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Reproductive Biology of Greenback Mullet, *Planiliza subviridis* (Valenciennes, 1836) from Segara Anakan Cilacap

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Abstract. Greenback Mullets (*Planiliza subviridis*) are a commercial commodity and are fished daily in the Segara Anakan Cilacap, although has not been overfished. Continuous and unregulated fishing might harm the Greenback Mullets population. Therefore, sustainable fisheries of this species are still needed. Reproductive biology is among the essential aspect that supports sustainable fisheries. This research aims to obtain information on the reproductive biology of Greenback Mullet in Segara Anakan Cilacap. The research used a survey method with a purposive random sampling technique. The observed parameters were the gonad maturity stage, gonad maturity index, and fecundity. The data were analyzed descriptively. The female individuals have gonad maturity stages III and IV, gonad maturity stage was 16.81±2.59 and 16.92±2.51, and fecundity was 37,208-183,661 and 38,468 - 272,835 in August and October, respectively. Conversely, male individuals have a low gonad maturity stage and gonad maturity index. Based on the data, Greenback Mullets in the Segara Anakan estuary have maximum gonad maturity stage, gonad maturity index, and fecundity in August and October, while in February they were in early gonadal development. These data are essential for sustainable fisheries management in the Segara Anakan estuary Cilacap, Central Java, especially for *Planiliza subviridis*.

Keywords: *estuary, fecundity, maturity stage, maturity index, mullets*.

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INTRODUCTION

Segara Anakan Cilacap is one of the estuary ecosystems, which is the meeting place of several rivers in the southwest of Central Java Province (Yuniarti et al., 2018). This estuary resides in the southern region of Cilacap Regency, Central Java, Indonesia (Rimadiyani et al., 2019; Kusbiyanto et al., 2020). It is a semi-open estuary because it is separated from the Indian Ocean by Nusakambangan Island (Redjeki et al., 2020). Segara Anakan estuary is connected to the open sea by two marine water inlets; an east opening and a west opening at both tips of the Nusakambangan Island (Rimadiyani et al., 2019). At the same time, the middle areas of Segara Anakan are highly affected by freshwater from several big rivers (Yuniarti et al., 2018). This condition makes Segara Anakan estuary a unique ecosystem in terms of its water salinity (Winarni et al., 2021).

Segara Anakan Estuary is a spawning ground and nursery ground of various aquatic fauna (Nuryanto et al., 2017; Pratiwi and Sukardjo 2018); Kusbiyanto et al., 2020). Moreover, it is also a habitat for various fish species, including Greenback Mullets (Suprastini et al., 2014: Setijanto and Rukayah, 2017). Greenback mullets (*Planiliza subviridis*) are among fish species that are highly tolerance to water quality changes, including water salinity, and consume a wide range of food items (Nuringtyas et al., 2019). These fish species migrate to the ocean for spawning and return to the estuary until the adult stage is reached (Saha and Kabir, 2014). Therefore, it is reasonable that Greenback Mullets could be found in the Segara Anakan estuary, Cilacap (Dawood et al., 2020, Suprastini et al., 2014). Greenback Mullets are also reported to live in other coastal regions in the south and north of Java Island (Herawati et al., 2020; Fitriah et al., 2021; Ratnaningsih et al., 2021; Afiyatillah et al., 2022).

Greenback Mullets have essential economic value and are widely consumed by the public (Ratnaningsih et al., 2021). According to the Ministry of Marine Affairs and Fisheries Statistics (2019), in 2019, Mullet fish caught in Cilacap waters amounted to 94.7 tons, while in 2020, it was 3.11 tons (Statistics of Capture Fisheries at the

Cilacap Ocean Fisheries Port, 2020). It means that the fishing rate of Mullet fish in Cilacap has not exceeded the maximum limit according to the Ministerial Decree of the Ministry of Marine Affairs and Fisheries Republic of Indonesia No. 47 (2016). On the ministerial decree, the allowed catch volume (JTB) for Mullet fish is 235.2 tons per year.

In the case of the Segara Anakan estuary, fishing for Greenback Mullets still needs serious attention because the Segara Anakan estuary is under ecological pressure caused by sedimentation from several big rivers and land conversion (Yuniarti et al., 2018). Sedimentation and other human activities have reduced the areas of Segara Anakan from 4,000 ha become approximately 2,200 in 2010. The size of the Segara Anakan estuary is suggested getting narrower than previously reported because high rates of sedimentation are still occurring (Utami et al., 2022).

The ecological pressure and fishing of Greenback Mullets in the Segara Anakan estuary might harm its population and might threaten the sustainable fisheries of Greenback Mullets. Sustainable fisheries of this mullet species are needed to be supported by adequate scientific data, including reproductive biology. The previous study reported that reproduction is an essential fish characteristic for sustainable fisheries and conservation (Soborido-Rey and Trippel, 2013). Reproductive characteristics of fish can be assessed using several parameters, such as Gonad Maturity Index (GMI), gonadal maturity stage (GMS), and fecundity (Rahman et al., 2015; Ratnaningsih et al., 2021; Susatyo et al., 2022).

Recent data about the reproductive biology of Greenback Mullet from the Segara Anakan estuary, Cilacap do not available. Previous studies reported reproductive characteristics of Greenback Mullets. Those studies reported different reproductive characteristics of *P. subviridis* among regions (Fitriah *et al*., 2021; Ratnaningsih *et al*., 2021). It is assumed that the reproductive characteristics of Greenback Mullets from the Segara Anakan estuary are also different from such characteristics reported in previous studies. However, this assumption must be proven through research.

This study aims to evaluate the gonad somatic stage, gonad somatic index, and fecundity of Greenback Mullets (*Planiliza subviridis*) from Segara Anakan Cilacap, Central Java, Indonesia. The data are essential for fisheries management in the Segara Anakan estuary, especially for Greenback mullets.

METHODS

Sampling location and time

Greenback Mullet's samples were collected in Karangsalam areas of the Segara Anakan estuary Cilacap, Central Java. The geographic position of the sampling sites was in the coordinates of 7°42'52.4"S and 108°56'09.2"E (Figure 1). Field trips were carried out in August and October 2021 and February 2022. Fish sample identification and reproductive characterization were carried out at the Animal Taxonomy Laboratory Faculty of Biology, Jenderal Soedirman University. These steps were performed from March to June 2022.

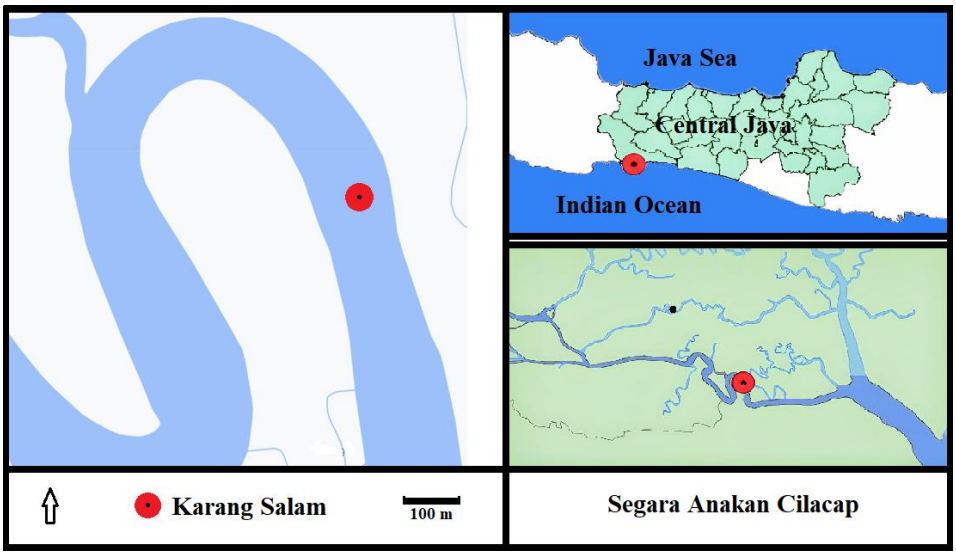

Figure 1. Research Location Map Showing Sampling Site (Sources: Geospatial Information Agency, Indonesian Sea Shapefile, Google Maps)

Research Parameter

Three main parameters were observed during the research to evaluate the reproductive characteristics of Greenback Mullets (*Planiliza subviridis*). These parameters were Gonad Maturity Stage (GMS), the Gonad Maturity Index (GMI), and fecundity. We measured also some additional parameters, i.e., total body weight, gonad weight, the weight of the gonad sample, and the number of eggs in gonad samples.

Research procedures

Fish collection

Fish samples were collected at the selected areas using gill nets that are commonly operated by the fishermen in the Segara Anakan estuary Cilacap (Figure 1). Sampling was carried out with help of the local fishermen. The fresh samples were directly placed in an ice box filled with ice and transported to the laboratory. In the laboratory, the samples were washed in running water before the identification step.

Fish Identification

Taxonomic status is the most essential information and the first step to be done before other steps in biology and ecology research. In this research, fish samples were identified by comparing their morphological characteristic with species characters in literature (White et al., 2013). The validity of the species' name was checked in Eschemeyer's Catalog of Fishes (Fricke et al., 2022).

Body weight measurement and fish dissection

Fish body weight was measured before dissection. The measurements were carried out using an electric balance with an accuracy of 0.01 g. Afterward, each specimen was dissected using a scalpel. Dissection procedures were as follow. Dissection was started from the anus to the anterior part of the body behind the operculum following ventral areas of the abdomen. Next step, dissection was continued from the anus to the dorsal areas following the body cavity. Then the scalpel was turnover to the anterior direction following the dorsal region of the body cavity up to the operculum. Finally, the flesh was cut off to expose the gonads (Susatyo et al., 2022).

Gonad maturity stage measurement

The gonad maturity stage (GMS) of fish is observed to know morphological changes of both male and female gonads during reproductive cycles. The changes were observed based on naked-eye observation. The gonad maturity stages of Greenback Mullets were determined based on the male and female gonad characteristics as described by Ahmad et al. (2018) (Table 1).

Table 1. Classification of Gonad Maturity Stage (GMS)

GMS	Female	Male
I	The ovaries are like threads, extending from the hind part to the anterior part of the body cavity, colorless, and smooth surface.	Testicles are like short threads visible at the hind part of the body cavity and colorless.
II	The size of the ovary is larger than in GMS I with a yellowish color. Eggs are not yet clearly visible visually.	The size of the testes is larger than GMS I. The color is white like milk. More prominence shape than GMS I.
III	Ovaries are yellow. Eggs are visible to the naked eye.	Testes become larger than GMS II with creamy white and translucent. It breaks easily when preserved.
IV	Have larger ovaries, eggs are yellow, and egg granules are visible and easily separated from one granule to another.	The testes have a larger size than GMS III and are milky white and solid which occupy most of the abdominal cavity.
V	The ovary has thick walls and is wrinkled; residual eggs are located near the release point. Lots of eggs like in stage II.	Testes size becomes reduced, and testicles become flattened and shrunk. Spermatozoa are located close to the release point.

Gonad maturity index measurement

The gonad maturity index (GMI) describes the proportion of gonads to body weight. Gonad weight was also measured using an electric balance with an accuracy of 0.01 g. The GMI of Greenback Mullets from the Segara Anakan estuary was estimated following the formula from Flores et al. (2015).

$$GMI = G/W$$

Note:
GMI= Gonad maturity index
G = gonad weight
W = Total body weight (includes gonad)

Fecundity estimation

The fecundity of Greenback Mullets was calculated using the gravimetric method. A small portion of the female gonads was taken and weighed using electric balance to estimate fish fecundity. The number of eggs in the gonad samples were calculated manually. Fecundity was estimated using the following formula after Islam et al. (2012).

$$F = n \times \frac{G}{g}$$

Note:
F: Fecundity (pcs)
n: Number of eggs in gonad sample
G: Weight of total gonad (g)
g: Weight of gonad sample (g)

Data Analysis

Data on the gonad maturity stage, gonad maturity index, and fecundity were analyzed descriptively by comparing gonad characteristics of Greenback Mullet's samples with standard characters in literature (Islam et al., 2012; Flores et al., 2015; Ahmad et al., 2018).

RESULT AND DISCUSSION

Specimen number and description

This study used a total number of 99 Greenback Mullets samples consisting of male and female individuals. The total body length of the fish samples ranges from 13.58 to 30.34 cm. Morphological observation proved that all the samples have the following characteristics; dark green dorsal, yellowish pectoral, white ventral, 4 - 5 spines with 8 - 9 soft rays in dorsal fins, and three hard spines with nine soft rays in the anal fin.

Planiliza subviridis has 4-5 dorsal spines, 8-9 dorsal rays, 3 anal spines. Additional characteristics of this species are dark green dorsally and white ventrally White et al. (2013) and Froese & Pauly, 2022). The observed characteristics of Greenback Mullets from the Segara Anakan estuary were fit well with the characteristics of *Planiliza subviridis* as described in the literatures. Therefore, this study determined that all mullet samples from the Segara Anakan estuary were taxonomically identified as *P. subviridis* (Figure 2). The validity check to Eschemeyer's Catalog of Fishes (Fricke et al., 2022) proved that *P. subviridis* is the valid name.

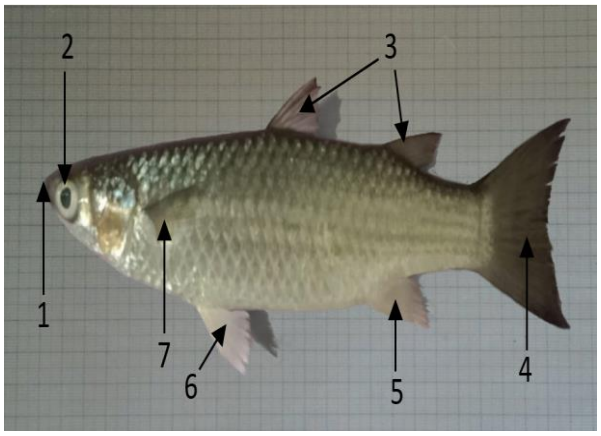

Figure 2. Morphological appearance of Greenback Mullets, *P. subviridis* (Valenciennes, 1836) from Segara Anakan estuary.

Note: (1) Mouth, (2) Eye, (3) Dorsal fins, (4) Caudal fin, (5) Anal fin, (6) Ventral fin, and (7) Pectoral fin

Gonad maturity stage (GMS)

After dissection, 75 out of 99 individuals of Greenback Mullet's samples were females. The remaining 24 individuals were identified as male (Table 2). Sexuality was determined based on the gonad morphology.

Table 2. Number of female and male individuals of Greenback Mullets with their GMS

Sampling period	Female GMS		Male GMS	
	III	IV	III	IV
August 2021	8	29	2	-
October 2021	7	23	-	-
February 2022	8	-	22	-
Total	23	52	24	

It could be observed in Table 2 that gonad maturity was faster on female than male individuals. It is shown by GMS IV that only observed in female individuals during period between August and October but none was observed in male individuals. This finding was in accordance to previous report that gonad development in female individuals of fish is faster than in male individuals (Parker et al., 2018)

Planiliza subviridis has ovaries fused anteriorly. This type of ovaries was also reported in wide range of fish species; such in *Puntius sarana* (Ahmad et al., 2018), *Schizothorax plagiostomus* (Pasha et al., 2016), and *Squalius platyceps* (Krivokapic 2015). Moreover, similar ovarium type was also reported in *Pomadasys stridens* (Amtyaz et al., 2013). Therefore, the ovarium of Greenback Mullets from the Segara Anakan shows the general type of fish ovary.

Careful and detailed observation of the gonad morphology of each Greenback Mullets (*P. subviridis*) individual proved that 23 female individuals had yellow ovaries with clearly visible egg granules. Ovaries filled out about two-thirds abdominal cavity. The remaining 52 female individuals have ovaries with yellow color and larger egg granules than the ovaries of the 23 female individuals (Figure 3). These gonads morphology indicated that 23 individuals of the female Greenback Mullets were in GMS III, while 52 female individuals were in GMS IV stage. These were because the gonad characteristics were similar to characteristics of GMS III and GMS IV as described by Ahmad et al. (2018) that GMS III of female gonad fish is characterized by yellow visible eggs, while GMS IV is characterized by similar color ovaries with GMS III but has a larger size than in GMS III.

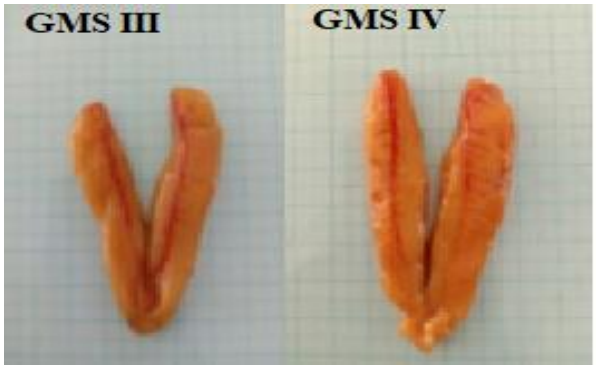

Figure 3. Morphology of female Greenback Mullet ovaries on GMS III (left) and IV (right)

According to the data in Table 2, most parts of the female individuals of Greenback Mullets from Segara Anakan estuary had GMS IV in August and October 2021, while no female individuals with GMS IV in February 2021. These phenomena indicate that female individuals matured and possibly spawn from August to October, while in February the female individuals were suggested in the early stages of gonadal development before the next spawning season in April and May. According to Djumanto et al. (2015), the spawning season of Mullet species is between April to May and September to October. However, it needs further observation of Greenback Mullets from the Segara Anakan estuary by collecting Greenback Mullets monthly over the year.

The present result was similar to the previous study that Greenback Mullets in the Cengkok coastal waters of Banten also in GMS IV in August. We could not make a further comparison in the following month, such as October and February because the previous study did not collect samples in those two months (October and February). However, we assumed that there were different spawning times and periods between Greenback Mullets in Segara Anakan and Cengkok coastal waters because we observed a high percentage of GMS IV both in August (78.37%) and October (76.66%), while previous study (Fitriah et al., 2021) observed maximum GMS IV were occurred in July and become significantly decreased in August. We suggested Greenback Mullets in the Segara Anakan spawn start in August to October, while from Cengkok bay start earlier in July but possibly finished in August. The difference in pawning times was also suggested between Greenback Mullets from the Segara Anakan estuary and the Northern coast of Indramayu West Java (Ratnaningsih et al. 2021). We did not observe female Greenback Mullets in GMS IV during sampling in February in the Segara Anakan estuary, while Ratnaningsih et al. (2021) found that most female Greenback Mullets in Indramayu coastal waters were in GMS IV February. These phenomena are assumed due to differences in ecological parameters among locations which need further clarification.

A careful observation proved that 24 male individuals had white color and translucent gonads but do not occupy most parts of the abdominal cavity. These characteristics were similar to the testes characters on GMS III described by Ahmad et al. (2018). Therefore, this study determined that most of the male individuals of Greenback Mullets in Segara Anakan estuary were in GMS III (Figure 4).

Figure 4. Morphology of Male Greenback Mullet testes on GMS III

According to the data in Table 2, two male individuals of Greenback Mullets were in GMS III but no specimen was in GMS IV in August. In October, none of the Greenback Mullets were either in GMS III or GMS IV was observed. These phenomena might indicate that male Greenback Mullets finished spawning in August and October. All fished Greenback Mullets had GMS III during sampling in February. This data become a strong indication that males Greenback Mullets in Segara Anakan estuary were in early gonadal development during February. It was assumed that male individuals will mature in March or April and be ready to spawn during these two months. Our assumption was made based on the result of a previous study that mullet species spawn from April to May and September to October (Djumanto et al., 2015). We could not make a comparison to the GMS of Greenback Mullets from the Cengkok coastal waters because Fitriah et al. (2021) did not collect samples in February. Further compared to Ratnaningsih et al. (2021) we found that there were differences in spawning time between male individuals of Greenback Mullets from Segara Anakan and Indramayu coastal waters because we only observed GMS III in February, while Ratnaningsih et al. (2021) reported that majority of male individuals of Greenback Mullets from Indramayu coastal waters were in GMS IV during February.

Gonad maturity index (GMI)

The other component of reproductive biology is the gonad maturity index. The value of the gonad maturity index of Greenback Mullet during sampling in the Segara Anakan estuary is presented in Table 3.

Table 3. Gonad Maturity Index (GMI) of Greenback Mullet

Year	Month	GMI (%)		Average ± Standard Deviation	
		Male	Female	Male	Female
2021	August	10.27 - 10.93	11.58 - 19.79	10.60 ± 0.46	16.81 ± 2.59
	October	-	11.07 - 19.84	-	16.92 ± 2.51
2022	February	8.15 - 12.85	10.98 - 14.05	10.45 ± 1.54	12.45 ± 1.00

The results show that the gonad maturity index (GMI) in the female Greenback Mullet is higher than in male individuals (Table 3). The data indicated that female individuals were mature first and followed by male individuals (Parker et al., 2018). The phenomena were also reported to occur on the Golden Gray Mullet (*Liza aurata*) in the Gulf of Gabes, Mediterranean, Tunisia (Abdallah et al., 2013) and Greenback Mullet (*Planiliza subviridis*) in Karangsong Coastal Waters, Indramayu (Ratnaningsih et al., 2021).

According to the data in Table 3, it can be assumed that the GMI of male and female individuals is less than 25%. According to Ridho (2021), GMI will be raised to maximum proportion until spawning has occurred. The spawning period occurred when GMI reach 25% of body weight. Therefore, the observed GMI values indicated that Greenback Mullets in the Segara Anakan estuary were not ready to spawn during sampling times. The observed GMI values support the GMS data that Greenback Mullets in the Segara Anakan estuary are most probably spawned from August to October because the highest GMI values were observed since August. However, the assumption needs to be strengthened by adding data obtained in other months such as June-July and September-November. Nevertheless, the highest GMI values observed in the current study were similar to the maximum values observed in the same species from southeast coast India (Rahman et al., 2015).

Fecundity

The fecundity of Greenback Mullet from the Segara Anakan estuary calculated during the study is presented in Table 4.

Table 4. Fecundity of Greenback Mullet

Year	Month	Fecundity	Average ± Standard Deviation
2021	August	37.208 - 183.661	73.765 ± 32.299
	October	38.468 - 272.835	99.749 ± 50.773
2022	February	19.674 - 93.706	64.908 ± 28.934

It can be seen in Table 4 that high fecundity was observed in August and October, while in February the fecundity was considerably lower than in August and October. The value indicated that some individuals almost spawned in both August and October. The data strengthen previous GMS and GMI data that Greenback Mullets in Segara Anakan estuary most probably spawned from August to October. This data indicated that the spawning time of Greenback Mullets is different among places (Rahman et al., 2015; Fitriah et al., 2021; Ratnaningsih et al., 2021). However, our result was similar to the report by Djumanto et al. (2015), that the spawning season of Mullet fish is between April to May and September to October.

One of the important components in reproductive biology is fecundity. It is essential for estimating fish production, stock-recruitment, and stock management (Qadri et al., 2015). Therefore, the present results can be used as a basis for policy makers in the Cilacap Regency to make a regulation of Greenback Mullets fisheries in the Segara Anakan estuary. Fishing activities of Greenback Mullets (*Planiliza subviridis*) in the Segara Anakan estuary should be restricted or even prohibited from August to October and April to May to ensure that the recruitment of Greenback Mullets running and safe.

CONCLUSION

Based on the result and discussion, Greenback Mullets, *Planiliza subviridis* in the Segara Anakan estuary have maximum gonad maturity stage, gonad maturity index, and fecundity in August and October, while in February they were in early gonadal development. These data are essential for sustainable fisheries management in the Segara Anakan estuary Cilacap, Central Java, especially for *Planiliza subviridis*. This is the first study about reproductive character of Greenback Mullets (*P. subviridis*) in the Segara Anakan estuary. However, the research was only covered narrow areas of the estuary (Karangsalam) and only short sampling periods. Further study with broader areas covering all areas of the Segara Anakan estuary and extending research periods are still needed to obtain comprehensive information about reproductive biology of Greenback Mullets (*P. subviridis*).

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