

Spawning season estimation for bullet tuna (*Auxis rochei* Risso, 1810) in southern waters of Bali: a postovulatory follicles - based approach

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Abstract. Bullet tuna is a crucial neritic tuna species in Indonesia that plays a significant role in small-scale and commercial fisheries. Regulation of seasonal fishing is an example of fishery resource management to prevent negative impacts on fishery resources due to fishing pressure. Postovulatory follicles (POFs) are a potential characteristic for determining spawning season in adult fish that have already spawned. The objective of this study was to determine the spawning season of bullet tuna (*Auxis rochei*) in the presence of POFs. This study employed purposive stratified sampling techniques, which were obtained from gill net fishing boats operating in the Kedonganan Fish Landing Base, Bali. Sampling was conducted from June to November 2021, and 151 ovaries were collected including the morphometric such as fork length (cm FL). Fresh ovary samples were fixed for histological analysis using the paraffin method and Harris-Hematoxylin and Eosin staining. The spawning season was determined by the presence of POFs at the spawning gonad development stage in the length range of 22-32 cm FL, indicating that peak spawning occurred in August. These findings can be used as a reference for formulating the management of bullet tuna in Bali.

1 Introduction

Neritic tuna is an important fishery commodity because it ranks as the second-largest catch of small pelagic fish in Indonesia [1]. One group of neritic tuna is bullet tuna (*Auxis rochei*), which is listed by the International Union for Conservation of Nature (IUCN) as Least Concern (LC). It is possible that species with an LC status still face the risk of extinction and will become a conservation concern if there is continuous fishing pressure. Reproduction data, including information on spawning season, size at maturity, and fecundity, are crucial for developing effective management strategies for bullet tuna. Understanding these reproductive patterns will help in setting appropriate fishing regulations, such as size limits,

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seasonal closures, and marine protected areas, to ensure sustainable populations and prevent overexploitation.

Bullet tuna exhibit an asynchronous spawning pattern, where the ovaries contain oocytes in all stages without a dominant oocyte. Ovaries have a mix of oocyte phases, allowing spawning to occur throughout the year [2-4]. Determining spawning patterns can be assessed macroscopically and microscopically by identifying the level of gonadal development and differentiation. Microscopic observation involves histological analysis of the gonadal tissue. Microscopic gonadal development is determined based on the composition of oocyte phases through the Most Advanced Group of Oocytes (MAGO) and classification of gonadal developmental stages, including postovulatory follicle (POF) availability [5]. The postovulatory follicle (POF) is a key indicator of gonadal development in fish. POF is a follicle in the ovary where the egg is released during ovulation [5]. POF is used to estimate spawning frequency by calculating the number of eggs spawned per breeding season [6]. The POF also indicates a peak in the annual spawning season [7]. This study enhances our understanding of reproductive biology, which can be linked to fishery resource management.

Spawning season estimation for bullet tuna, when observed microscopically, is considered accurate and detailed in determining the peak spawning of bullet tuna, allowing for identification of the size of the bullet tuna that is considered suitable for capture. The aim is to avoid overfishing [8]. Several studies have mentioned that the capture of bullet tuna in the waters of Nusa Penida Island, Bali, has reached overfishing status [9]. Regulations on fishing months based on peak spawning seasons are an example of fishery resource management, which can be established through government regulations to prevent negative impacts on fishery resources.

The aim of this study was to describe the histological characteristics of POF in bullet tuna, and to use this observation to calculate the frequency of POF, analyzed in relation to estimating the peak spawning season based on the presence of POF.

2 Materials and Methods

1.1 Data Collection and Histological Preparation

This study used a purposive stratified sampling technique [10] targeting 20 adult female fish per month. The samples were taken from gill net fishing boats (neritic gill nets) operating at the Kedonganan Fish Landing Base, Bali. Data collection and sampling of bullet tuna was conducted from June to November 2021, and 151 samples were collected. The morphometric aspects measured included forked length (cm FL), and the preparation of the female gonad samples in the laboratory followed the histological technique, which used the paraffin method with Harris-hematoxylin and eosin staining [11].

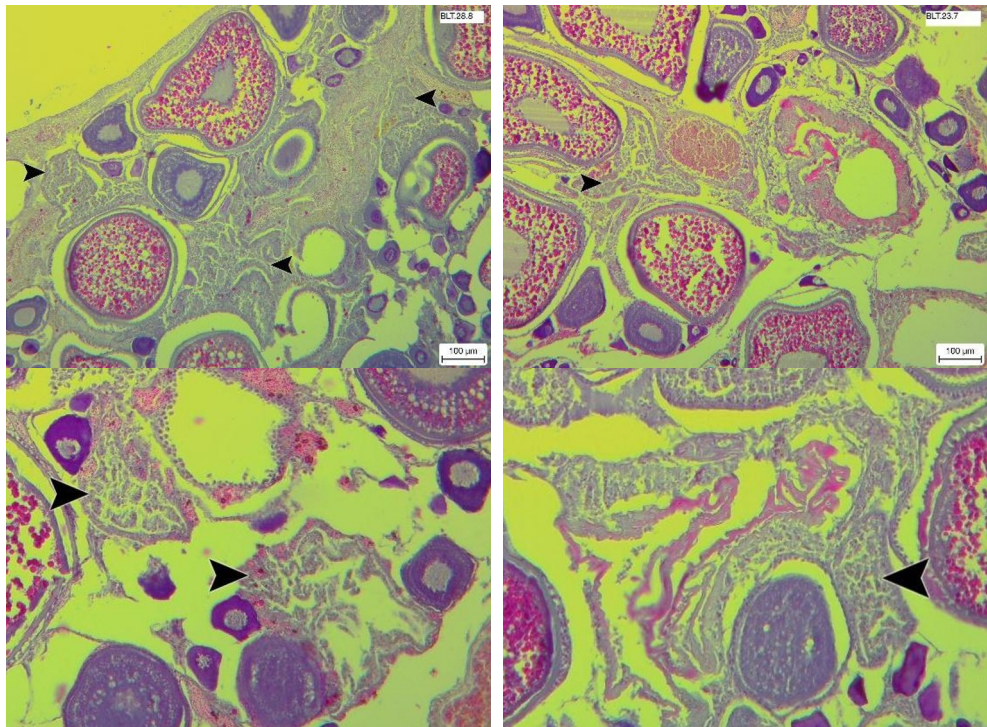
Samples were taken from the middle section of both the right and left lobes of the ovary, with a thickness of 2-3 mm, and fixed in 10% formalin. The histological preparation process included: (1) dehydration using graded alcohol (70%, 90%, and 96%), (2) washing with xylene, (3) infiltration with liquid paraffin, (4) embedding the tissue in liquid paraffin, (5) sectioning the tissue with a microtome to obtain intact sections 4-6 μm thick (as preparations), (6) staining with hematoxylin and eosin (HE), and (7) covering with cover glass and entellan.

1.2 Analysis of POF

The presence of POF was determined through the microscopic classification of gonadal development, which also described POF [4]. A POF is a follicle in the ovary that originates from hydrated oocytes that have undergone ovulation. POF is typically found in mature fish during spawning gonadal development [12]. POF analysis was conducted after identifying the level of gonadal development, where gonads containing advanced yolked oocytes, migratory nuclei, and hydrated oocytes required further observation to locate the POF. This approach aims to reduce the analysis time per histological preparation, as gonads identified with POF usually indicate active spawning conditions.

3 Results

The total number of female samples collected from June to November 2021 was 151, with lengths ranging from 15,7 to 33,5 cm FL. According to a previous study [13], histological preparations of 151 gonad samples of bullet tuna revealed eight stages of gonadal development: immature (26%) and developing (3%), while mature fish included spawning capable (10,5%), spawning (51,8%), regressing-potentially reproductive (2%), and regressed (5,1%). Postovulatory follicles (POF) were found in fish with active spawning status. Microscopically, signs indicating spawning were emphasized by the presence of oocytes, such as migratory nuclei and hydrated yolk, as well as the presence of POF (Figure 1).



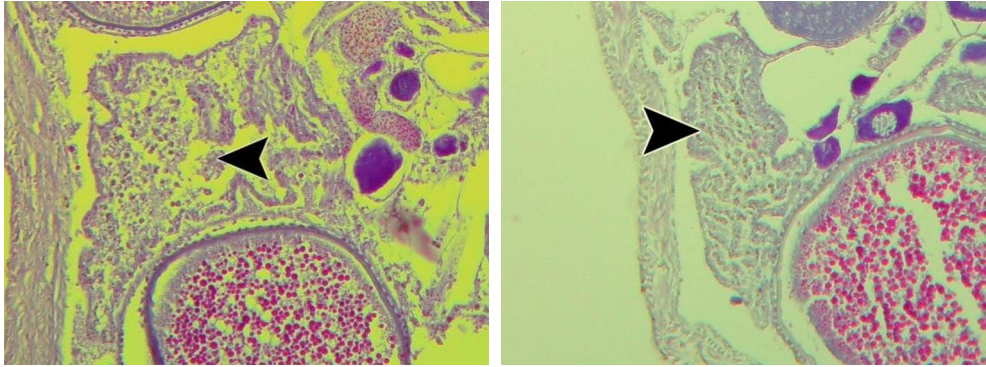


Fig. 1. The ovaries histological structure of the bullet tuna (*A. rochei*) showing the presence of POF (black arrow).

The results indicate that 22 female fish gonad samples showed the presence of postovulatory follicles (POF). The highest proportion of gonad samples identified with POF occurred in July and August, the lowest proportion with POF occurred in June and September, and with no POF in October and November (Figure 2). POF was found in fish with forked lengths ranging from 22 to 32 cm FL, with the majority ranging from 23 to 24 cm FL.

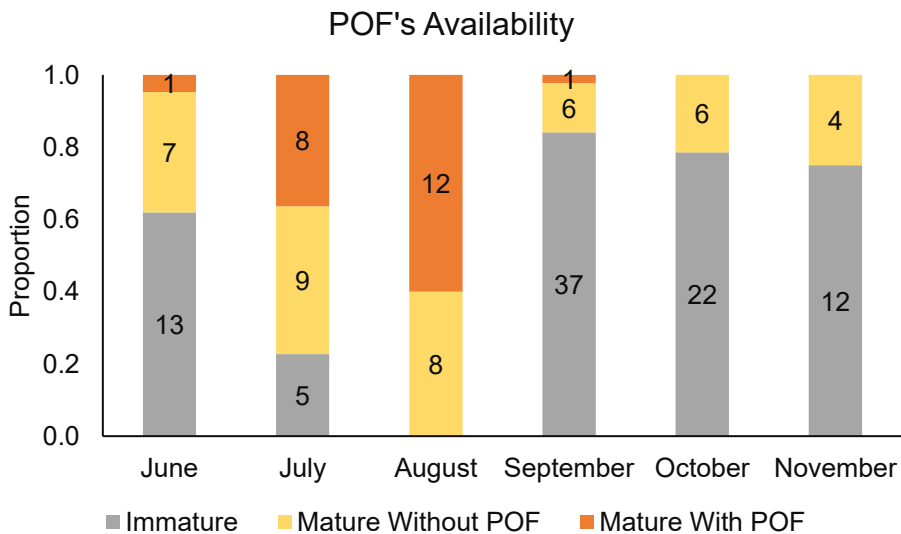


Fig. 2. Proportion of POF presence in bullet tuna (*A. rochei*) during June-November 2021.

4 Discussion

Microscopic observations indicate that bullet tuna spawn throughout the year, as spawning gonad development levels with postovulatory follicles (POF) were found nearly every month, except in October and November. This demonstrates that bullet tuna underwent multiple spawning phases in each season, with a peak spawning period in July and August. This finding is supported by a previous study on spawning seasons based on sex ratios and GSI values [13]. The sex ratio approached the ideal value of nearly 1:1 in August and the GSI peaked in July and August.

Spawning occurs concurrently with ovulation, during which hydrated oocytes are released from the surrounding follicles. Follicles, which are POF, remain unfragmented and do not exit the ovary along with hydrated oocytes. Initially, POF have a wrinkled shape, but over time, these follicles shrink and are reabsorbed [14].

4.1 Linking to Spawning season

The use of POF classification to determine spawning times was introduced by Hunter and Goldberg [15], which indicated that anchovies can spawn approximately 20 times a year. Determining spawning season can support the implementation of sustainable management strategies. The need for sustainable management was based on a previous study that showed that

The length at first capture was lower than the length at first maturity, indicating that young fish (immature and never spawned) were dominant in the catch and would be an overfishing status [16]. On the other hand, fishing pressure on bullet tuna is indicated by an exploitation rate above 0.5, which means overexploitation (overfishing) [9, 17]. Therefore, responsible and sustainable fishery management is essential for ensuring that fishery resources remain viable.

Responsible and sustainable fisheries management is based on research findings, particularly regarding the spawning seasons. Restrictions on fishing days or months and areas during spawning seasons are also necessary, as exemplified by the policy outlined in Indonesian PERMEN-KP Republik Indonesia No. 26, which limits the capture of yellowfin tuna (*Thunnus albacares*) in spawning and breeding areas in WPP RI 714 from October to December. Customary laws, local wisdom, and community involvement also support the enforcement of seasonal fishing restrictions.

This consideration is reflected in the Undang-Undang Republik Indonesia (UU RI) No. 31 Pasal 6 ayat 2 (2004) regarding fishery management. An example is Nyepi Segara on Nusa Penida Island, Bali, where marine activities, such as fishing and transportation to and from Nusa Penida, are halted for one day, typically observed in September or October [18].

Another example is the traditional "kenduri adat laut" or "hari pantang laut" in Pidie Jaya Regency, Aceh, which is held at least every three years, depending on the agreement and willingness of local fishermen. Fishing was halted for three days, from sunrise on the day of the feast until sunset on the third day. In addition to the "kenduri adat laut," there are other days of abstinence, including Fridays, from sunset on Thursday until sunset on Friday, as well as during the celebrations of Idul Fitri and Idul Adha, on Independence Day, August 17, and on December 26 to commemorate the tsunami that occurred on December 26, 2004 [19].

In addition, similar spawning seasons have been observed in studies on bullet tuna in the waters of Mejane in August [20], Tunisia from June to August [21], Indian waters from July to August [22], Mediterranean waters of Turkey from July to August [23], and Ligurian waters starting in July [24].

5 Conclusion

The spawning season for bullet tuna (*Auxis rochei*) in the presence of POF occurred in July and August, with lengths ranging from 22 to 32 cm FL. Spawning occurs concurrently with ovulation, during which hydrated oocytes are released from the surrounding follicles. Follicles, which are POF, remain unfragmented and do not exit the ovary along with hydrated oocytes. Determining spawning season can support the implementation of sustainable management strategies. Responsible and sustainable fisheries management is based on research findings, particularly regarding the spawning seasons.

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