

## Reproduction Characteristics of Rice Field Eel (*Monopterus albus* Zuiewu) on Several Functionally Changed Lands in Banyumas Regency

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### ABSTRACT

Functional change of agricultural lands into non-agricultural lands will obviously have an impact on the diminished or loss of habitat of the rice field eels (*Monopterus albus* Zuiewu). The aim of this research was to understand the difference between (1) reproduction parameters (fecundity, gonadal maturity level (GML), and gonadal maturity index (GMI); (2) histological features of female and male gonadal development (oogenesis and spermatogenesis); (3) range of body length in each sexual phase; and (4) range of body length in each GML stage of captured eel from 4 sub-districts of Banyumas regency, Central Java (Ajibarang, Sumpuh, Banyumas, and Sumbang). The total number of captured subjects were 631 eels; which consisted of 155; 227; 157; and 92 eels from Ajibarang, Sumpuh, Banyumas, and Sumbang, respectively. We observed oogenesis in the stages of GML-I (chromatin nucleolar stages and perinucleolar stage); GML-II (cortical alveolar formation stage or globular yolk stage), GML-3 (late globular yolk stage), GML-IV (mature or ripe stage), and follicle atretic stage. Also, we observed male gonadogenesis and spermatogenesis in the intersex. The range of GMI of captured eels was between 0.023 and 0.686. Eel in GML-I, GML-2, GML-3, and GML-IV had GMI within the range of 0.023-0.096; 0.096-0.425; 0.427-0.686; and >0.686, respectively. Eels achieved stage of GML-IV in body length range of 22.6-34.5 cm in Sumpuh, 22.6-38.5 cm in Ajibarang, and 26.6-34.5 cm in Banyumas. There was no eel with the status of GML-IV in Sumbang, which had the most residential or functionally changed lands in its area. Based on these findings, can be concluded that the condition of rice fields or habitat of eel in functionally changed lands may affect eel's growth and gonad maturation.

**Keywords:** Rice field eel (*Monopterus albus* Zuiewu), Gonad Maturity Level (GML), Gonad Maturity Index (GSI), Oogenesis

### INTRODUCTION

Functional change of agricultural lands (rice fields or green farming area) into non-agricultural lands (residential or industrial area) are currently inevitable [1]. This condition may threaten the habitat of living things within the area and cause environmental imbalance. One of the living creatures, which are currently threatened to lose their habitat is rice field eel (*Monopterus albus* Zuiewu) [2].

Rice field eel is the original fish from Indonesia (Figure 1). This fish can exploit oxygen from the atmosphere. Therefore, it can survive in limited water condition. It also has a tolerance of temperature and salinity [3]. Also, eel can pass through wet soil for migration [4]. We can find Eels in other Asian countries, including India, China, Japan, and Malaysia [5].



Figure 1. Rice field eel (*Monopterus albus* Zuiewu)

In China, eel is the most important and economically valuable fish [6]. Yin and Liu [6] reported that eel production in China in 2004 was 137,486 tons. Eel protein is rich in several essential amino acids, such as lysine and methionine. Also, it contains phosphorus, crude fiber, and calcium. Eel can give benefit either as

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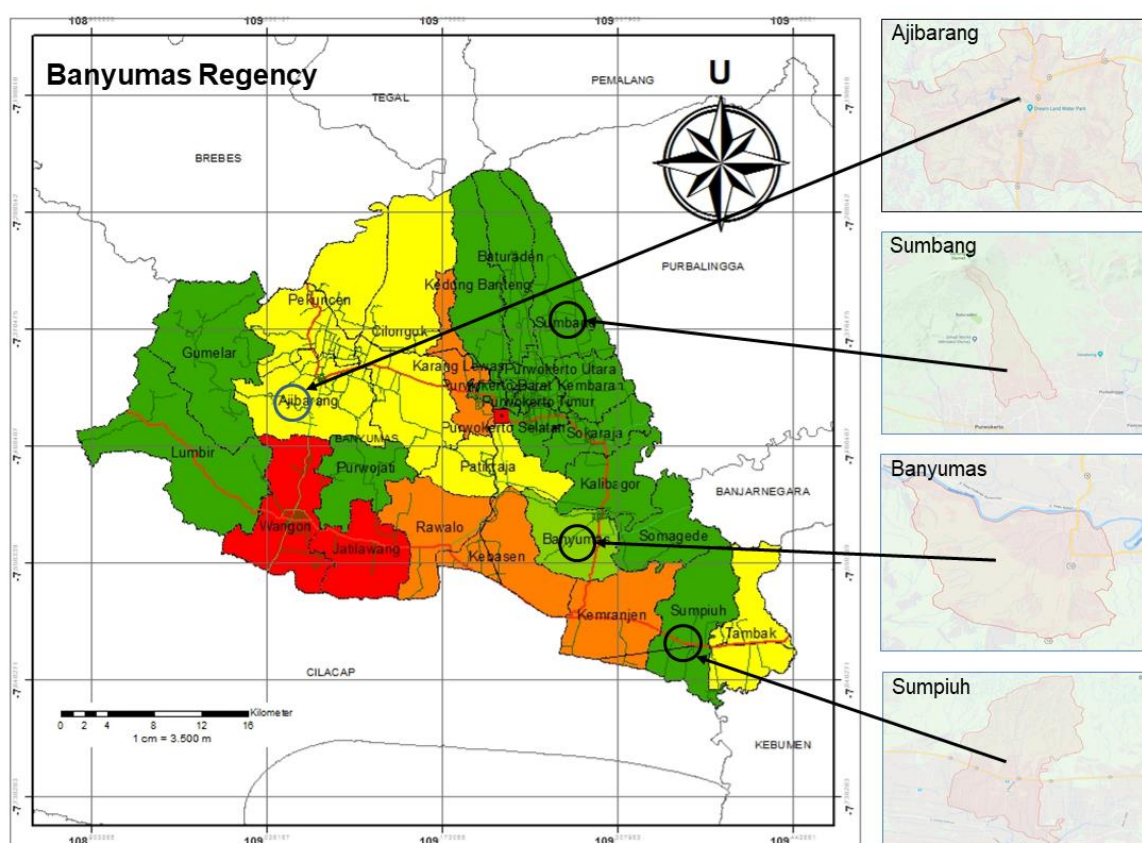


Figure 2. Location of sampling area at Ajibarang sub-district ( $7^{\circ}25'28.380''$ LS  $109^{\circ}4'8.751''$ BT), Sumbang sub-district ( $7^{\circ}21'50.795''$ LS  $109^{\circ}16'16.531''$ BT), Banyumas sub-district ( $7^{\circ}31'43.898''$ LS  $109^{\circ}16'16.531''$ BT) and Sumpiuh sub-district ( $7^{\circ}35'10.976''$ LS  $109^{\circ}21'59.125''$ BT) in Banyumas Regency ( $7^{\circ}36'42''$  S,  $109^{\circ}21'13''$  E)

food for human or as a mixture of chicken feed [7]. Despite its importance, there are no intensive cultivation of eel in Indonesia.

The aim of this research was to understand the difference between (1) reproduction parameters (fecundity, gonadal maturity level (GML), and gonadal maturity index (GMI)); (2) histological features of female and male gonadal development (oogenesis and spermatogenesis); (3) range of body length in each sexual phase; and (4) range of body length in each GML stage of captured eel from Banyumas regency of Central Java.

## MATERIALS AND METHOD

### Research materials

The material used in this research were male and female eels with a body length of 10-29 cm for female and >30 cm for male [8].

### Research method and study sites

This research was using a stratified random sampling method to determine four sub-districts as repre-

sentative of total 27 sub-districts in Banyumas Regency, Central Java, Indonesia. The selected sub-districts were Ajibarang, Sumbang, Sumpiuh, and Banyumas. We chose three villages from each sub-district as the location for capturing eel. These chosen villages were as follows: Tipar Kidul, Kalibenda, and Pancasan of sub-district Ajibarang; Karanggintung, Tambaksogra, and Banteran of sub-district Sumbang; Nusadadi, Karet, and Selanegara of sub-district Sumpiuh; and Pekunden, Kejawar, and Dawuhan of sub-district Banyumas (Figure 2).

The four sub-districts were representing four different conditions of land functions. Sumpiuh and Banyumas sub-districts represented natural rice field area. On the other hand, Sumbang and Ajibarang sub-districts represented a residential and industrial area with a change of function of rice field into plantation (orange, chili, maize and others).

### Morphometric identification of eel sex

Referring to research by Riani and Ermawati [8] in

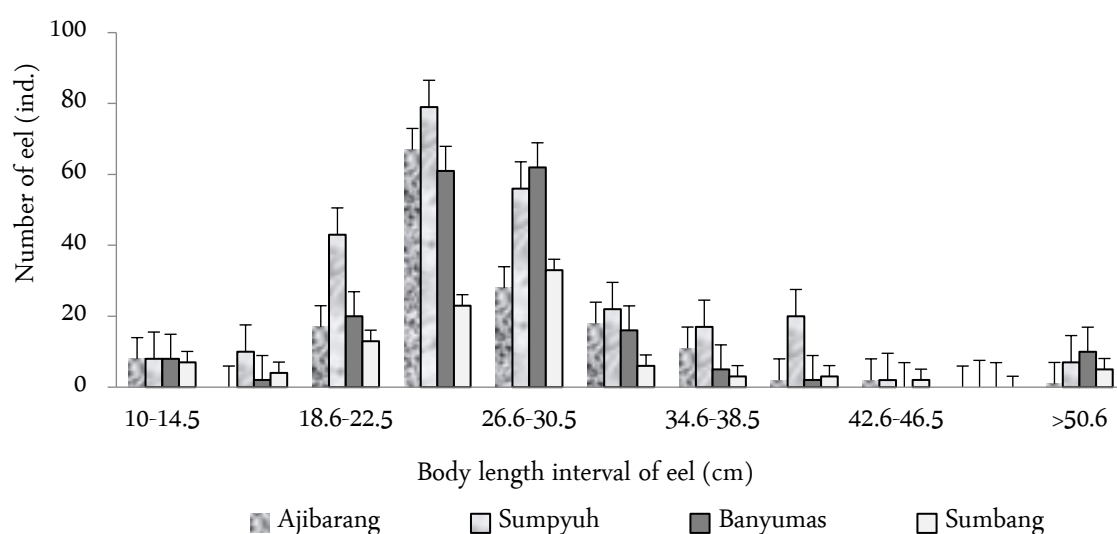


Figure 3. Number of captured eels in each body length interval group

Central Java, Yogyakarta, and West Java, we can use body length of eel for sex differentiation. Eel with body length < 29 cm is a female, while eel with body length > 30 cm is a male.

#### Gonad Maturity Index (GMI)

The GMI was calculated by comparing the weight of the gonads with the body weight of the fish and multiplied by one hundred percent by Effendie's formula [9]:

$$GMI = \frac{GW}{BW} \times 100\%$$

Note:

GW: Gonad Weight

BW: Body Weight

#### Gonad Maturity Level (GML)

The GML was identified according to the description of GML by Taufik and Saparinto's Method [10].

#### Identification of eel's gonad histology

The preparation of gonad histology of male and female eel was using paraffin method with Haematoxylin-Eosin staining according to Suntoro Method [11], which has been modified by our Structure and Development Laboratory. The slide was observed using a light microscope with 400× of magnification.

#### Ricefields superficial water quality

The supporting data was water quality during the research. Physical analysis of water was including tem-

perature and pH. Water temperature was measured using mercury thermometer (°C). The degree of acidity was measured using calibrated pH meter.

#### Data analysis

Monthly data of eel's GMI, Fecundity, and GML from 12 villages of four sub-districts in Banyumas Regency were presented in table and bar chart. The histological features of the gonads were analyzed descriptively.

## RESULTS AND DISCUSSION

#### Number of captured eels

The total number of captured eels were 631; which were consisted of 155; 227; 157; and 92 eels from Ajibarang, Sumpyuh, Banyumas, and Sumbang, respectively (Figure 3). We divided the body length data of eels into eleven body length intervals. Majority of captured eels in 4 sub-districts were in the 22.6-26.5 cm of the body length interval group.

In May 2017, the majority of sub-district in Banyumas Regency had entered the rice planting season with adequate water on the surface of rice fields. The captured eels were still available, although the planting season had been begun in February 2017. However, in June and July 2017, there was no adequate water on the surface of rice field, and eels were more difficult to obtain.

In Sumpyuh, the beginning of the rice planting season was at the end of March or in early April. Therefore, in May, June, and even July 2017 eels were still easy to obtain. Eels from Sumpyuh sub-district dominated both

in the number, body length, and body weight. In contrast, eels from Sumbang sub-district had the lowest number among four sub-districts. Furthermore, there were no eels with a body length between 30.6 and 34.5 cm in Sumbang. In Sumpyuh sub-district, there was female eel with 51 cm of body length, 121 g of weight, and 5.89 g of gonadal weight (GMI = 0.48). There was also male eel with 71 cm of body length, 298 g of body weight, and 0.48 g of gonad weight. These findings were different from the data from Susatyo and Sugiharto [12], which stated that the female eel from the Banyumas region had 15 – 20 g of body weight and 20 cm of body length. Natural feature of rice fields in Sumpyuh sub-district, which adequately provide the nutritional needs for eel's growth, might be the cause of these differences. Also, the soil of rice fields in Sumpyuh sub-district contained the highest clay contents among four sub-districts.

Clay contents of the soil represent the nutritional source for eel's growth [13, 14]. Clay content in Sumpyuh, Ajibarang, Banyumas, and Sumbang sub-districts were 41.75%, 38.56%, 35.58%, and 28.3%, respectively (Primary data, results of the Agronomy Laboratory Analysis, Faculty of Agriculture Unsoed, 2017).

Furthermore, in Banyumas and Sumpyuh sub-districts, there were many rice milling factories, which activities cause high noise and vibration. However, these factories were located quite far from rice field area, where eels were captured. Therefore, the life of eels in the rice fields was not significantly disturbed.

### ***Gonad maturity level and sex change***

The GML of captured eels in this research can be seen in Figure 4A, 4B, 4C, and 4D. This result was different from research by Riani and Ernawati [8], which stated that eels with body length of 16.9-20.8 cm have GML-II and GML-III. There were eels with a body length of 16.9 – 20.8 cm, which already underwent transition period or sex change. Eels with GML-IV were only found at a body length interval of 24.9 – 28.8 cm. In addition, Ani and Ernawati [8] explained that female eels had body length of < 29 cm and changed into a male at a body length of > 29 cm. Body length interval 32.9 – 36.8 cm and 40.9 – 44.8 cm were only found in male eel. Therefore, in their research, eel with a body length of 32.9 cm had been started to change into a male.

Chadijah [15] explained that the female sex phase of eel was achieved at GML-III. At the GML-III stage, the weight of gonads reaches its maximum and the eggs

were started to be released. The value of GIS in the transition phase (GML-V) were decreased because of the gonad weight in that phase was zero. The highest score of GSI of male eels was achieved on GML-VIII, because of the gonad weight in that phase reached its maximum.

Environmental factors such as substrate and alkalinity might be the cause of the differences in data between sub-districts in this research. These factors can accelerate the conversion of eel's sex into a male. In this state, eel is already old. Sex change occurs because the eel is a protogynous hermaphrodite with multiple genitals, which will be changed from female to male in its older age [16].

Eel life cycle begins with the differentiated juvenile period. Furthermore, it develops into females and subsequently undergoes an intersex or transitional period. After this period, the eel will turn into a functional adult male [17]. Also, reproduction status or sex of eel also can be determined by its body length.

Rice fields along Ajibarang and Wagon highway have now become an industrial area (e.g., Bima cement, recreation, sand mining, rock mining, and mineral water industry). This functional change of lands might cause habitat reduction of eel. The waste product might also contaminate its source of life. The impact can be seen in the delayed of achievement of the GML-IV stage. However, the result of pH measurement on Ajibarang rice fields was still within the normal range for growth of organism according to Dewi *et al.* [18], which was 6 – 7. The results of pH measurement on the Ajibarang rice field (around the location of the Bima cement industry) were within the range of 6.2 – 6.6.

The temperature of the water surface of rice fields in 4 sub-districts was within the range of 26.2 – 27.6°C. According to Kordi [19], the optimal temperature for eel's growth was within the temperature of 25 – 32°C. A similar result also found by Fujiani [20], which stated that temperature of 20 – 27.8°C and pH 7 is a proper medium for eel's growth.

District was achieved faster than eel from Ajibarang sub-district. It was achieved at 10-14.5 cm of body length interval. The GML-IV of eel from Sumpyuh sub-district was achieved at 26.5 – 30.5 cm of body length interval. It was also faster than the achievement of eel from Ajibarang sub-district.

Sumbang sub-district had the lowest number of captured eels. Furthermore, another factor that affects the number of captured eel in this sub-district was clay content. In their original habitat, eel consumes invertebrates

and vertebrates including fish [13, 14]. Eel also consumes Mollusca, frog, fish egg [21, 22].

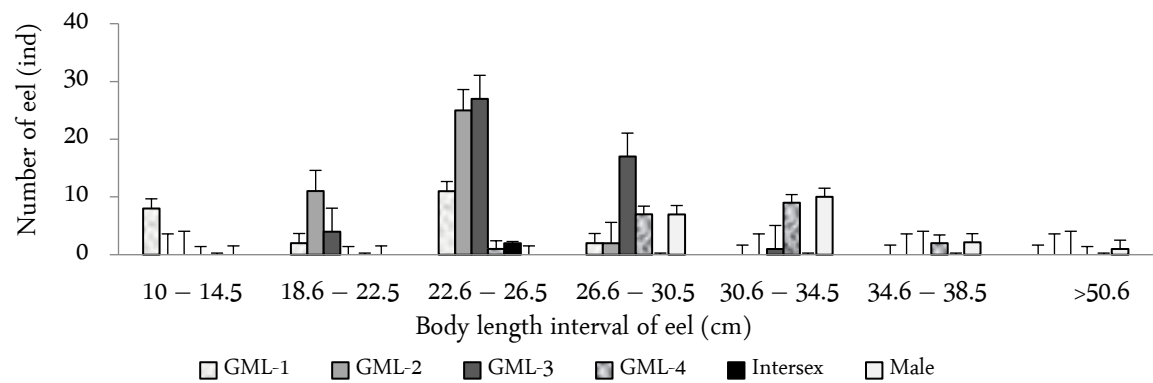
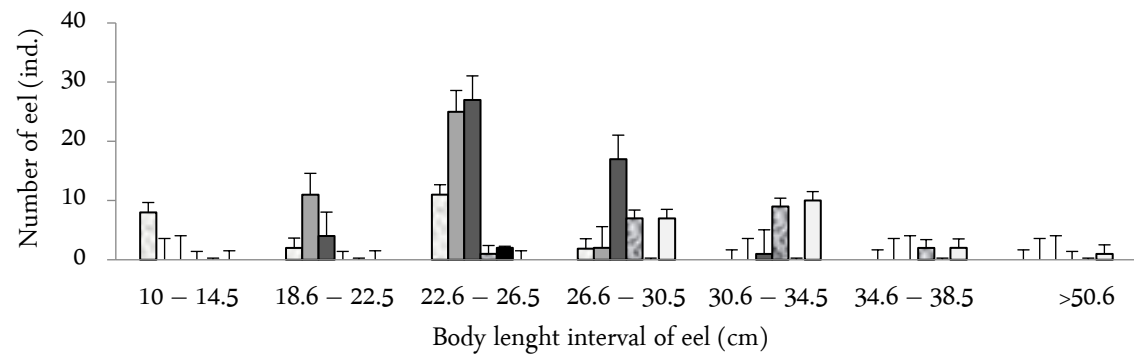
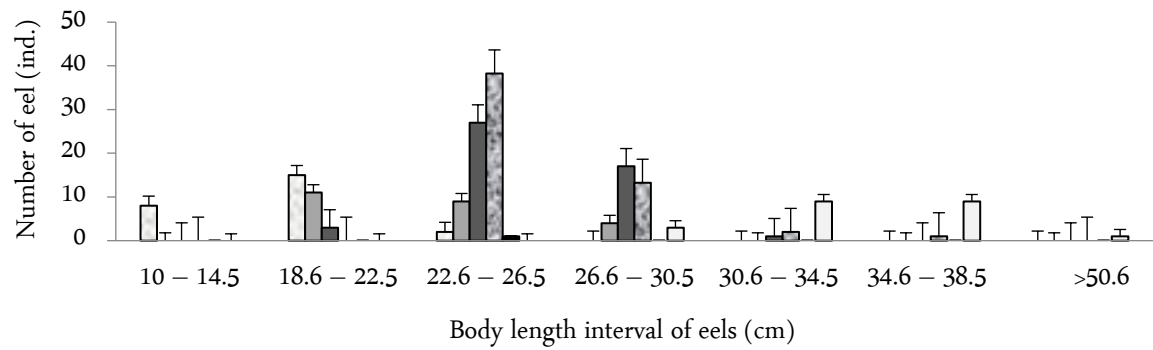
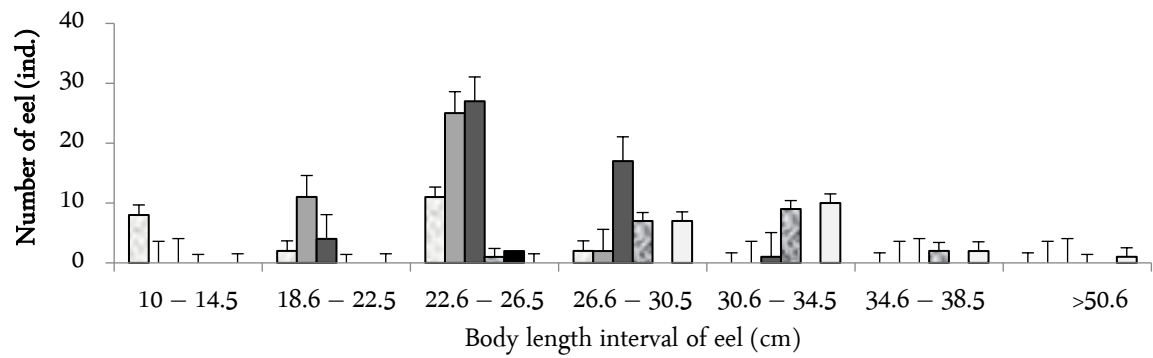


Figure 3. The number of captured eels and Sex versus GML on Specific Body length intervals in A) Ajibarang; B) Sumpyuh; C) Banyumas; and D) Sumbang sub-district



On the location of eel capturing in the three sub-districts (except Sumbang), eels were found in the mature form of sex (the female with GML-IV) at 16.9 cm of body length. These eels underwent sex change on varied body length. There was eel in intersex stage at 20.8 cm of body length. At body length interval of 27.4 – 35.8 cm, eel underwent sex changing into a male. These results are different with research by Riani and Ermawati [8], which stated that eel with 10 – 29 cm of body length has female sex, while eel with > 30 cm of body length has changed into male. Rice field eel species is a protogynous hermaphrodite, which starts its reproductive stadium and mature as a functional female. Subsequently, it will differentiate into intersex individual and grows larger until it becomes a functional male [22].

These data showed that sex change was not determined by eel's body length. It tends to be determined by eel's age. Eel body length in certain age can be varied. It depends on genetic, nutrient, and environmental factors [23].

Chadijah [15] observed the relationship between body weight and body length of eel at Sidenreng Lake in Sidenreng Rappang Regency, South Sulawesi. She stated that female eel had body length within the interval of 33.1 – 66.2 cm, body length average of 42.4 cm, body weight within 38.113 – 294.884 g, and body weight average of 92.242 g. The intersex eel had body length within 36.5 – 67.5 cm, body length average of 45.3 cm, body weight within 53.630 – 311.121 g, and body weight average of 105.157 g. Eel reached male sex stage when its body length within 44.5 – 70.0 cm, body length average of 48.5 cm, body weight within 100.110 – 343.889 g, and body weight average of 135.869 g.

Sex change on eel happens because of its nature as the protogynous hermaphrodite, which undergoes sex change from female into a male. This change happens because, in the gonad of eel, there are both ovary and testes. During their lifetime, the eel will undergo several changes. In juvenile stage, it is undifferentiated. As the ovary developing, it becomes female. After becomes female, its ovary will eventually shrink and eel enter the intersex stage. Finally, the testes will be developed and eel becomes a functional male [24].

Figure 4A, 4B, 4C, and 4D illustrate the influence of a functional change of the lands (as the habitat of eel) and activities (farming, constructing, and waste product disposing of an industry) from the four sub-districts. As a result, there was a low number of captured eels, a delayed maturity of the gonad, and extreme cases of a female with body length above 50 cm and male with body

length above average.

### Gonad maturity index

Gonad Maturity Index (GMI) is the value in percentage as the result of comparison between gonad weight and eel body weight. The number of eels captured and their relationship with GMI on each sub-district can be seen in Table. Furthermore, it is confirmed on GML data in each sub-district at Figure 4A, 4B, 4C, and 4D.

In Ajibarang, gonad maturation happened in body length interval of 26.6 – 30.5 cm. As the example, eel with a body length of 29.2 cm had GMI 0.6835. These data showed that in the interval of 26.6 – 30.5 cm, eel was prepared to spawn with a large size of the gonad. In Sumpuyuh sub-district, female eel with mature gonad had body length intervals of 22.6 – 26.5 cm and 26.6–30.5 cm with the highest GMI within 0.835 – 2.32. In Banyumas sub-district, female eel with mature gonad had body length intervals of 22.6 – 26.5 cm and 26.6–30.5 cm with the highest GMI of 0.425. On the other hand, there was no mature gonad in eels from Sumbang sub-district. Eel in Sumbang sub-district had the highest GMI of 0.275.

According to Figure 4, there was female eel with 5.89 g of gonad weight, 121 g of body weight, and 51 cm of body length from Nusadadi Village in Sumpuyuh sub-district. Therefore, its calculated GMI was 0.48. This female eel Gonad Maturity Index (GMI) or Gonad Somatic Index (GSI) was 0.48. After its fecundity being calculated, there were 363 eggs of this female with the diameter between 1.3 mm and 3 mm. Although the GMI was not above 0.6, this female was not ready to spawn. Whereas, the eel with the highest body weight (298 g) and the highest body length (71 cm) had 0.4 g of gonad weight and male sex. This large male eel was also captured at Nusadadi Village in Sumpuyuh Sub-district.

Gonad Maturity Index was correlated with Gonad Maturity Level. The GMI was increasing in female sex phase and was decreasing on GML-IV. On GML-V, eel had entered the intersex phase. The GMI of male eel was increasing on GML VI-IX and was decreasing on GML-X. On research by Chadijah [15], a female phase was achieved in GML-III with maximum gonad weight. Therefore, indication for egg release was high. Value of GMI on GML-V was decreasing because of zero weight of the gonad. The highest GMI on male eel was achieved on GML-VIII because of maximum weight of the gonad at this stage.

Table 1. Number of eels in four sub-district According to Gonad Maturity Index (GMI)

Interval of GMI	Number of Eels (individuals)			
	Ajibarang Sub-district	Sumpyuh Sub-district	Banyumas Sub-district	Sumbang Sub-district
0.0236 – 0.0445	13	16	19	12
0.0446 – 0.0655	4	13	16	11
0.0656 – 0.0965	6	7	18	11
0.0966 – 0.3245	18	43	26	18
0.3245 – 0.4275	20	29	29	21
0.4275 – 0.5285	11	26	13	6
0.5285 – 0.6865	18	27	16	5
> 0.6866	20	57	11	0

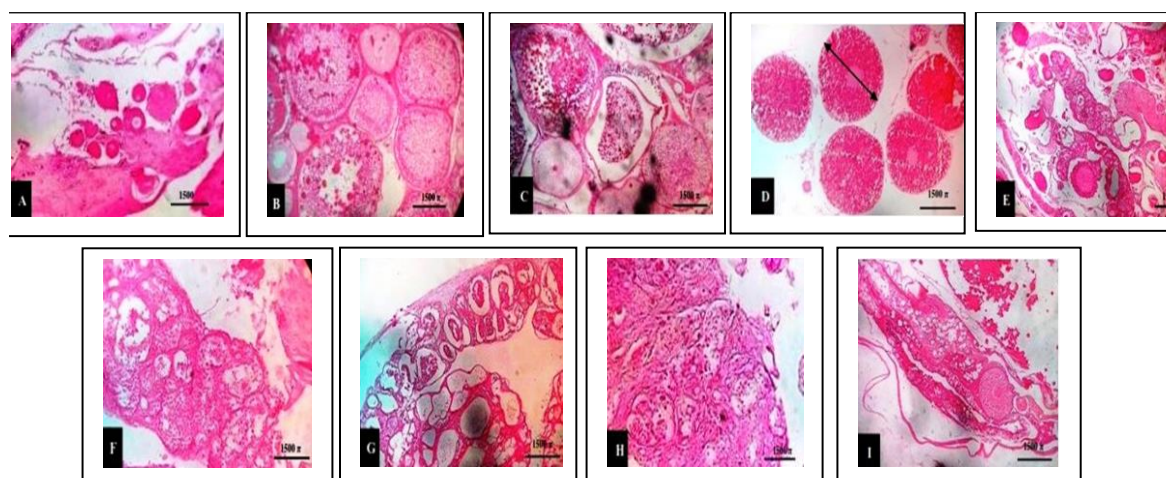


Figure 5. Micrograph / Histology of Gonad Development (Gonadogenesis) of Female, Intersex, and Male Eel. Note: (A) GML-I (10-14.5 cm and 14.6-18.5 cm of body length interval); (B) GML-II (18.6-22.5 cm of body length interval); (C) GML-III (22.6-26.5 cm of body length interval); (D) GML-IV (26.6-34.5 cm of body length interval); (E) intersex (26.5-34.5 cm of body length interval); (F) pre-functional male from intersex; (G) Functional Male (30.6–40 cm of body length interval); (H) Lobulation of Testes; I. Atretic stage

### ***Development of gonad (gonadogenesis) in female, intersex, and functional male***

Sex change of rice field eel can be determined from its body length. It is confirmed by histological observation of eel's gonad. Morphologically, female eel had brownish-black back, yellowish-white abdomen, small head, and long sharp-end tail. The male eel had greenish-brown back, brownies yellow abdomen, large head, and short blunt-end tail. Microscopic figure of eel's gonad development showed that gonadogenesis or oogenesis in female eel was observed in the stages of GML-

Microscopic figure of eel's gonad development 1 (chromatin nucleolar stages and perinucleolar stage); GML-II (cortical alveolar formation stage or globular yolk stage), GML-3 (late globular yolk stage), GML-IV (mature or ripe stage), and follicle atretic stage. This result was similar to identification by Ravaglia and

Maggese [25]. Gonadogenesis or spermatogenesis in male eel is the reproduction process, which involved sexual differentiation process, regulation of germinal cell, and development of gonad somatic cell [26]. This process was observed in pre-functional and functional male stage.

### ***Fecundity and diameter of female eggs***

Fecundity measurement is important to understand the dynamic of the population and life history of fish [17]. Fecundity is the amount of eggs, which were released from the ovary before spawning. It affects the number of produced tillers. Fecundity is strongly related to body weight, body length, age, diameter of egg, and the egg protection manner. Fish with high fecundity had no egg protection habit. It also had small eggs. Current



Table 2. The diameter of egg of captured eel in the four sub-districts

Interval of Egg Diameter	Ajibarang Sub-district		Sumpuyuh Sub-district		Banyumas Sub-district		Sumbang Sub-district	
	GML-III	GML-IV	GML-III	GML-IV	GML-III	GML-IV	GML- III	GML-IV
0.0625 - 0.1624	193	11	152	22	121	0	83	0
0.1625 - 0.2624	90	33	76	30	68	0	76	0
0.2625 - 0.3624	11	11	2	0	4	0	2	0
0.3625 - 0.4624	19	21	33	21	5	0	5	0
0.4625 - 0.5624	5	25	11	22	2	0	2	0
0.5625 - 0.6624	3	6	0	11	0	0	0	0
0.6625 - 0.7624	2	4	0	13	0	0	0	0
0.7625 - 0.8624	0	2	0	0	0	0	0	0
0.8625 - 0.9624	0	1	0	12	0	0	0	0
0.9625 - 1.0624	0	6	0	17	0	0	0	0
1.0625 - 1.1624	0	15	0	3	0	0	0	0
1.1625 - 1.2624	0	13	0	0	0	0	0	0
1.26225 - 1.46554	0	0	8	1	0	0	0	0
1.46555 - 1.7654	0	0	7	3	0	0	0	0
1.7655 - 2.2555	11	0	6	9	0	0	0	0
2.2556 - 3.1455	19	20	59	32	0	0	0	0
<b>Total of Eggs</b>	<b>353</b>	<b>168</b>	<b>363</b>	<b>194</b>	<b>200</b>	<b>0</b>	<b>168</b>	<b>0</b>

research showed that fecundity of eel was within the range of 36 – 363 eggs and had 95 eggs on average. This low fecundity might be caused by its habit of protecting eggs. It is similar to the result of a research by Bozkurt and Ögretmen [27], which showed that fish with eggs protecting habit had low fecundity.

According to Damsgaard [28], fertilized egg of rice field eel will be swallowed into male's mouth. Furthermore, the male eel will spread it out in its new hiding hole in the soil. The eggs will be left by the male eel after 15 days post-hatching. The diameter of eel's egg in current research is presented in Table 2.

Table 2 showed that in the certain stadium, there were several diameters of eggs. This finding showed that development of egg cells in eel's gonad was relatively not similar. Therefore, these eggs cannot be released at the same time, which was resulting in the different size of eggs. In Sumbang sub-district, there was no eel with GML-IV. Even in gonad that was ready to spawn, GML-IV eel showed the multilevel peak of eggs diameter. Type of eel spawning can be determined either by its eggs diameter or its histological features [29]. Based on these parameters, a fish can be divided into total spawner or partial spawner type. In total spawner type fish, diameter and histological figure of eggs in its gonad are relatively similar. On the other hand, in partial spawner type fish, there are different size of egg's diameter. In the current research, the eggs had more than one peak in diameter and the different size on histological examination. Therefore, eel is a partial spawner type [30].

In a total spawner fish, diameter and histological figure of eggs in its gonad are relatively similar. In contrast, partial spawner fish had different size of eggs [31]. Generally, among all of the parameters in this research, environmental factors, availability natural feed, and nutrition effect on life sustainability, growth, sex maturation, and another period [32].

Laboratory examination result of clay contents of soil in Sumpuyuh, Ajibarang, Banyumas, and Sumbang sub-districts were 41.75%, 38.56%, 35.58%, and 28.3%, respectively (Primary data of Agronomy Laboratory Analysis of Faculty of Agriculture, Unsoed, 2017). Clay content of eel habitat facilitates the availability of eel's natural feed. In Sumpuyuh sub-district, as the best location, the soil was contained earthworms and snails. Earthworms are one of the feeds that contains proteins. It contains 67 – 76% of protein [33].

Generally, among all of the parameters in this research, environmental factors, availability natural feed, and nutrition have an effect for life sustainability, growth, sex maturation, and another period.

## CONCLUSION

Based on the discussion section below, can be concluded that:

1. Sex change of eel from female to male occurred at 29 cm of body length. Sex change was determined by eel's age. Eel is a fish, which had eggs protecting habit. It was resulting in low fecundity, which was within the range of 36-363 eggs. Eel is a partial

spawner fish. The highest number of captured eel was within the body length interval of 22.6 - 26.5 cm. Stage of GML-IV was achieved in eel's body length range of 22.6-34.5 cm in Sumpyuh, 22.6-38.5 cm in Ajibarang, and 26.6-34.5 cm in Banyumas. There was no eel that reached the status of GML-IV in Sumbang. The GMI was within the range of 0.023 - 0.686.

2. Oogenesis was observed in the stages of GML-1 (chromatin nucleolar stages and perinucleolar stage); GML-II (cortical alveolar formation stage or globular yolk stage), GML-3 (late globular yolk stage), GML-IV (mature or ripe stage), and follicle atretic stage. Male gonadogenesis and spermatogenesis were observed in the intersex.
3. Eel in GML-I, GML-2, GML-3, and GML-IV had GMI within the range of 0.023-0.096, 0.096-0.425, 0.427-0.686, and >0.686, respectively. The number of captured eel with GML-IV was obtained in Sumpyuh, Ajibarang, Banyumas, and Sumbang sub-districts were 57, 20, 11, and 0, respectively.

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