

**Updated gonadal information and analysis of southern bluefin tuna  
collected by Taiwanese scientific observer program**

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**ABSTRACT**

Gonadal samples were collected by Taiwanese scientific observers dispatched on the longline vessels operated in the Indian Ocean. There were 1260 gonad samples in total collected from 2010 to 2023 including 599 females and 661 males. The range of fork length of samples were concentrated between 90 and 150 cm in females and males. According to the trends of the monthly gonado-somatic index (GSI) analyses, the trend of monthly GSI of females indicated that higher values occurred during April to July and gradually decreased after the peak in April, the second higher values of GSI was revealed in July. For the monthly GSI of males, it decreased gradually after the highest value in March. The trends of monthly GSI of both female and male showed no obvious changes with previous results.

According to the histological results, a total of 947 gonad samples in the collection period of 2010-2022 were analyzed for the sexual maturity stages determination including 469 females and 478 males with updated 78 samples in 2022. The majority of these samples were diagnosed as immature stage, and about 10.9% samples designated as mature but at reproductively inactive status. Also, most mature females were identified at regressed or regenerating stages from April to August, and most mature males were founded at regressing or regenerating stages during March to August.

**1. INTRODUCTION**

The researches related the reproductive biology of the southern bluefin tuna (SBT, *Thunnus maccoyii*) were conducted among different basins including the waters of Australia and Indian Ocean. Multiple index of SBT such as age-at-first-maturity, gonad

index, ovary size-frequency and fecundity had been investigated in the waters off the south eastern and southern Australia (Thorogood, 1986). And Farley and Davis (1998) studied the spawning dynamics of SBT using ovaries obtained from the spawning ground and the main feeding ground in the Indian Ocean. Also, the study related the sexual maturity of SBT have been investigated with the histological observations of the gonad samples collected by Taiwanese observers program in the southwest Indian Ocean (Chen et al., 2013). However, there is no specific standard for determining the maturity stages in SBT. Establishing the guideline of the determination maturity stages is essential and helpful for further understanding of reproductive index of SBT.

Therefore, developing an independently estimated maturity criteria were supported by the ESC, and also listed as a high priority in the Scientific Research Plan (SRP) since 2015 (Farley et al., 2013a, 2014). For improving the integrity of the reproductive studies and the continuity of the project, we conducted the scientific data collection of SBT including biological samples such as gonads, otoliths, and muscle tissues as essential objectives in the Taiwanese scientific observers program annually. Here, we presented the updated the latest gonad information including the results of GSI analyses and maturity status of SBT collected by Taiwanese scientific observers program.

## 2. MATERIALS AND METHODS

The SBT gonad samples were collected by scientific observers deployed on Taiwanese longline vessels operated in the Indian Ocean. The biological information including the fork length, body weight, sex, sampling date and location were recorded for each specimen.

For the calculation of gonado-somatic index (GSI), we adopted a length-based GSI (Chen et al., 2013) in this study:

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

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

For the histology section, the gonad samples were fixed in 10% buffered formalin for the further histological processes. And histological sections were processed and stained with Harris' haematoxylin and eosin for the preparation of the histological slides. We conducted the histological classification for diagnosing sexual maturity stages of gonad samples. The criteria of histological classification for gonadal developmental

stages of SBT were needed further discussed specifically. Therefore, here, we followed the criteria of Farley et al. (2013b), which were used for albacore in the southern Pacific Ocean, and adopted to categorize the gonadal developmental stages for SBT. Seven categories of the developmental stages were classified as the (1) immature stage, (2) developing stage, (3) spawning capable stage, (4) spawning stage, (5) regressing - potentially reproductive stage, (6) regressed stage, and (7) regenerating stage. Individuals were identified as mature if the most advanced oocytes were indicative of  $\geq$  stage 3. Stages 3 and 4 are reproductively active stages, and stages 1-2 and 5-7 are reproductively inactive stages. The details of the criteria were listed in Table 1. adopted from Farley et al (2013b).

### 3. RESULTS AND DISCUSSION

A total of 1260 gonad samples of SBT were collected during March to September from 2010 to 2023 including 599 females and 661 males. 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). The range of fork length of female and male samples were from 73 to 182 cm and 60 to 194 cm, respectively. The majority of samples' fork length were distributed between 90 and 150 cm in both female and male (Fig. 2).

The gonad weights revealed increasing pattern with the growth of fork lengths and presented relative larger variation in larger size specimens especially were over 150 cm in fork length (Fig. 3). The similar patterns of the relationship between fork length and GSI were also presented in both females and males, which the GSI showed increasing pattern as fork length increasing. However, in some cases, the values of GSI presented lower levels and didn't follow the increasing pattern (Fig. 4). It might be related to the maturity status of those samples and needs to be further investigated.

In order to understand the monthly variation of GSIs and gonad maturity status, we calculated the average of GSIs by month in both sexes. First, the monthly GSIs of females revealed highest value in April and remained higher values from April to July. Then it started the decreasing trends after July and revealed the lowest value in September. For the monthly GSIs of males, it revealed highest value in March and then decreased gradually reached the lowest value in September with updated data to 2023 (Fig 5). The monthly trends of GSI for females and males remained the similar trends as the past. The samples were collected with limitation only from March to September, monthly trend of GSI would not be demonstrated for the entire year, because the limited fishing season of Taiwanese SBT longliner fishery in the Indian Ocean (Fig. 5).

For the identification of maturity stage with histological method, there were some samples not qualified for further histological processes, due to the difficulties of processing frozen samples in the sample preservation. Therefore, a total of 947 gonad samples including 469 females and 478 males were collected from 2010 to 2022 were successfully examined histological sections, and the sexual maturity stages were determined using the criteria of developmental stages in Farley et al (2013b). Based on the results of the histological sections of both sexes, the majority of samples were determined as immature stage and some samples were identified at developing stage. And there were about 10.9% of samples designated as mature but most of these samples were reproductively inactive (regressed or regenerating stages) (Figs. 6-8).

Furthermore, we demonstrated the proportion of maturity stages by fork length with 5 cm intervals. The majority of gonadal samples of females and males were identified as immature specimen with the newly updated information to 2022. The smallest fork length of mature females and males were 97 and 93 cm, respectively (Figs. 9-11). Although the gonad weights and GSIs generally showed the increasing pattern with the fork lengths increased, there is no obvious separation boundary between mature and immature individuals could be identified. Most immature and mature samples overlapped in the ranges of the fork lengths, gonad weights and GSIs. And there was no overlapped for the samples with fork length less than about 90 cm (Figs. 10 and 11).

According to the proportion of gonadal developmental stages by months from March to September (Fig. 12), most mature females and males were at inactive reproductive status. And, most mature females which identified as regressed and regenerating stages (stage 6 and stage 7) were found during April to August, and most of mature males were regressing and regenerating stages (stage 5 and stage 7) during March to August. Based on the results of histological sections, the monthly variation in the proportion of maturity stages indicated that mature fishes might migrate to the fishing ground of Taiwanese SBT fishery after reproductive activity. Due to the limitation of sample collection and coverages of Taiwanese SBT longliner fishery, we could not fully understand the reproductive activity of SBT with entire year. Therefore, it would be essential to collaborate with others researcher for developing the criteria of histological classification for gonadal maturity stages and improving the reproductive biology of SBT.

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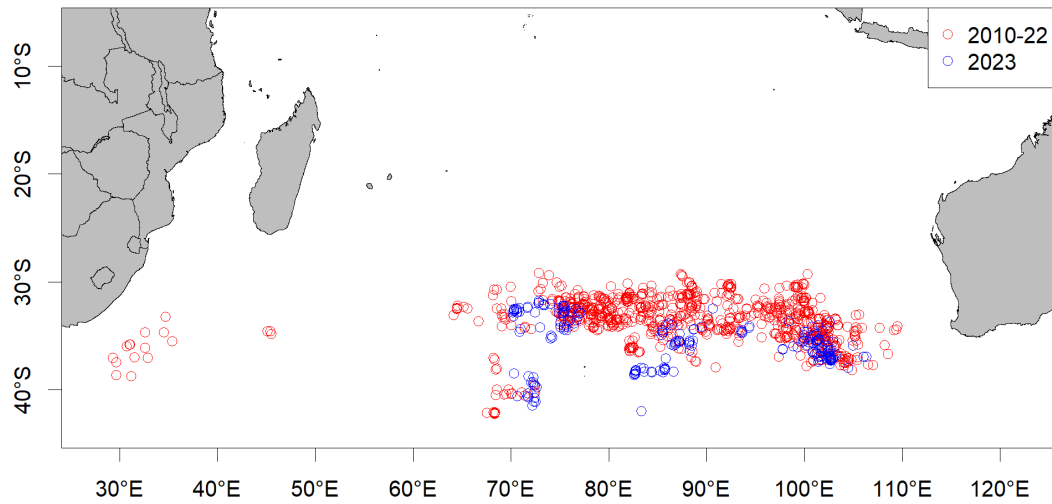


Fig. 1. Sampling locations of southern bluefin tuna collected by Taiwanese scientific observer program from 2010 to 2023.

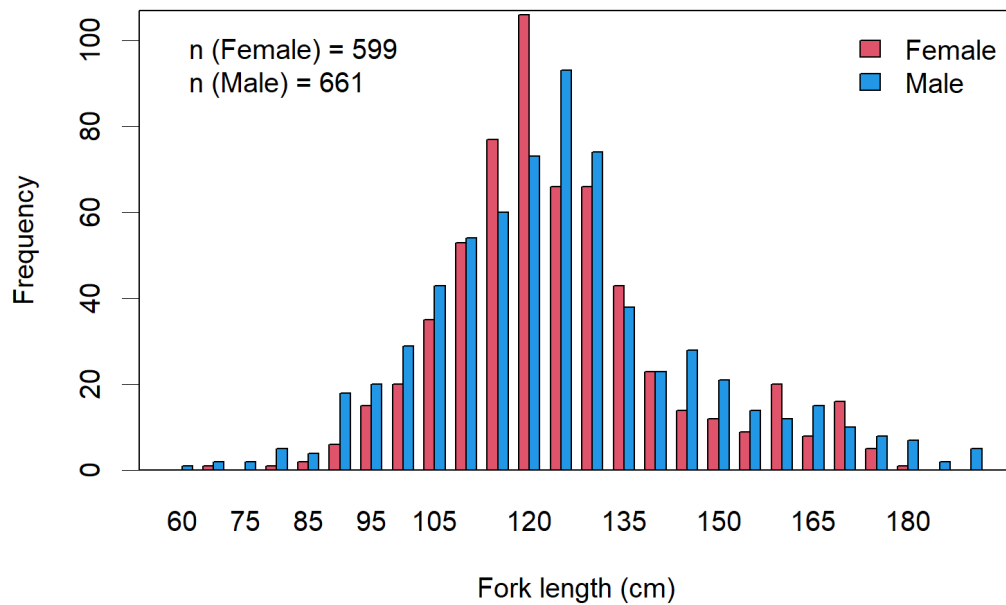


Fig. 2. Length frequency distributions (5 cm intervals) for gonad samples of southern bluefin tuna collected by Taiwanese scientific observer program from 2010 to 2023.

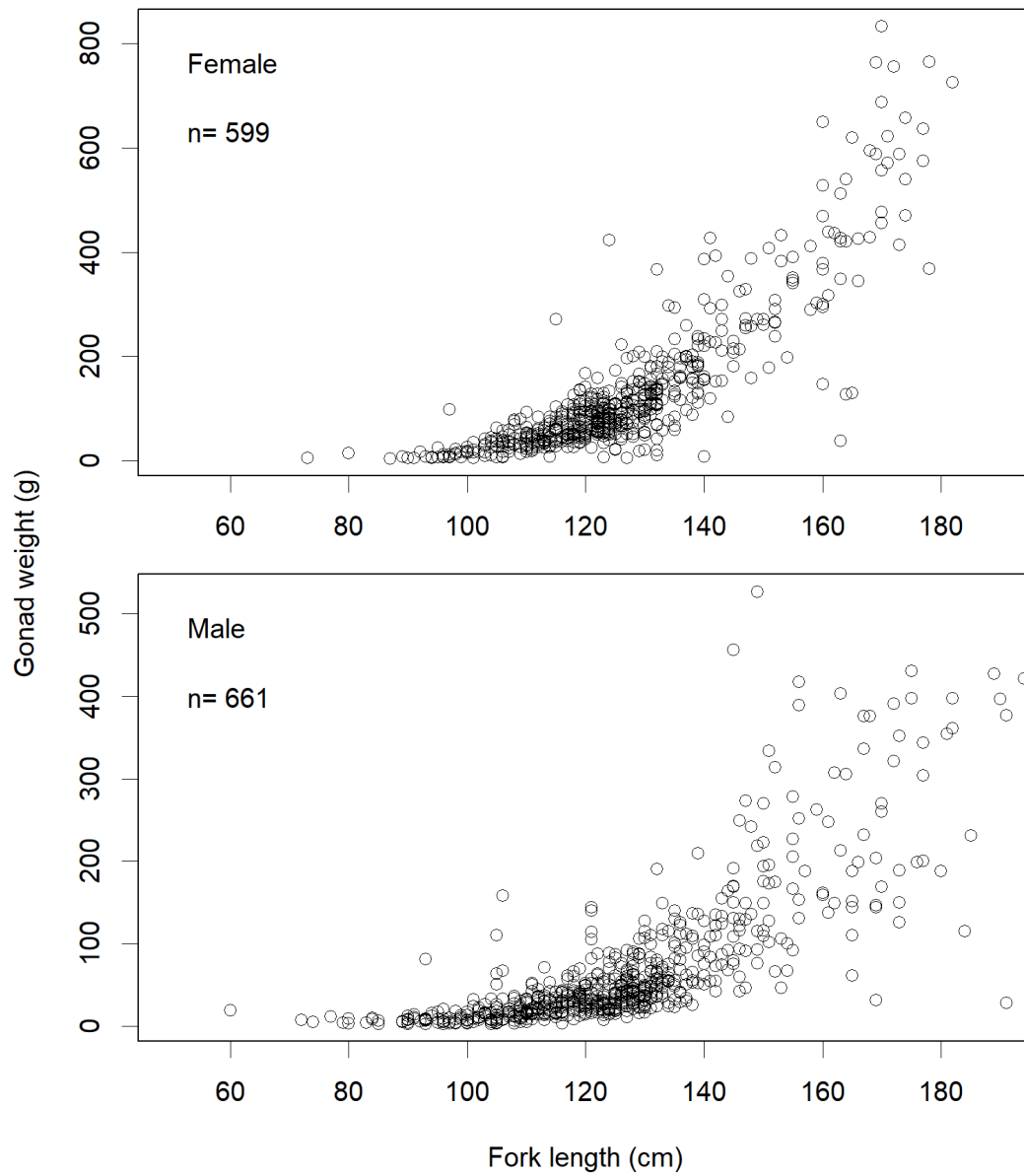


Fig. 3. Relationship between fork length and gonad weight of samples of southern bluefin tuna collected by Taiwanese scientific observer program from 2010 to 2023.

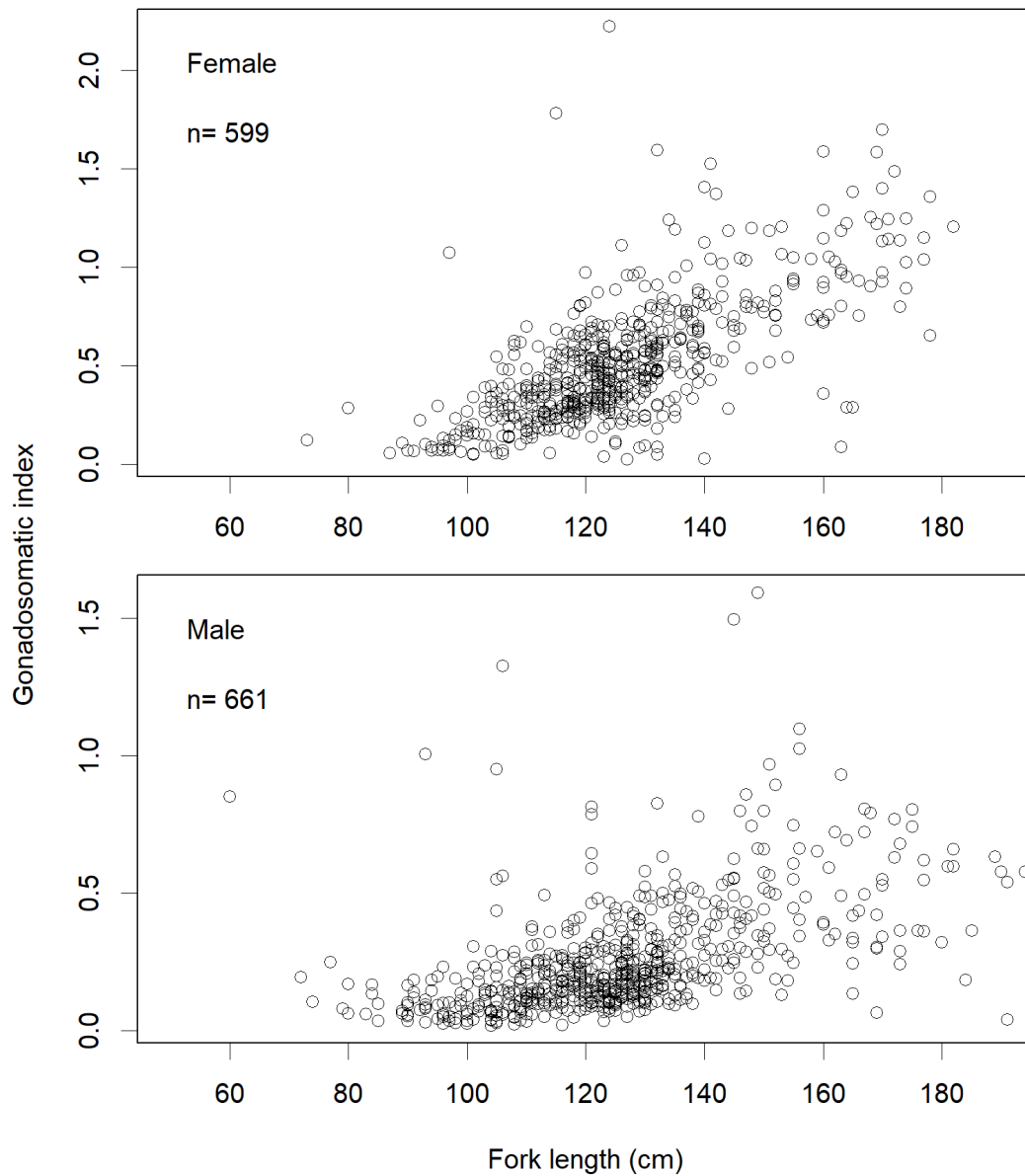


Fig. 4. Relationship between fork length and gonadosomatic index (GSI) of samples of southern bluefin tuna collected by Taiwanese scientific observer program from 2010 to 2023.



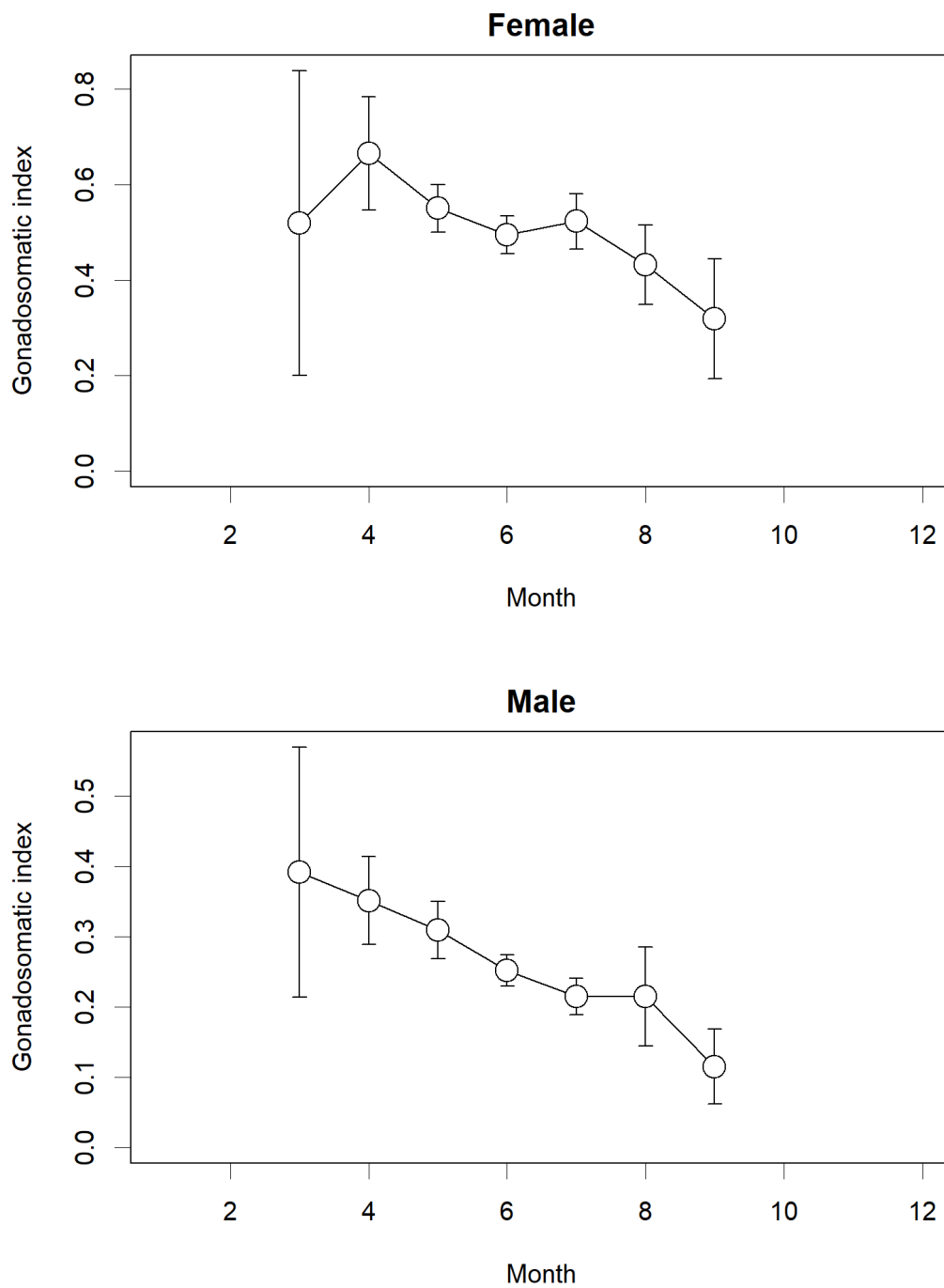


Fig. 5. Monthly trends of gonadosomatic index (GSI) for gonad samples of SBT collected by Taiwanese scientific observer program. Vertical bars represent the 95% confidence interval for means during 2010-2023.

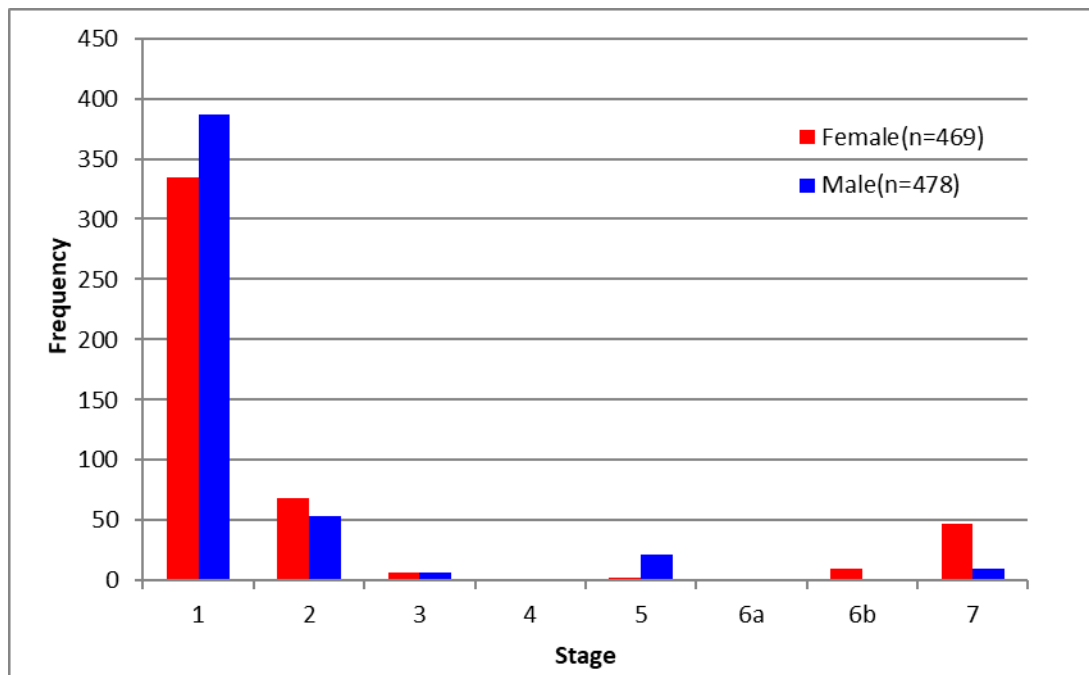
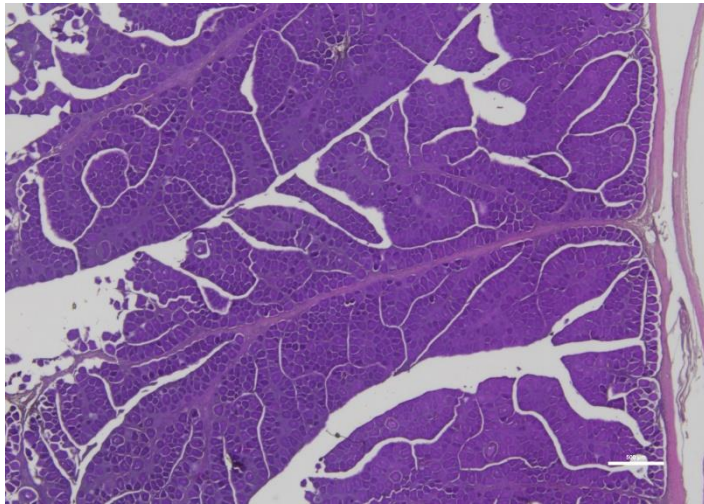
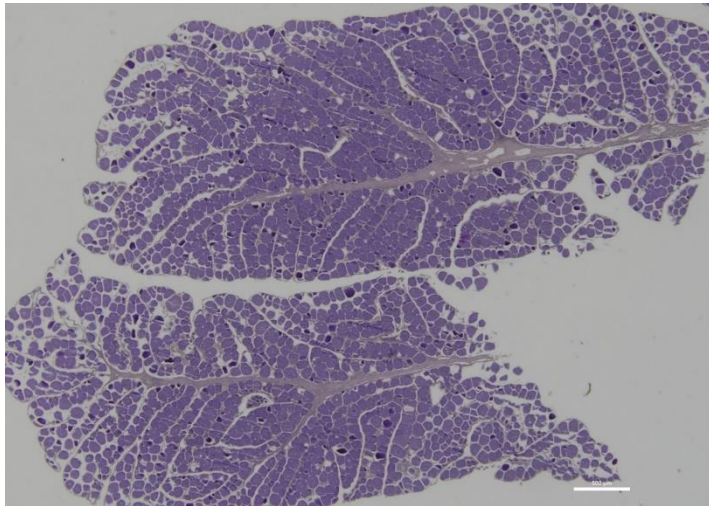


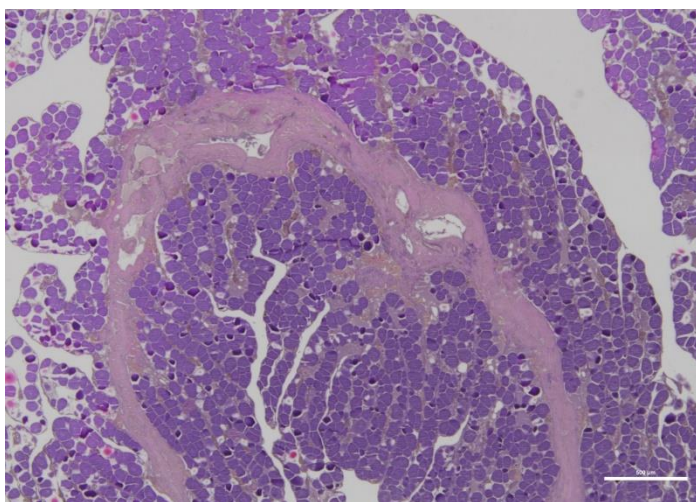
Fig. 6. Number of samples by maturity classes for gonad samples of SBT collected by Taiwanese scientific observer program during 2010-2022.



FL: 114cm, GW: 76.34 g (Stage 1, immature status)

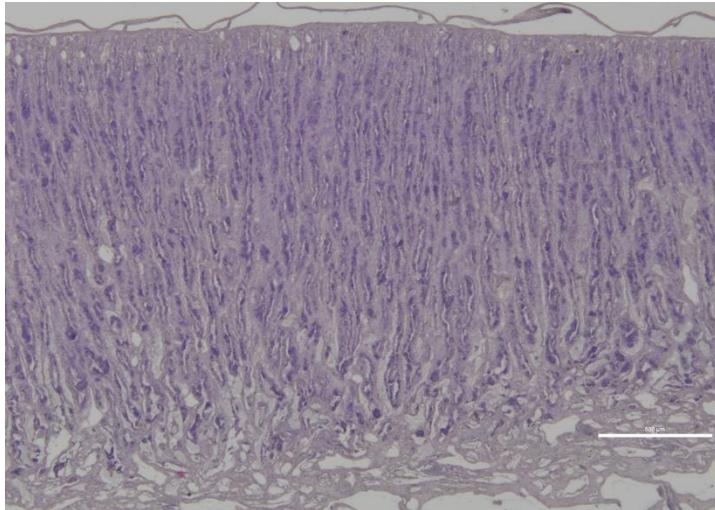


FL: 142cm, GW: 151.55g (Stage 2, developing status)

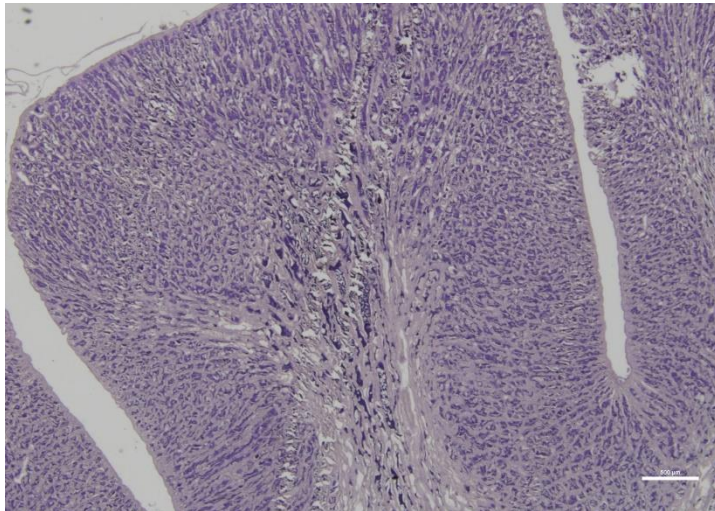


FL: 177cm, GW: 574.0 g (Stage 7, Regenerating status)

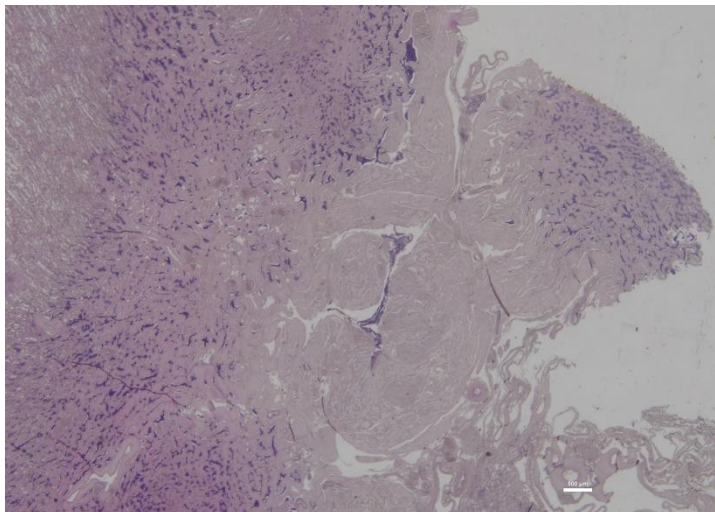
Fig. 7. Histological sections and measurements of oocytes for gonad samples of female SBT collected by Taiwanese scientific observer program in 2022.



FL: 128cm, GW: 54.97g (Stage 1, immature stage)



FL: 137cm, GW: 210.0g (Stage 2, developing stage)



FL: 181cm, GW: 354.50 g (Stage 5, Regressing stage)

Fig. 8. Histological sections and measurements of oocytes for gonad samples of male SBT collected by Taiwanese scientific observer program in 2022.

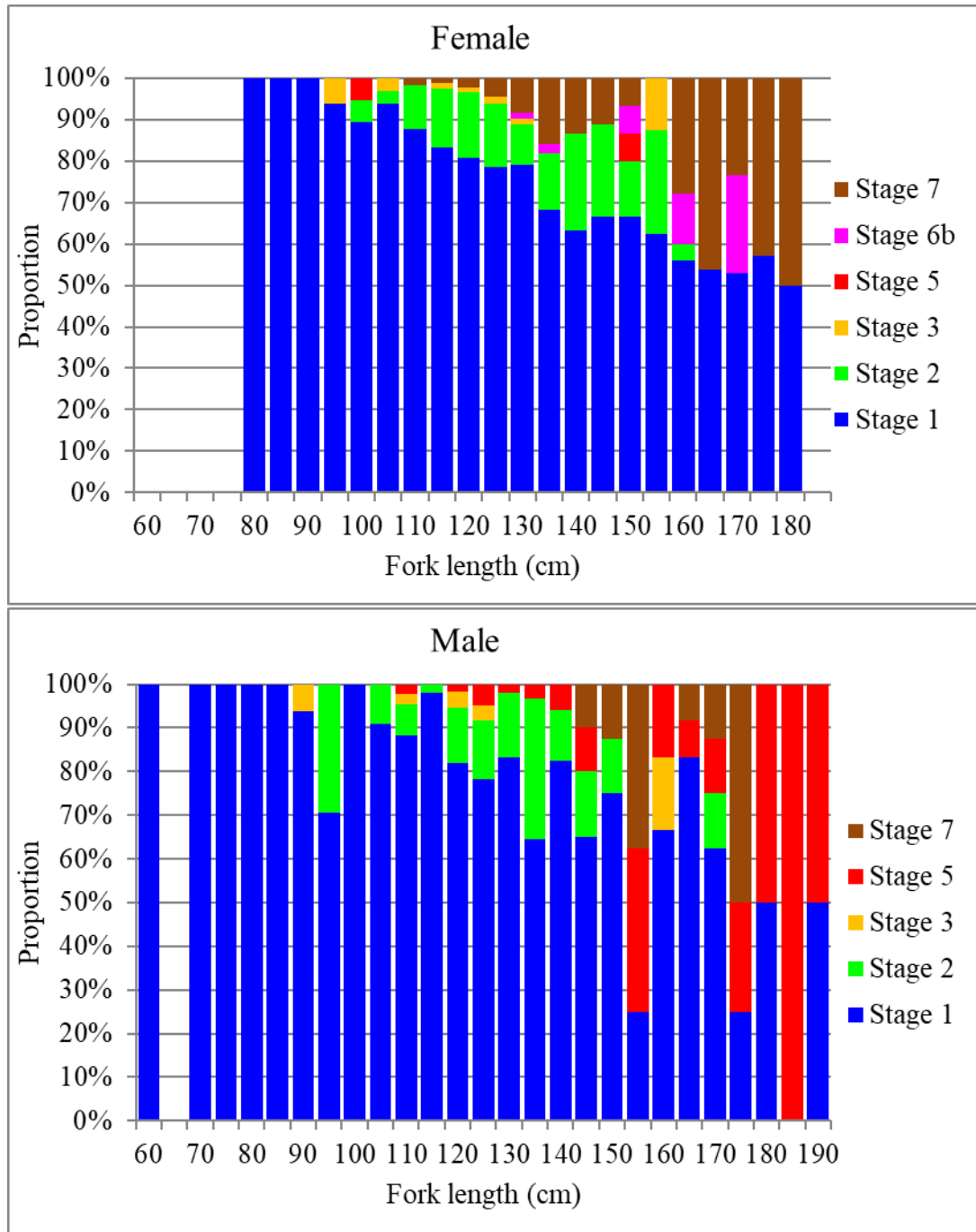


Fig. 9. Proportion of maturity stages by fork lengths with 5 cm intervals for gonad samples of SBT collected by Taiwanese scientific observer program during the period of 2010-2022.

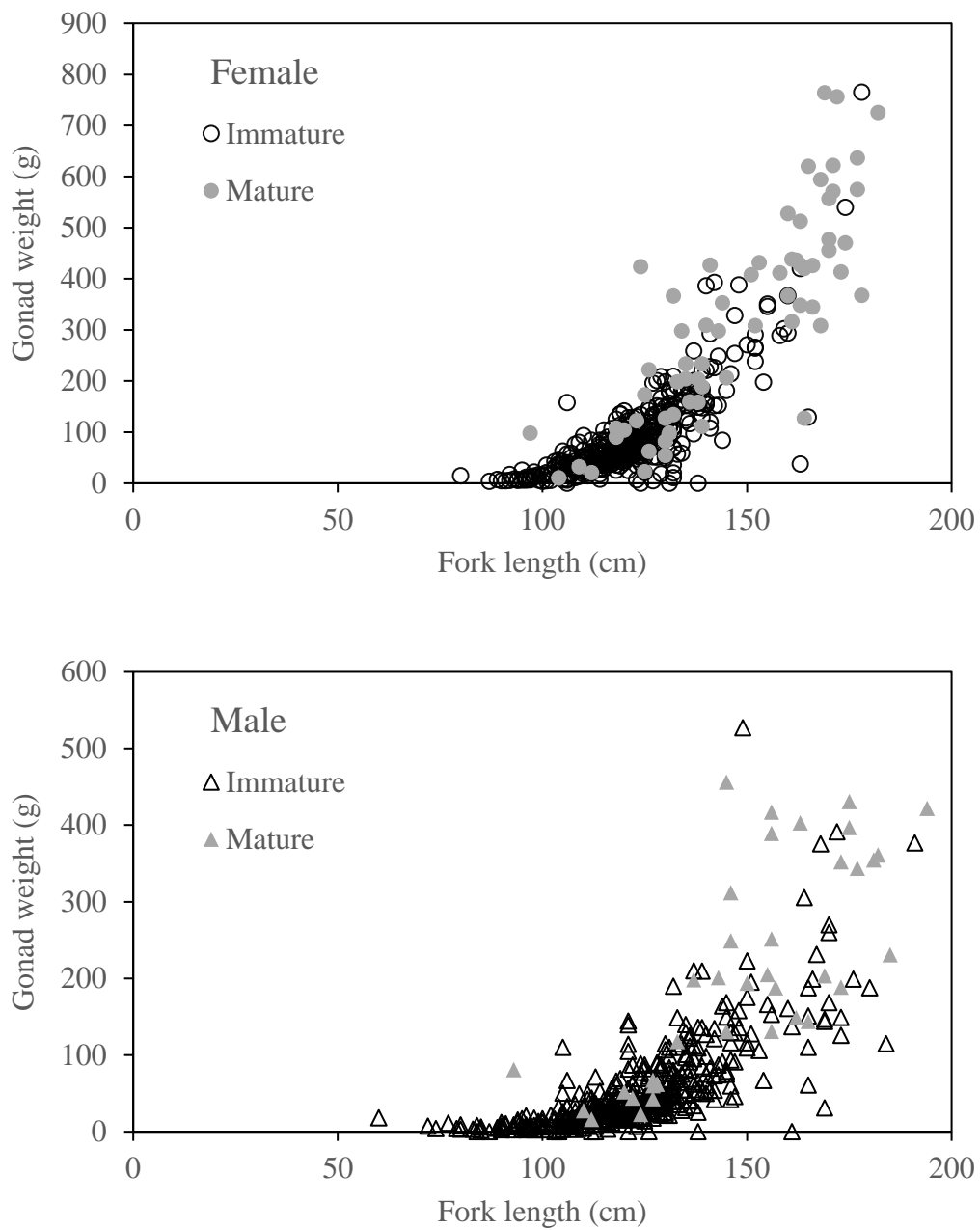


Fig. 10. Relationship between fork length and gonad weight by maturity status for female and male gonad samples of SBT collected by Taiwanese scientific observer program during the period of 2010-2022.

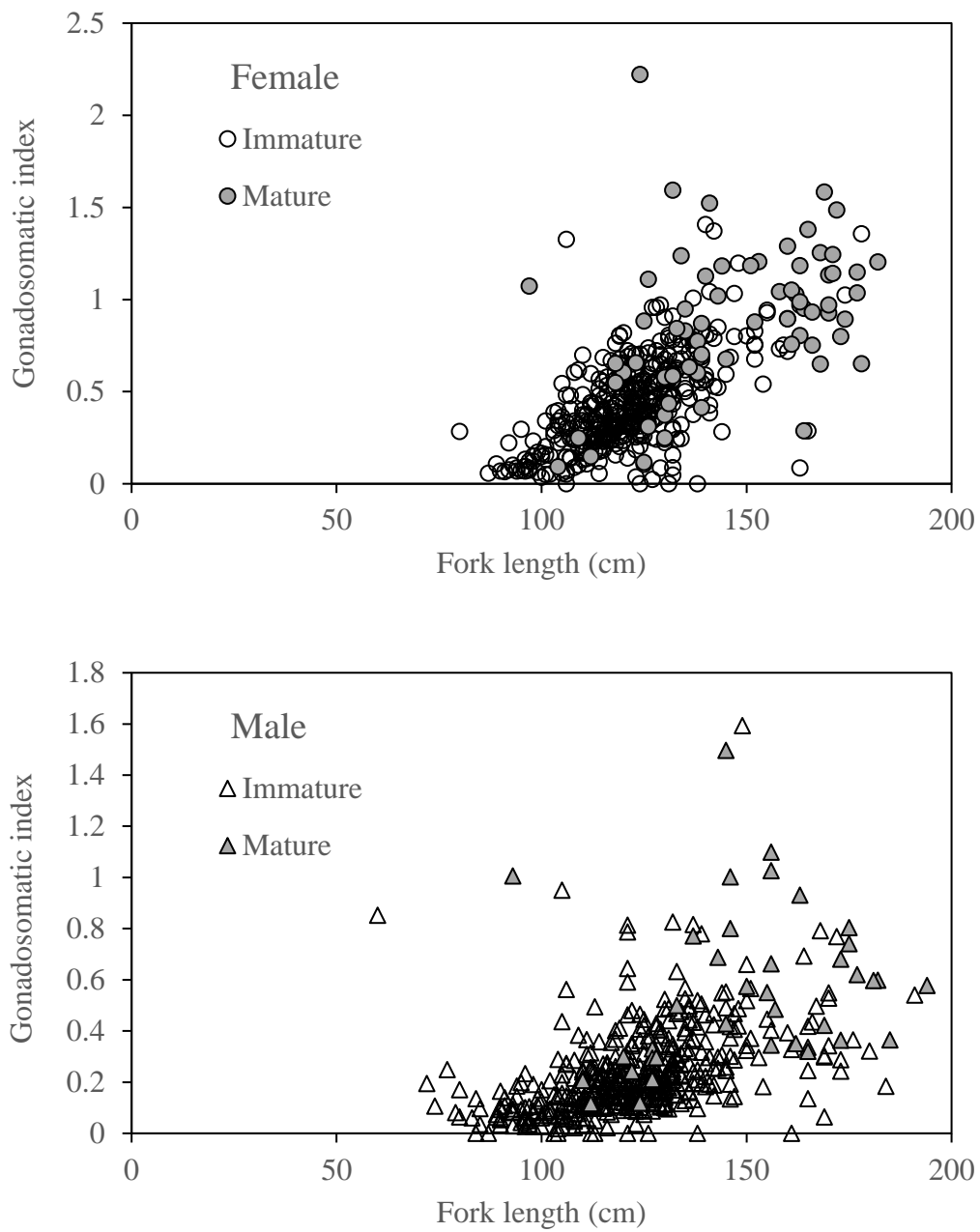


Fig. 11. Relationship between fork length and gonado-somatic index (GSI) by maturity status for female and male gonad samples of SBT collected by Taiwanese scientific observer program during the period of 2010-2022.

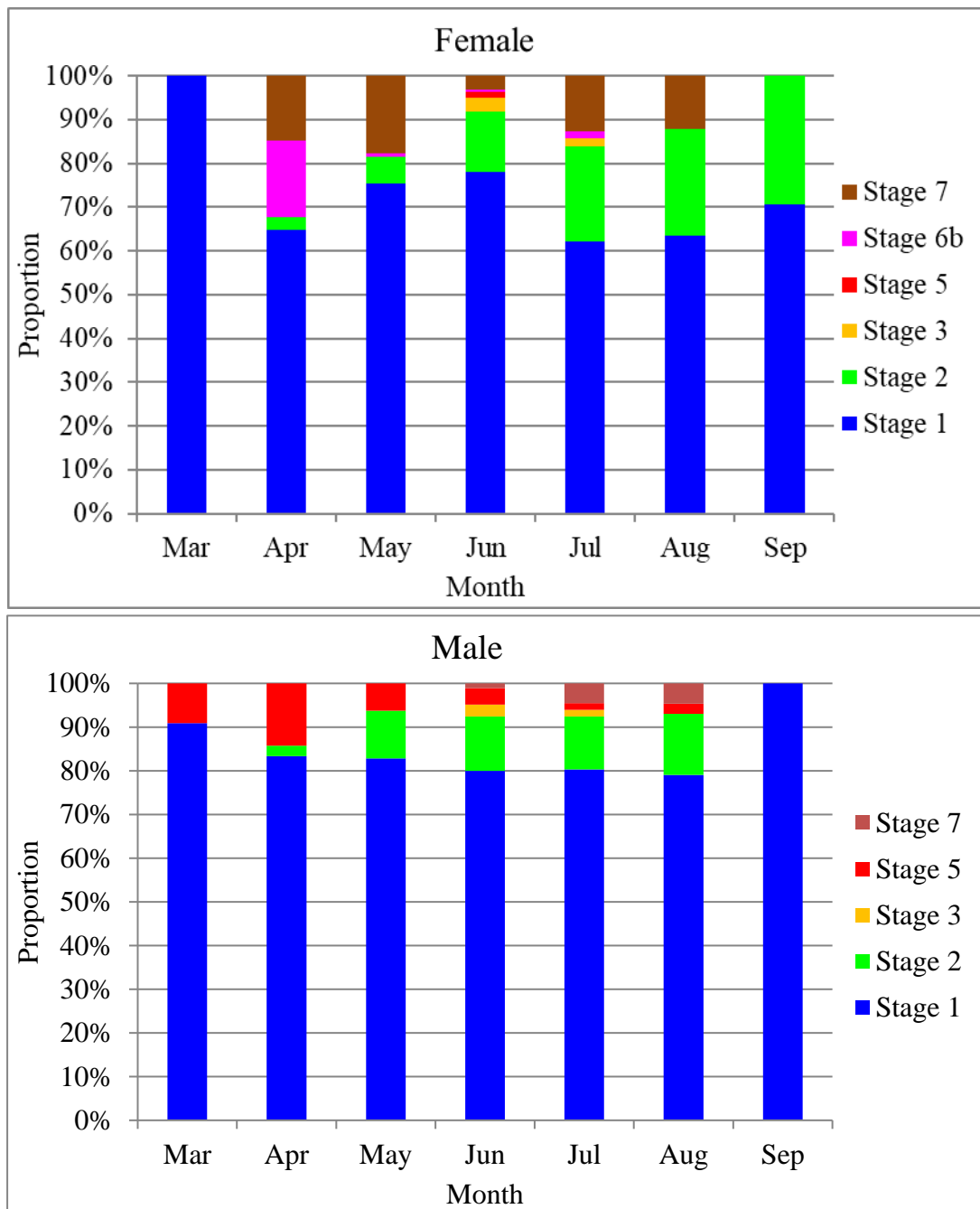


Fig. 12. Proportion of maturity stages by monthly category for female and male gonad samples of SBT collected by Taiwanese scientific observer program during the period of 2010-2022.



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	$\alpha$ and $\beta$ atresia of yolked oocytes
1	Immature	Inactive	Immature	Unyolked,no POFs	Absent
2	Immature	Inactive	Developing	Early yolked,no POFs	Absent
3	Mature	Active	Spawning capable	Advanced yolked,no POFs	<50% $\alpha$ and $\beta$ atresia may be present
4	Mature	Active	Spawning	Migratory nucleus or hydrated and/or POFs	<50% $\alpha$ and $\beta$ atresia may be present
5	Mature	Inactive	Regressing-potentially reproductive	Advanced yolked,no POFs	$\geq$ 50% $\alpha$ and $\beta$ atresia present
6a	Mature	Inactive	Regressed 1	Unyolked or early yolked, no POFs	100% $\alpha$ and $\beta$ atresia may be present
6b	Mature	Inactive	Regressed 2	Unyolked or early yolked, no POFs	No $\alpha$ and $\beta$ atresia present
7	Mature	Inactive	Regenerating	Unyolked or early yolked, no POFs	Absent