Characteristics of productive broodstock based on body length and gonad histology of eel (*Monopterus albus*) in Semarang

N Setiati*, Partaya, S Ngabekti, B Priyono, and S. Rabiha

Department of Biology, Faculty of Mathematics and Natural Science, Universitas Negeri Semarang, Indonesia

*Corresponding author: ningsetiati@mail.unnes.ac.id

Abstract. The purpose of this study is to describe the characteristics of productive broodstock based on body length and histological gonad description of eels in Semarang. Samples of catched eels in various sizes, each measured its length and weight, determined its sex, recorded morphologically and made microtechnic histological description of gonads. Based on description of gonad histology, the eels body length is 28-32 cm for female and consists of egg cells set, which are in 32-36 cm is intersex, the egg cell is decreased sperm cells begin to develop and those are in 37-45 cm. Based on IKG eels obtained in four subdistricts, the egg size cannot be measured because they are in 1.7 to 2.3 mm. Based on gonads microscopic view and visual body length at TKG II, TKG III and IV consisted of eggs and sperm. While eels IKG obtained the most are 25 eels in IKG class 2.829943-3.829943. Conclusion of this research showed that productive female eels have an average body length of 26 - 34 cm with a morphology characteristic sharp head, greenish skin color on the back and yellowish white on the abdomen. Productive male eels have a body length in 54 - 60 cm with a morphological characteristic is greenish brown back, brownish yellow belly, large head and nape size, slightly short tail and blunt ends.

1. Introduction

The use of natural resources and environment is meant provide economic benefits, safety and welfare for community also continuity preservation [1]. The increase in economic growth and development in a city in Central Java, especially in Semarang is one of the reasons, so it require development for infrastructure in all aspects. Demand for agricultural land has become quite large, and causing the decrease of its area and quality in Semarang [2]. Ecologically, eels that live in rice fields can be used as indicators for environmental pollution because these animals are easy to adapt. The disappearance of eels indicates that serious environmental damage has occurred, even though eels are fish that contain a lot of nutrition and also have high economic value [3].

Until now, the commodity of eels is obtained by utilizing eels that are available in nature, whereas the eels are potential enough to be cultivated. If this is allowed it will cause a reduction in eels population in nature [4] and technology for providing productive broodstock is needed based on histological description of gonads and body length. This study presents a histological description of eel gonads from rice field in Gunungpati, Tembalang, Pedurungan and Genuk. The first problem faced by eel cultivators is seeds. Beside the rare of eel seeds, currently 90% of eel seeds on market are from natural catches, so their quality is not guaranteed. Eel catchers in nature usually fight with other catchers, so that various
methods are used to catch eels, such as electrocuted, poisoned and so on. Eel seeds that are caught by electrocuting or poisoning are certainly not good for cultivation [3].

The specific purpose and urgency of this study is to describe the characteristics of productive broodstock based on body length and description histological gonad of eels in Semarang. Central Java is one of provinces in Java Island, which supplies about 4.4% (average production/province of 2.94%) fishery production in Indonesia, both from freshwater and marine fisheries [5]. Especially for freshwater fisheries, this province has been selected as one of eels center cultivation in Indonesia in line with its achievements as a national rice barn with potential for wide agricultural land (rice fields) as a native habitat for eel [6].

Eels production in Indonesia is increase from 12.000 tonnes in 2014 to 17.000 tonnes in 2016. Utilization of natural resources and environment for rice fields to fullfill needs of eel consumption in Indonesia is mostly (± 94%) obtained by seeds supply from nature and developed technology are not advanced yet (intensive) [7]. Massive exploitation of eel seeds has led to significant decline in population, therefore it is necessary to conduct research on various aspects of eel biology and its habitat in rice fields. One aspect of biology is quite interesting to research is aspect of reproduction. On this occasion, the natural source and environmental study center conducted a research on characteristics of productive broodstock based on body length and histology of eel gonads and gonad maturity level (TKG). The results of this study are expected to be used as information about the provision of seeds for cultivation in the future.

2. Methods

Eels sample collection are from rice fields in Gunungpati, Pedurungan, Genuk and Tembalang. Continued by identification to make sure the species of *Monopterus albus* based on body length, this step was assisted by field workers (students). Water on rice field samples were analyzed ecologically with parameters which measured were temperature, soil acidity (pH), water pH, and alkalinity. Tools that used in this research are microtome, preparatory glass, thread, name label, xylol basket, water heater, gauze, film bottle, oven, hotplat, stabiliger, template, cassette, light microscope and its components, books and stationery. The materials used were eel gonads, fixative solutions, alcohol (80%, 90%, 95%, 100%) and 100%), xylol (1, 2, and 3), paraffin, hematoxylin, BNF (Buffer). Netrach formalin), 70% alkool, auxin, distilled water, cosin, and entellan [8].

Research Implementation. Eels were taken as sample included all sizes. The eel that has been obtained, measured body length, body weight, gonad length, gonad weight and determined morphological characteristics of head shape, back color, stomach color and tapered tail shape or not. Next surgery are conducted for its gonad collection [9].

To determine the level of gonad maturity, we used criteria according to Bahri's research (2000), the characteristics of eel gonad maturity level (TKG) are as follows TKG I: Egg grains cannot be seen visually, the proportion of eggs is greater than the proportion of males. TKG II: Visually the eggs are very clear, the large egg grains between egg grains are still sticky so it is rather difficult to separate, the proportion of eggs is about 95% of gonad content. TKG IV: Egg seen very clear, egg granules are large, difficult to separate between egg grains, gonads are almost entirely filled with a very small proportion of sperm. Intersex: A condition where the proportion of eggs and sperm is equal [10].

Gonad maturity index is a value in percent as a result of the ratio of gonad weight to eels body weight including gonads multiplied by 100% with the formula: $I_{KG} = W_g / W \times 100\%$, where $W_g$ is gonad weight and $W$ is final body weight - gonad weight [11]. The parameters measured were temperature, water pH, soil pH, COD, BOD and air humidity from eel habitat in rice fields.

Provision of histological gonads. The first step to take the tissue is prepare the tested fish for gonad removal. Then cut, put it in BNF (Neutral Formalin Buffer) for 1 hour, then dip in 70% alcohol for 2 hours. Dehydration and Clearing, the tissue that has been taken is immersed in a fixative solution for 48 hours. The selected tissue was immersed in 70% alcohol for 48 hours, then continued to be soaked.
during 2 hours in 80%, 90%, 95%, 95% alcohol and 12 hours in 100% alcohol and 30 minutes in 100% alcohol [9]. According to [5] that the making of preparations such as Figure 5 is a flow diagram, cutting the tissue from eel gonads. Then it was fixed with 10% formalin solution and cut into pieces with a microtome to produce 5-10 micron ribbon. Declassification, dissolving calcite in coral tissue using 10% acetic acid solution dissolved with fresh water and 10% formalin. Preparation staining, is done to differentiate the parts of cell. The solutions used were Hematoxylin (blue) and Eosin (red). The adhesion of preparations was carried out to preserve the tissue. This glue is usually embalmed [8].

3. Results and Discussion

Eel that catched during the study are shown in Table 1. Most of them have a body length of 54 - 60 cm. Eels from Sekaran and Gunungpati is relative in the same size, while eels from Kuripan is different because it has various size. Based on sex, female eels were 26-32 cm in length. While for size more than 32 cm is dominant to male.

Based on body length measurements, gonad length and sex determination of 108 eels caught in four subdistrict in Gunungpatis, as in Table 1 below:

| No | Size | Length (cm) | Gunungpati | Tembalang | Genuk | Pedurungan |
|----|------|-------------|------------|-----------|-------|------------|
|    |      |             | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± |
| 1. | 26-32 | 8.7-8.8 | 11 | 0 | 0 | 4 | 1 | 0 | 13 | 2 | 0 | 0 | 0 | 2 |
| 2. | 33-39 | 8.8-9.0 | 2 | 3 | 1 | 0 | 2 | 0 | 5 | 3 | 0 | 1 | 0 | 0 |
| 3. | 40-46 | 10.1-10.4 | 0 | 0 | 5 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 1 |
| 4. | 47-53 | 11.3-12.2 | 0 | 0 | 5 | 0 | 0 | 3 | 0 | 0 | 2 | 0 | 6 | 0 |
| 5. | 54-60 | 16.0-16.5 | 0 | 0 | 1 | 0 | 0 | 12 | 0 | 0 | 3 | 0 | 0 | 0 |
| 6. | 61-67 | 16.8-16.9 | 0 | 0 | 2 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7. | 68-74 | 17.1-17.6 | 0 | 0 | 2 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| 8. | 75-81 | 18.0-18.3 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| Total | | | 13 | 3 | 33 | 4 | 3 | 19 | 18 | 5 | 5 | 1 | 9 | 2 |

I : Intersex

Based on the research, ecological parameters as shown in Table 2 that support the eels catching include the number, body length, gonad length and sex. The numbers show ideal conditions according to the needs of eel habitat in a rice fields in Gunungpati, Tembalang, Genuk and Pedurungan. The results of measurement of ecological parameters in four sub-districts are still suitable as a habitat for eels to live because their pH is not too acidic and a temperature of > 26 °C is needed by eels [12]. The four districts have relatively the same ecological parameters. As for the results of previous research, it is known that the preferred habitat for rice field eels is rice fields that have enough water, are rich in organic material and have a relatively high temperature more than 27°C.

Factors that influence the number, caught eels body length and gonad length depend on environmental conditions of rice fields including food availability, temperature, water pH, soil pH, COD, BOD and 25% humidity. The results of this study are listed in Table 5. The number of eels in Kuripan Village is the largest based on observation that rice fields condition which consist of 80% of rice fields and 20%, can support eels, live and breed better. In contrast to that condition, rice fields in Sekaran and Gunungpati villages been damaged and contaminated with other chemicals. The number of eels were 11 for Gunungpati and 7 from Sekaran. The factors of temperature, water pH, soil pH BOD, COD and air humidity were suitable for eels’s habitat, but the rice field area was getting smaller. Quoting from wikipedia, eels can be used as environmental indicators because they are easy to adapt, so if eels are not found in rice fields, it means that the rice field has been damaged. Beside environmental factors
above, genetic factors, differences in habitat conditions, geographic position can also affect the diversity of morphological features [1].

Table 2. Parameter of Eels Ecology

| City     | Sub-district | Temperatur | Water pH | Soil pH | COD  | BOD   | Humidity |
|----------|--------------|------------|----------|---------|------|-------|----------|
| Semarang | Gunungpati   | 28         | 6.9      | 7       | 0    | 0.125 | 25%      |
| Tembalang| 26           | 6.6        | 6.4      | 1       | 0.125| 27%   |
| Genuk    | 27           | 6.6        | 7        | 5       | 0.125| 23%   |
| Pedurungan| 33-39       | 6.9-7.2    | 6.9-7.2  | 0-2     | 0-0.125| 29%   |

According to [13], environmental factors that also affect the growth of fish in waters are temperature, salinity, sunlight intensity, shade, turbidity, depth, current velocity and the accumulation of bottom organic matter [14], stated that an increase in water temperature by 10°C would increase the metabolic rate of fish to 2-3 times. An increased metabolic rate will increase oxygen need for fish, meanwhile increase temperature will cause dissolved oxygen in water decrease, thus causing respiration difficulties for fish. Other factors that affect the number of organisms in water are Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD). Biological Oxygen Demand is the need for oxygen to oxidize chemical compounds or waste materials in water by microorganisms. Chemical Oxygen Demand is the amount of oxygen needed to oxidize all organic materials (which are easily decomposed and difficult to decompose) chemically using strong oxidator [15].

The sex of caught eels in this study were body length, gonad length and sex ranging from 26 -32 cm, 8.7-8.8 cm, consist of egg cells is female, 23-39 cm, 8.8-8.9 cm and consists of egg cells and immature sperm are intersex, 40-81 cm, 8.8-18.3 cm and which gonads consist of sperm are males. The results of this study are the same as the results of research [5], that eels which less than or equal 29 cm are female, while which more than 29 cm have already got sex change into male and between classes 27.4 - 31.5 cm, eels is in transition period or intersex. And according to [16] to determine broodstock from morphological characteristics, it is also based on estimated age, which is about 3-6 months for female and 6-9 months for male.

From Table 2 it can be seen that image on intersex contains some parts, (1) sperm storage lobe, (2) sperm, (3) ovum, (4) radiata zone. Histological image of male gonads contains sperm cells and for females there are egg cells. In male eels, egg is inactive and only sperm pockets. Eels that are in transitional or intersex period have equal proportion between eggs and sperm, while in females, only eggs are seen. Body length has effect on gender because eels are hermaphrodite fish. At a certain length of female's body develops intersex and develops into a male. The development of oogonia of female eel consists of five stages, each of which is determined by the size of its oogonia development. Stage one indicates early oogonia development, while stage five represents oogonia development that is ready to hatch. According to [17], factors that can affect reproductive function in fish species consist of external and internal factors. External factors include rainfall, temperature, sunlight, plants, and the presence of male fish. In general, fish in natural waters will spawn at the beginning of rainy season or at the end of rainy season because at that time there will be a change in environment or water conditions that can stimulate fish to spawn. The success of spawning cannot be separated from body internal conditions and production of reproductive hormones.

The genitals or gonads of eels have eggs and sperm. During their life, the eel will changes, on juvenile period it is indifferentiated, then the ovaries will develop so the eel will be female. After become female, the eels will switch to transitional period (intersex), which is marked by ovaries shrinking and testes development. After that the eel will turn into male [6]. Morphologically, female eel has a blackish brown back, yellowish white belly, small head, and long tail with a sharp tip. Meanwhile, the male has a greenish brown back, brownish yellow belly, a large head, and a slightly short tail with a blunt tip (Table 3). The Calculation Result of Gonad Maturity Level (TKG) as in Table 4.
Table 3. Body Length, Structure View of Gonad Tissue and Eel Morphological Characteristic

| Sex   | Histological view | Morphological Characteristic.                           |
|-------|-------------------|--------------------------------------------------------|
| Female| ![Female Histology](image) | The head is slightly tapered, the body length ranges from 26-32 cm, the back color is greenish-brown, the belly is yellowish, the size of the head and nape is small, the tail is elongated and the tip is sharp. |
| Intersex| ![Intersex Histology](image) | The body length ranges from 26-39 cm, its color is dark brown backs belly tawny color, the head size and neck rather large when compared to females, a little bit long section rather blunt ends. |
| Male  | ![Male Histology](image) | The shape of the head is more blunt, body length ranges from 40-81 cm or more, the color of the back is greenish brown, the color of the stomach is brownish yellow, the size of the head and nape is large, the tail is rather short and the ends are blunt. |

Table 4. Gonad Maturity Level

| No. | Female Length (cm) | TKG I | TKG II | TKG III | TKG IV | Intersex |
|-----|--------------------|-------|--------|----------|--------|----------|
| 1   | 26-26.9            | 13    | 0      | 0        | 0      | 0        |
| 2   | 27-27.9            | 0     | 5      | 6        | 0      | 0        |
| 3   | 28-28.9            | 0     | 0      | 0        | 4      | 0        |
| 4   | 29-29.9            | 0     | 7      | 0        | 0      | 0        |
| 5   | 30-30.9            | 0     | 0      | 0        | 0      | 0        |
| 6   | 31-31.9            | 0     | 0      | 0        | 0      | 3        |
| 7   | 32-32.9            | 0     | 0      | 0        | 5      | 0        |
| 8   | 33-33.9            | 0     | 0      | 0        | 0      | 4        |
| 9   | 34-34.9            | 0     | 0      | 0        | 0      | 3        |
| 10  | 35-35.9            | 0     | 0      | 0        | 0      | 5        |
| Total|                   | 13    | 12     | 6        | 4      | 20       |

As shown in Table 5, eels at size 31-31.9 cm are already include got sex change or start to switch from female to male or are in intersex phase with 20 eels and most are at body length 32-32.9 and 35-35.9 cm, for sizes after 35.9 more are considered to male phase. Meanwhile, the length of 28-28.9 already has perfect gonad maturity for females with 4 eels in TKG IV. For the length after 28.9 cm the eels have spawned so the level of gonad maturity in table, it is noted that there are 12 eels have TKG II because they have spawned or do have an immature gonad state. Then for TKG I there are 13 eels with immature gonads and not ready to spawn.
Table 5. Index Maturity Gonad based on body weight and gonad weight

| No. | Body Weight (gr) | Grup of IKG (%) | Total |
|-----|------------------|-----------------|-------|
| 1.  | 58-107           | 0.5-3.5         | 0.829943-1.829943 | 23   |
| 2.  | 108-157          | 3.5-6.5         | 2.829943-3.829943 | 55   |
| 3.  | 158-207          | 6.5-9.5         | 4.829943-5.829943 | 18   |
| 4.  | 208-257          | 9.5-12.5        | 6.829943-7.829943 | 13   |
| 5.  | 258-307          | 12.5-15.5       | 8.829943-9.829943 | 6    |

Based on the results of research [6], the level of gonad maturity correlates with gonad development, it is shift of the egg core (nucleus) towards the pinggi and abundance of egg yolk granules on histological view and the increase in diameter of eel’s eggs. According to [5] the level of IV gonad maturity was mostly found in range 21-30 cm of length.

As research from IKG number achieved 0.6095, the researchers stated that eels were ready to spawn, because their gonads were large in size. Meanwhile for all research results, the IKG of eel already achieve that number so the IKG number is high. If the IKG tends to be low because of gonad weight and body weight are also low, and vice versa. It can be seen in table 5 that IKG eels obtained in 4 Sub Districts in Semarang, the highest IKG is in IKG class of 2.829943-3.829943 with 55 eels from 4 sub-districts while the lowest is in IKG 8.829943-9.829943 with 6 eels only.

4. Conclusion

The results showed that productive female eels have average body length 26 - 34 cm with the morphological characteristics are pointed head, greenish skin color on the back and yellowish white on the abdomen. Meanwhile, in males more than 31 cm with morphological characteristics are the head is more blunt, and the skin color is grayish. Productive male eels have a body length 54 - 60 cm with a morphological characteristic of greenish brown back, brownish yellow belly, large head and nape size, slightly short tail and blunt ends. The IKG value of the eel obtained is in IKG class between 2.829943 to 3.829943.

References

[1] Hasan M, Norhan N A S, Wahab W, Melad A A N, Kismiyati K, Aziz M F H A, Husin N M, Zakariah M I 2019 Sci. J. Fish. Mar. 2 11
[2] Li W, Sun WX, Fan J, Zhang CC 2013 Biologia. 36 821
[3] Covain R., Ready J, Zuanon J 2019 Nat. Comun.
[4] Besirovic H, Alic A, Prasovic S & Drommer W 2010 Turk. J. Fish. Aquat. Sci. 10 255
[5] Khanh NH, Ngan HTB 2010 Aquac. Asia Mag. 15(3) 26
[6] Reenamole G R 2018 India Int. J. Sci. Res. (IJSR)
[7] Kanjuh T, Mrdak D, Piria M, Tomljanović T, Joksimović A, Talevski T and Milošević D 2018 Acta Adriatica. 59(1) 91
[8] Dehghani M, Kamrani E, Salarpouri A & Kamali E 2015 J. Fish. Livest Prod. 32
[9] Milošević D & Mrdak D 2016 J. Appl. Ichthyol. 32 1331
[10] Sow A Y, Ismail A, Azmal M N A 2018 Malay. Nat. J. 70(1).
[11] Lei L, Feng L, Jian TR, Yue GH 2012 Conserv. Genet. Resour. 4 363
[12] Khanh NH, Ngan HTB 2010 Res. inst. Aquac. 15 26
[13] Nova T S D, Adiputra Y T, Adiputra Y T 2020 J. Perikan. 10(2) 167
[14] Kocovsky P M, Adams J V & Bronte C R 2009 Fish. Soc. 138 487
[15] Fitzgerald D G, Nanson J W , Todd T N, Davis B M 2002 J. Aquat. Ecosyst. Stress Recovery 9 115
[16] Ravaglia MA, Maggese MC 2002 Biocell 26(3) 325