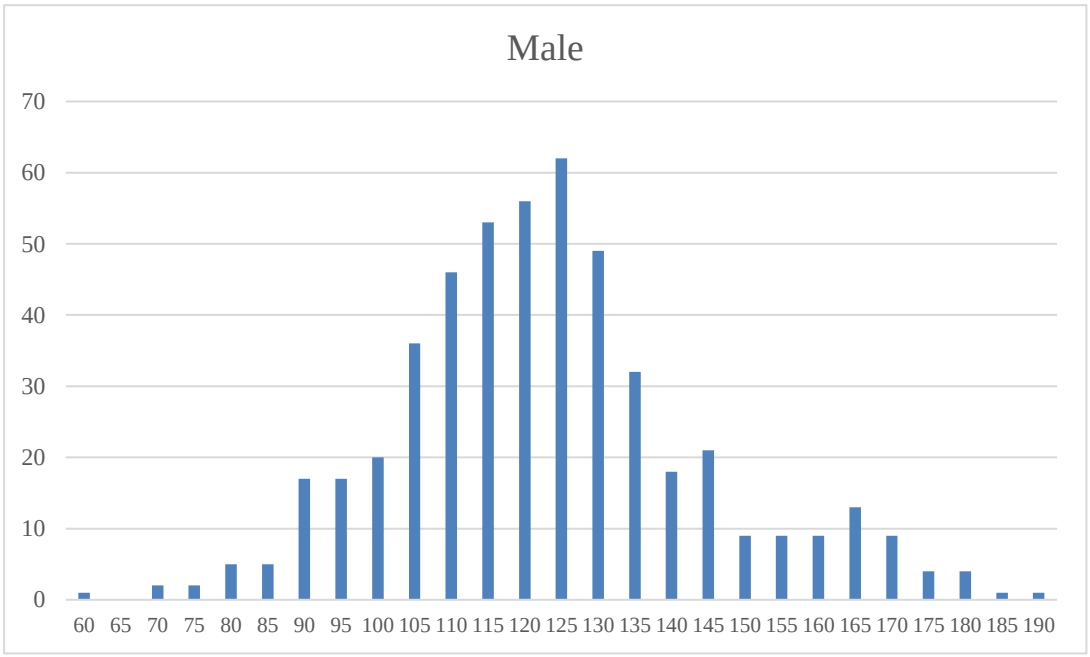


Fig.102. Proportion of maturity stages by fork lengths with 5 cm intervals for gonad samples of SBT males collected by Taiwanese scientific observer program during the period of 2010 to 2024.

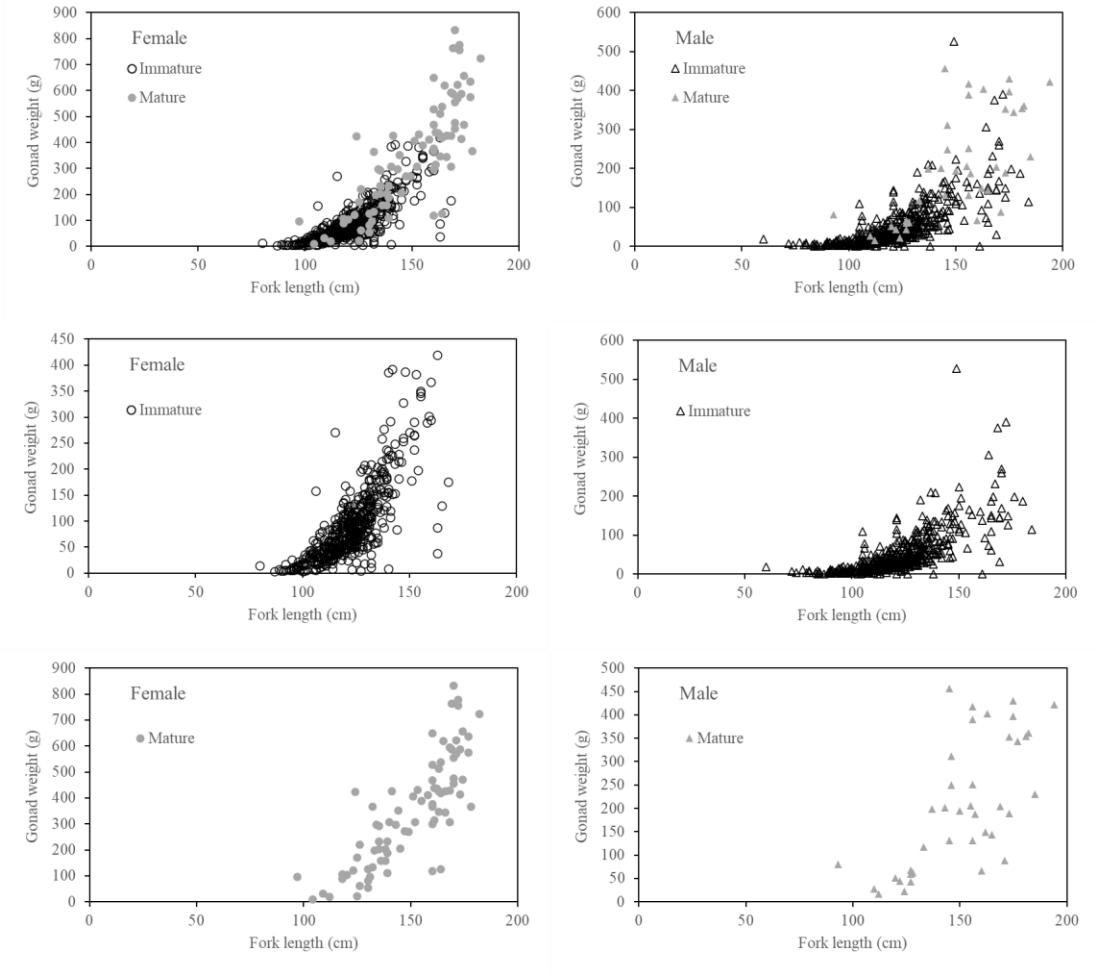


Fig. 11. Relationship between fork length and gonad weight by maturity status for gonad samples of SBT females (left) and males (right) collected by Taiwanese scientific observer program from 2010 to 2024.

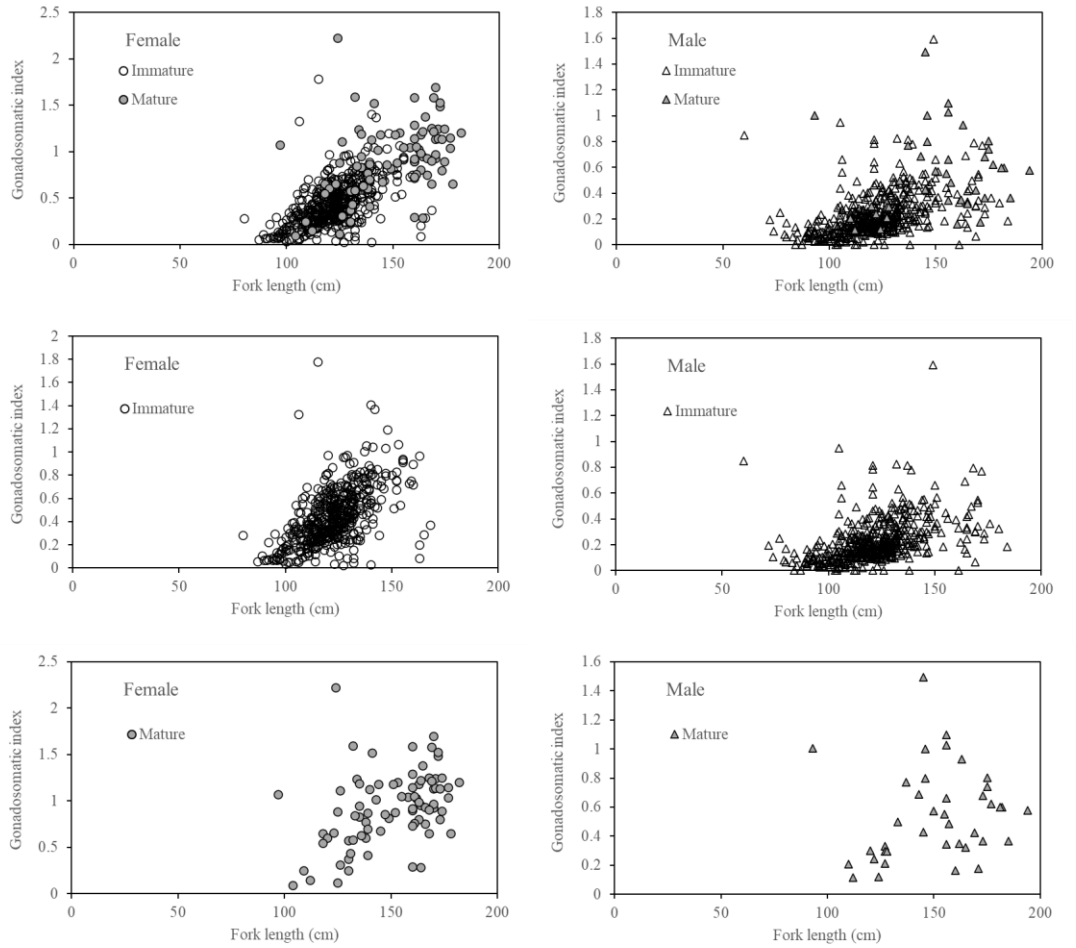


Fig. 12. Relationship between fork length and gonado-somatic index (GSI) by maturity status for gonad samples of SBT females (left) and males (right) collected by Taiwanese scientific observer program from 2010 to 2024.

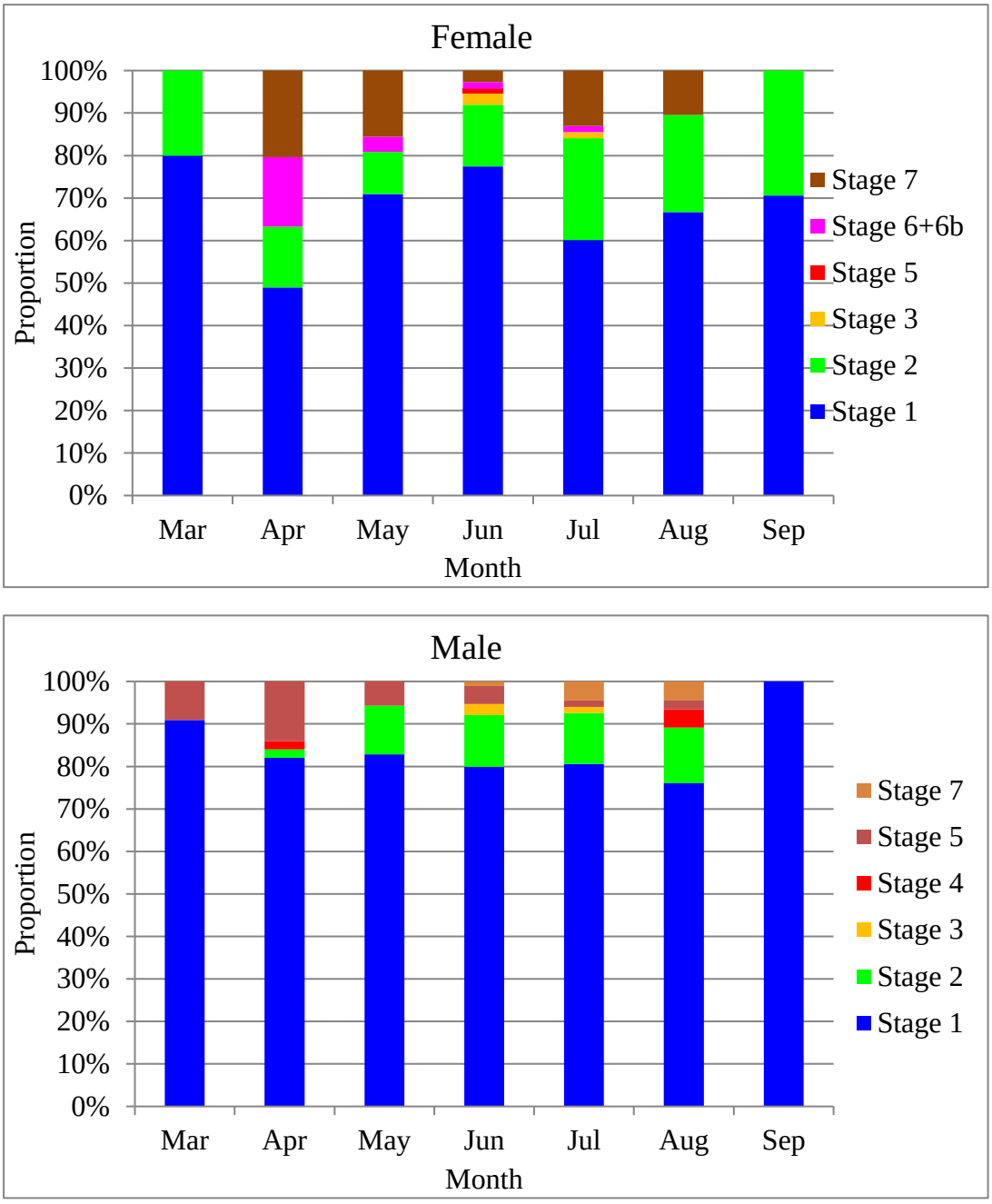


Fig. 13. Proportion of maturity stages by monthly category for gonad samples of SBT females (top) and males (bottom) collected by Taiwanese scientific observer program from 2010 to 2024.

Table 1. The criteria of gonadal developmental stages for albacore in the south Pacific Ocean (Adopted from Farley et al., 2013b).

Class	Maturity status	Activity	Development class	MAGO and POF stage	α and βatresia of yolked oocytes
1	Immature	lnactive	Immature	Unyolked,no POFs	Absent
2	Immature	lnactive	Developing	Early yolked,no POFs	Absent
3	Mature	Active	Spawning capable	Advanced yolked,no POFs	<50% α and β atresia may be present
4	Mature	Active	Spawning	Migratory nucieus or hydrated and/or POFs	<50% α and β atresia may be present
5	Mature	lnactive	Regressing-potentially reproductive	Advanced yolked,no POFs	≧50% α and β atresia present
6a	Mature	lnactive	Regressed 1	Unyolked or early yolked, no POFs	100% α and β atresia may be present
6b	Mature	lnactive	Regressed 2	Unyolked or early yolked, no POFs	No α and β atresia present
7	Mature	lnactive	Regenerating	Unyolked or early yolked, no POFs	Absent