Australian Journal of Basic and Applied Sciences
2019 August; 13(8): pages 112-117
DOI: 10.22587/ajbas.2019.13.8.15
Original paper
AENSI Publications
Journal home page: www.ajbasweb.com

The Primary Sex Organs Development Comparison of Tiger Grouper, *Epinephelus fuscoguttatus* and Coral Trout, *Plectropomus leopardus*

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Received date: 19 June 2019, Accepted date: 24 August 2019, Online date: 29 August 2019

Abstract

Grouper is one of the marine fish commodities which have economic value and often found in Indonesia. A change in sex from females to males after reaching a certain age and size make these fish belong to the protoginous hermaphrodite group. The purpose of this study was to provide a described comparison of the primary sex organ differentiation phase in tiger grouper, *Epinephelus fuscoguttatus* (species A) and coral trout, *Plectropomus leopardus* (species B). Undifferentiated phase was found in 20-35 days after hatching for both species A and species B, characterized by the histological representation of germ cell groups surrounded by somatic cells. The differentiated phase was characterized by the somatic cell division, especially at the end of the somatic cell that made the somatic cell become extended. The differentiated phase was found in 40-50 days after hatching for species A and species B. Along with the increasing days, 55-60 days after hatching for species A and Species B, the extension of both ends of the somatic tissue met each other and formed ovarian cavity. The presence of oogonia in the ovary was found in fish 60 days after hatching for both species. Oogonia frequently increased in number and size in the fish above 60 days for both species. To sum up, the differentiation process of primary sex organs in tiger grouper and coral trout did not show significant differences.

Keywords: Groupers, histological figure, gonad differentiation, protoginous hermaprodite

INTRODUCTION

Approximately 0.66% of the total fish species in the world are Serranidae family or better known as groupers. These group of fish are often found in the tropical oceans to the subtropic coral reefs (Devlin and Nagahama, 2002; Kim et al., 2013; Oh et al., 2013). Indonesia, an archipleago country in South East Asia, comprises with a tropical climate and about 80,000 km coastline rich in marine resources, including fish. Indonesia is incorporated in Life Reef Food Fish (LRFF) serves as the leading provider for groupers. Two of the most common genus found are *Epinephelus* and *Plectropomus* (Pet et al., 2005; Aumeeruddy and Robinson, 2006; Afero, 2009; Palm et al., 2015).

Most of the grouper are female during juvenile and turn into male after reaching the size at a certain age. This sex change is known as protoginous hermaphroditism (Ruiz-Carus 2002; Bhandari et al., 2006; Sao et al., 2012). The morphological differences of the primary sex organs in the coral trout, *Plectropomus leopardus* aged 27 months which forms sperm granules in testes, oogonia, oocytes in ovaries, and the combination of both sexes were successfully identified through histological observation (Sembiring et al., 2014). However, there is lack information regarding the primary sex organs development from the initial phase until completely formed into the main organs (Murata et al., 2009). This study aimed to describe the primary sex organs differentiation that occurs in Indonesia's endemic grouper, namely tiger grouper, *Epinephelus fuscoguttatus* and coral trout, *Plectropomus leopardus*.

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2. MATERIALS AND METHODS

Sample Preparation

The fish used was acquired from Institute for Mariculture Research and Development (IMRAD), Bali. The research materials were the tiger grouper broodstock, *Epinephelus fuscoguttatus* labeled as species A and the coral trout broodstock, *Plectropomus leopardus* labeled as species B. The selection must be conducted carefully to obtain the matured male characterized with the pale operculum and more aggressive swimming to the opposite sex in both species. In contrast, the matured female was characterized by the enlargement of the abdomen followed by the gonads reddening. Spawning process is done by spawning method naturally. The sample larvae were reared in a 3x3x1 m^3 concrete tank with the seawater recirculation and aeration added. The live feed used during the larval rearing was *Branchionus* sp. which was performed for 35 days, followed by *Artemia* sp. until the larvae reached the age of 45 days and afterwards the larvae were given pellet Otohime® A1 and A2 for supporting the larval growth.

Sample Collection

A total of 60-100 larvae from each species were collected on the 20th, 25th, 30th, 35th, 40th, 45th, 50th, 55th and 60th days after hatching. The total length and weight were measured. The fish was anaesthetized using MS222 (tricaine methanesulfonate) at a dose of 15 - 30 mg / L (Kathleen et al., 2011). A pair of organs that lies parallel to the kidney or known as gonads were then surgically removed from the larvae and fixed using Bouin's solution.

Histological Observation

Gonads that had been fixed for a day were dehydrated using ethanol and then embedded in liquid paraffin to form a cube. Paraffin cubes containing samples were sliced thin using a rotary microtome machine to produce a histological sample with 3-5 µm thickness. The histological samples should be colored first using haematoxylin and eosin to help clarify the image during observations under a light microscope (Olympus CH20). Each sample was coded based on the age of the fish dissected (e.g 20D, meaning twenty days after hatching). The observations of the histological gonads were then divided into three types: the undifferentiated phase, the differentiated phase, and the gonad phase.

3. RESULTS

Characteristics of the fish sample that were obtained through the artificial spawning activity in IMRAD was presented in Table 1. The fertilization rates were 85.45% and 76.50% with the hatching rates were 80.55% and 60.25% in species A and B, respectively. Total of 11.5% larvae of species A and 2.8% larvae of species B survived to be fry at 60 days after hatching. The survival rates of the fry in the nursery were 92.35% and 94.4% for species A and B, respectively, after 60 days rearing.

Histological observation of the primary sex organs from both fish species showed 3 significant stages differentiation, i.e., the undifferentiated phase; the differentiated phase and the gonad phase (Fig. 4). At the end of the research, the sex of the fish found were all females, so the gonad phase contained in the series of differentiation was called the ovarian phase. The results of histological observation of fish test gonads during the study were presented in Table 2.

Table 1: Fertility, hatching, larval survival and fry survival rates of fish samples produced by artificial spawning

Parameter	*Epinephelus fuscoguttatus*	*Plectropomus leopardus*
Fertility Rate (%)	85,45	76,50
Hatching Rate (%)	80,55	60,25
Larval Survival Rate (%)	11,5	2,8
Fry Survival Rate (%)	92,35	94,4

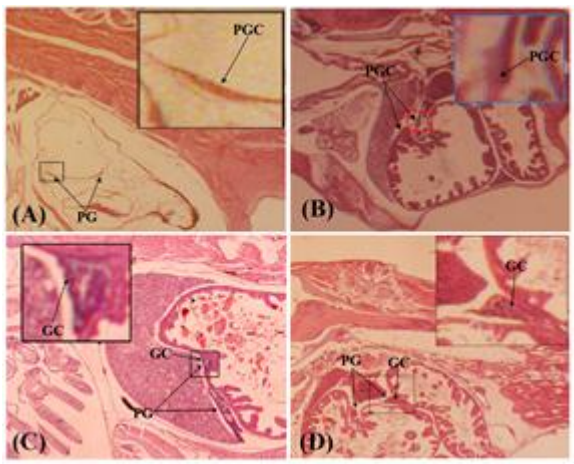

Figure 1. Tiger grouper larvae and coral trout larvae gonads on D20 - D25. **(A)** Tiger grouper gonad on D20, **(B)** coral trout gonad on D20, **(C)** tiger grouper gonad on D25, **(D)** coral trout gonad on D25.

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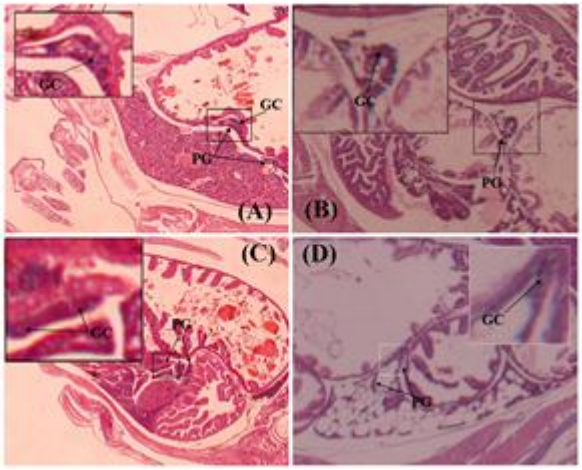

Figure 2. Tiger grouper larvae and coral trout larvae gonads on D30 – D35. **(A)** Tiger grouper gonad on D30, **(B)** coral trout gonad on D30, **(C)** tiger grouper gonad on D35, **(D)** coral trout gonad on D35.

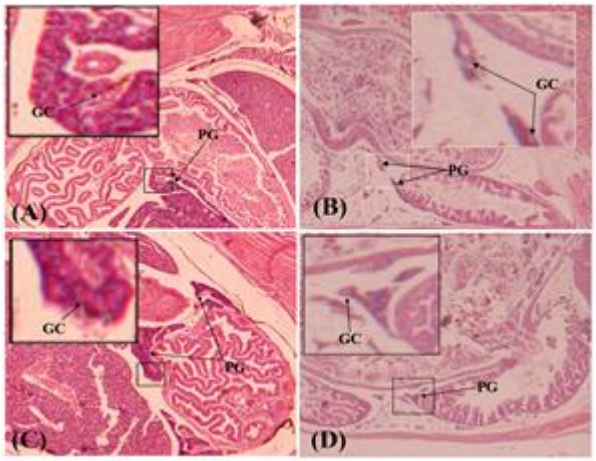

Figure 3. Tiger grouper larvae and coral trout larvae gonads on D40 – D45. **(A)** Tiger grouper gonad on D40, **(B)** coral trout on D40, **(C)** tiger grouper gonad on D45, **(D)** coral trout gonad on D45.

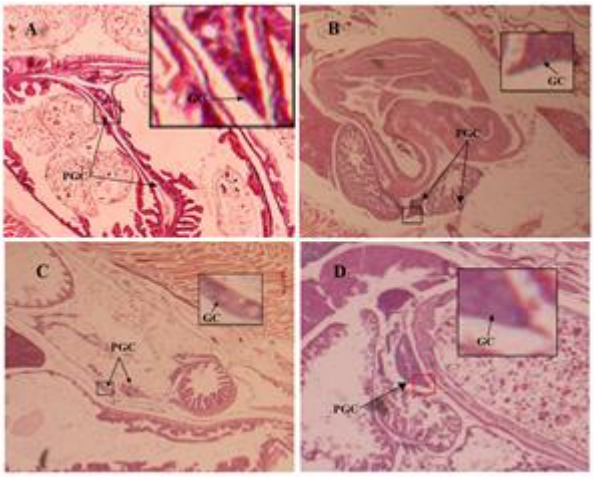

Figure 4. Tiger grouper larvae and coral trout larvae gonads on D50 – D55. **(A)**Tiger grouper gonad on D50, **(B)** coral trout gonad on D50, **(C)** tiger grouper gonad on D55, **(D)** coral trout gonad in D55.

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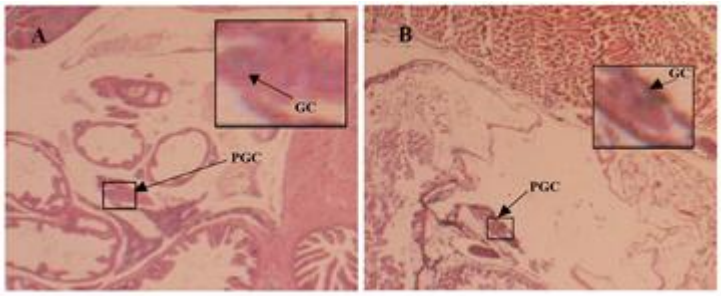

Figure 5. **(A)**Tiger grouper gonad on D60, **(B)** coral trout gonad on D60

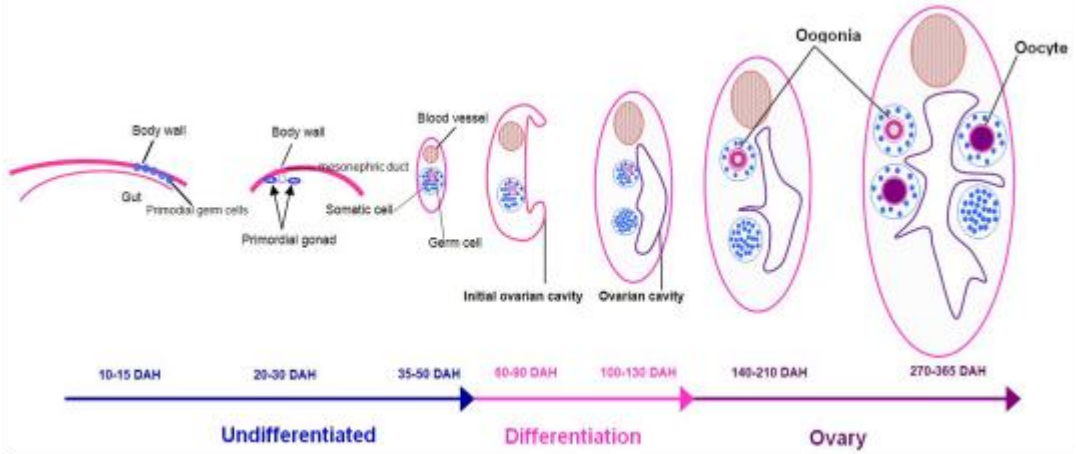

Figure 6. The main phase of primary sex differentiation in protoginous hermaprodite which consists of undifferentiated, differentiated, and ovary phase (Sao *et al*., 2012).

Table 2: The results of gonad histological observation in fish samples A, *Epinephelus fuscoguttatus* and B, *Plectropomus leopardus*

Age (Days After Hatching)		Fish Total Length Average (mm)		Sample Total (fishes)		Gonad Status (%)					
						Undifferentiated Phase		Differentiated Phase		Gonad Phase	
A	**B**	**A**	**B**	**A**	**B**	**A**	**B**	**A**	**B**	**A**	**B**
20	20	8,4	6,8	100	100	75	60	69	45	-	-
25	25	16,8	10,2	100	100	75	60	70	55	-	-
30	30	21,5	12,8	100	100	80	65	70	60	-	-
35	35	22,2	16,8	100	100	80	70	70	60	-	-
40	40	26,8	20,5	100	100	85	70	85	60	-	-
45	45	28,2	24,6	80	80	88	70	85	65	-	-
50	50	32,4	30,8	80	80	90	80	90	70	-	-
55	55	36,8	33,2	60	60	90	80	-	-	80	60
60	60	39,4	34,4	60	60	95	85	-	-	85	75

According to Table 2, the undifferentiated phase occurred because of the presence of gonads among the other organs was not very clear. This phase was found in sample species A and B on D20 - D60. Primordial germ cells were located above the intestinal organs, generally already showed by sample species A and B at the D20 (Fig. 1ab). When a primordial germ cell appeared to be surrounded by a little cytoplasm, it was known as a primordial gonad. This phenomenon was found in sample species A and B at D25-D35. The size of the primordial germ cell then enlarged with an increase in the number of cytoplasm and blood vessels (Fig. 2). This was the end of the undifferentiated phase.

Entering the differentiation phase found in the sample species A and B at D20-D50, the cytoplasm group was rapidly dividing the cells and filling the ends of somatic tissue walls so that it looked like an extension (Fig. 3ab (species A and B at 40D). This activity continued until the extension of somatic tissue became more apparent (Fig. 3cd, species A and B at 45D) and formed the ovary cavity (Fig. 4AB). The primordial gonads scattered in the somatic tissue continued to divide. Approaching the ovary cavity, the oogenesis stage was about to begin (Fig. 4ab).

The ovarian phase was characterized by the occurrence of mitotic division from oogonium to oocyte which was then distributed in the presence of the ovary cavity (Fig 4cd). At that age, the ovary was filled with oocytes and prepared for meiotic division (Fig. 5ab). The oocytes were located on the edge of the inner ovary cavity, while the outer portion of the ovary cavity or which was the result of somatic tissue extension generally did not contain germ cell (D60).

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4. DISCUSSION

The sampled fish obtained from the artificial spawning activity could be classified into good category, due to all the parameter values were higher than those reported from several studies (Table 3).

This research reviewed the comparison of gonad differentiation phase in sample species A and B both in terms of morphological and of time characteristics. If there was a time difference between ovarian formation and the appearance of oogonia, then this indicated that the two phases were regulated by the following mechanism.

Nakamura et al. (1998) and Nagahama (2000) suggested that steroid hormones played a role in controlling the development of gonads and sex differentiation in fish.

Table 3: Grouper Larvae and Fry Quality Range Parameters

No	Cited in	Parameter	Percentage (%)
1	James, *et al*. (1997)	Fertility Rate	90-100
2	Eileen, *et al*. (1997)	Hatching Rate	67,3-80,3
3	Boglione, *et al*. (2009)	Larval Survival Rate	0,2-17,5
4	Rimmer (2000)	Fry Survival Rate	1-10

In zebra fish, *Danio rerio*, which is also hermaphrodite protogini, the emergence of primordial germ cells began to be seen in fish aged 2 weeks after fertilization. A pair of gonads found in the fish's abdominal cavity were discovered at the 4th week after fertilization (Maack and Segner, 2003). According to Nakamura et al. (1998), in primary sex organs of fish that had not experienced genital differentiation, there were two somatic cells of the medulla and cortex. In protoginous fish, the formation of the ovaries is derived from somatic cells of the cortex type whereas the medulla is degraded and disappears.

It is reported that the presence of oocytes in the new ovaries was found one week after the appearance of gonads in zebrafish (87% of total samples) (Maack and Segner, 2003). This condition was also similar to the results of this research which showed that the formation of female genital cells in tiger grouper, *Epinephelus fuscoguttatus*) and coral trout, *Plectropomus leopardus* coincided with the discovery of the ovary cavity or after the formation of the ovary cavity.

Based on the results of previous research, the differentiation of primary sex organs of fish into males or females began to be observed when the fish gonads looked like transparent threads (Saeed et al., 2010). If the histology reading of the primary sex organs found a cell with a core on the inside, then the primary sex organ was an ovary. The formation of the genital cells occurred in the lamella which was attached to the ovary. Meanwhile, the appearance of a collection of dark dots was a marker that the existence of sperm in the primary sex organs. Sperm itself was produced by testes in the seminiferous tubules (Mackie and Lewis, 2001; Subagja and Gustiano, 2010).

ACKNOWLEDGEMENTS

We would like to appreciate the support from the Institute for Mariculture Research and Development (IMRAD) and its staffs involved in this study.

REFERENCES

Afero, F. 2009. Economic analysis for tiger grouper *Epinephelus fuscoguttatus* and humpback grouper *Cromileptes altivelis* in commercial cage culture system in Indonesia. *Depik*. **1**(1): 10-21.

Aumeeruddy, R. and J. Robbinson. 2006. Closure of the live reef food fish fishery in Seychelles. *SPC Live Reef Fish Information Bulletin*. **16,** 3-9.

Bhandari, R. K., M. A. Alam, K. Soyano and M. Nakamura. 2006. Induction of female to male sex change in the honeycomb grouper (*Epinephelus merra*) bay 11 ketosterone tratments. *Zoological Sciences*. **23**: 65-69.

Boglione, C., G. Marino, M. Giganti, A. Longobardi, P. D. Marzi and S. Cataudella. 2009. Skeletal anomalies in dusky grouper *Epinephelus marginatus* (Lowe 1834) juveniles reared with different methodologies and larval densities. *Aquaculture* (1-2): 48-60.

Devlin, R. H. and Y. Nagahama. 2002. Sex determination and sex differentiation in fish: an overview of genetic, physiological, and environmental influences. *Aquaculture* **208,** 191-364.

Eileen, P., O. Wade, C. E. Simon, J. Ginoza and A. Moriwake. 1997. Effect of turbulence, salinity, and light intensity on hatching rate and survival of larval Nassau grouper, *Epinephelus stratiatus*. *Journal of Applied Aquaculture* **7**(3): 33-43.

James, C. M., S. A. Al-Thobaiti, B. M. Rasem and M. H. Carlos. 1997. Breeding and larval rearing of the camouflage grouper *Epinephelus polyphekadion* (Bleeker) in the hypersaline waters of the red sea coast of Saudi Arabia. *Aquaculture Research* **28**(9): 671-681.

Kathleen M. Carter, Christa M. Woodley, Richard S. Brown. 2011. A review of tricaine methanesulfonate for anesthesia of fish. Fish Biol Fisheries, 21:51–59

Kim, S., C. Lee, Y. Song, S. Hur, H.Kim and Y. Lee. 2013. *Tissue and Cell*. **45,** 261-268.

Maack, G and H. Segner. 2003. Morphological development of the gonads in zebrafish. *Journal of Fish Biology*. **62**. 895-906.

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Mackie, M. and P. Lewis. 2001. Assesment of gonad staging systems and other methods used in the study of the reproductive biology of narrow-bared Spanish mackerel, *Scomberomorus commerson*, in western Australia. *Fisheries Research Report* (3): 1-32.

Murata, R., H. Karimata, M. A. Alam and M. Nakamura. 2009. Gonadal sex differentiation in the Malabar grouper, *Epinephelus malabaricus*. *Aquaculture*. **293**: pp 286-289.

Nagahama, Y. 2000. Gonadal steroid hormones: major regulators of gonadal sex differentiation and gametogenesis in fish. *In Reproduvtive Physiology of Fish* (Norberg, B.; Kjesbu, O. S.; Taranger, G. L.; Anderson, E. and Stefansson, S. O., eds). 211-222. Bergen: University of Bergen Press.

Nakamura, M., T. Kobayashi, X. T. Chang and Y. Nagahama. 1998. Gonadal sex differentiation in teleost fish. *Journal of Experimental Zoology*. **281**. 362-372.

Oh, S., H. Kang, C. Lee, S. Hur and Y. Lee. 2013. Sex reversal and masculination according to growth in longtooth grouper *Epinephelus bruneus*. *Dev. Reprod.* **17** (2): 79-85.

Palm, H. W., I. Yulianto, S. Theisen, S. Rueckert and S. Kleinertz. 2015. *Regional Studies in Marine Science*. pp 1-17.

Pet, J. S., P. J. Mous, A. H. Muljadi, Y. J. Sadovy and L. Squire. 2005. Aggregations of *PLECTROPOMUS AREOLATUS* and *Epinephlelus fuscoguttatus* (grouper, Serranidae) in the Komodo National Park, Indonesia: monitoring and implication for management. *Environmental Biology of Fishes*. **74** (2): pp 209-218.

Rimmer, M. 2000. Review of grouper hatchery technology. *SPC Live Reef Fish Information Bulletin* (7): 14-19.

Ruiz-Carus, R. 2002. Chomosome analysis of the sexual phases of protogynous hermaphrodites *Epinephelus guttatus* and *Thalassoma bifasciatum* (Serranidae and Labridae; Teleostei). *Carribean Journal of Science*. **38**(1-2): pp 44-51.

Saeed, S. S., I. M. Reza, A. F. Bagher and G. Saeed. 2010. Histological study of ovarian development and sexual maturity of kutun (*Rutilus kutum* Kamenskii, 1901). *World Applied Sciences Journal* (8): 1343-1350.

Sao, P. N., S. Hur, C. Lee and Y. Lee. 2012. Gonadal sex differentiation of hatchery-reared longtooth grouper (*Epinephelus bruneus*). *Dev. Reprod.* **16**(3): pp 185-193.

Sembiring, S. B. M., R. Andamari, A. Muzaki, I. K. Wardana, J. H. Hutapea dan N. W. W. Astuti. . Gonadal development of domesticated coral trout (*Plectropomus leopardus*) reared in floating net cage. *Jurnal Ilmu Teknologi Kelautan Tropis.* **6**(1): pp 53-61.

Subagja, J. and R. Gustiano. 2010. Uji keturunan jantan hasil pengalihan kelmain pada ikan nilem (*Osteochilus hasselti C.V*). *Omni-Akuatika*. **9**(11): 69-74.