

### **RESEARCH NOTE**

# Hermaphroditism in the white spot grouper *Epinephelus* coeruleopunctatus (Pisces: Serranidae) harvested from Padang City waters, Indonesia [version 1; referees: 2 approved, 1 approved with reservations]

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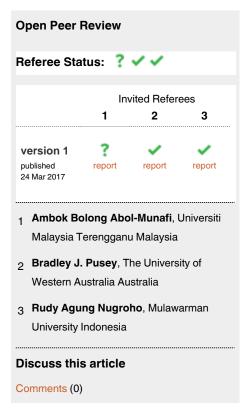


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### **Abstract**

The objective of the present study was to determine the length (mm) for sex transformation of hermaphroditism in white spot grouper *Epinephelus coeruleopunctatus* as a basis for developing breeding technology. Fish sampling was carried out between April and October 2013 in Padang City waters, Indonesia. A total of 56 white spot groupers were recorded during the study; of these 22 were male, 28 female and 6 samples were not recognized regarding sex preference. Sex differentiation was detected at a length of 183 mm, and at this size the fish are female. Sex transformation to male begun to occur at 302 mm total length.



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The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: No competing interests were disclosed.

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### Introduction

Groupers (family Serranidae) belong to 109 species and 11 genus<sup>1,2</sup>. Groupers are commercial marine fishes that have been harvested intensively from the wild, resulting in decreasing the population worldwide<sup>3,4</sup>. The white spot grouper, *Epinephelus coeruleopunctatus*, is one of the most popular groupers and has a high economic value among groupers in Asia-Pacific regions<sup>5,6</sup>. However, this species is rare and difficult to catch. According to local fishermen of Padang City, Indonesia, the population of *E. coeruleopunctatus* has been declining sharply over the last two decades<sup>7</sup>. According to Teixeira *et al.*<sup>8</sup> and Mariskha and Abdulgani<sup>9</sup> the decreasing fish population is caused by overfishing, habitat perturbation<sup>10</sup> and unfriendly fishing practices<sup>11</sup>. The International Union for Conservation of Nature<sup>12</sup> reports this species on the Red List as a threatened species.

Culturing of white spot grouper has been initiated in Indonesia; however, the fry (juveniles) are strongly dependent from the wild supply<sup>13</sup>. Therefore, it is very crucial to develop breeding technology of the white spot grouper. One of the problems in the development of breeding technology is hermaphroditism sex development, which is observed in this species<sup>14</sup>. Therefore, it is difficult to determine the sex differentiation between male and female. Hermaphroditism has also been reported in several other groupers, such as *E. tauvina*<sup>15</sup>, *E. aeneus*<sup>16</sup>, *E. rivulatus*<sup>17</sup>, *E. striatus*<sup>18</sup>, and *Plectropomus laevis*<sup>19</sup>. Hence, this paper reports on the size (length and body weight) of sex transformation in white spot grouper. This information is crucial to plan a better management strategy of fishery resources<sup>20</sup> and to develop breeding technology for the white spot grouper.

### **Methods**

All procedures involving animals were conducted in compliance with Bung Hatta University Research and Ethics Guidelines, Section on Animal Care and Use in Research. Fish were caught from Padang City waters, at GPS coordinates 0° 54' 55.34" S, 100° 10' 15.49" E (Figure 1), between April and October 2013. The fish were caught using hooks and hand line at the depth of 30-50 m. Fishing operations were carried out from 6.00 am to 16.00 pm. The sampled fish were anesthetized with MS222, prepared by dissolving 4g of MS222 in 5L tap water<sup>21</sup> and then transported to the Laboratory of Fisheries Resources of Bung Hatta University for further analysis. In the laboratory, the fish samples were measured for total length (mm) and body weight (g). The abdomen was dissected and the gonad was removed carefully and cleaned using a tissue paper and then weighed nearest to mg using a digital balance (ACIS: AD300; errors 0.01g). Sex differentiation by gonad was examined microscopically (100x magnification) and determined based on Muchlisin *et al.*<sup>22</sup>. The data were analyzed descriptively.

### Results

A total of 56 fish were recorded during the study, where 50 fish were recognized regarding sex differentiation by gonad, of which 22 were males and 28 females. A total of 6 samples were not recognized regarding their sex, due to being still in the early gonadal development stage. The sex ratio was 2:3 (male:female). The total length of the male fish ranged from 302–537 mm, while females ranged from 183–537 mm. The body weight ranged between 374–2107 g and 85–373 g for male and female fish, respectively. The total length of fish with undetermined sex ranged from 125–242 mm and 85–373 g body weight (Table 1 and Table 2).

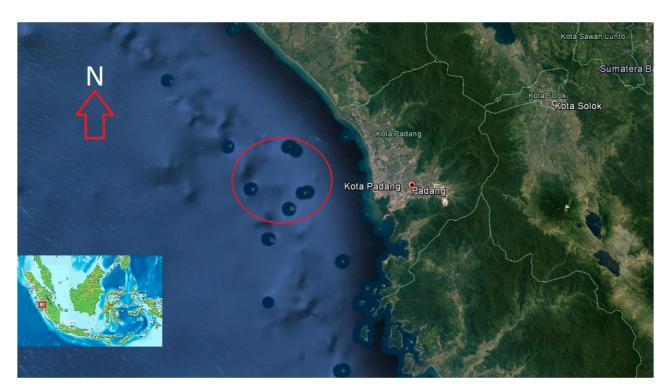


Figure 1. A map of Padang City waters showing the sampling location (red circle)7.

Table 1. Length frequency distribution of fish Epinephelus coeruleopunctatus.

Length class (mm)	N (%)			
	Male	Female	Unrecognized sex	
125–182	-	-	3 (50.0)	
183–242	-	4 (14.3)	3 (50.0)	
243–301	-	4 (14.3)	-	
302–360	2 (9.1)	5 (17.9)	-	
361–419	6 (27.3)	7 (25.0)	-	
420–478	5 (22.7)	5 (17.9)	-	
479–537	9 (40.9)	3 (10.7)	-	
Total	22 (100)	28 (100)	6 (100)	

Table 2. Weight frequency distribution of fish Epinephelus coeruleopunctatus.

Weight class (g)	N (%)			
	Male	Female	Unrecognized sex	
85–372	-	7 (25.0)	6 (100)	
374–662	1 (4.5)	7 (25.0)	-	
663–951	6 (27.3)	5 (17.9)	-	
952-1240	2 (9.1)	2 (7.1)	-	
1241–1529	4 (18.2)	2 (7.1)	-	
1530–1818	3 (13.6)	5 (17.9)	-	
1819–2107	6 (27.3)	-	-	
Total	22 (100)	28 (100)	6 (100)	

The study showed that the first sex differentiation of *E. coeruleopunctatus* occurred at a size above 183 mm; fish of this size were recognized as female and no male fish were detected in this size group. First sex differentiation is species dependent; for example, *E. bleekeri* occurrs at 170 mm<sup>23</sup> and *Plectropomus laevis* at 280 mm<sup>19</sup>.

The results revealed that the female white spot grouper begun to transform to male at 302 mm in length, indicating a protogynous hermaphroditism. However, the size at which all fish transform to male fish was unknown, since there were no fish sample more than 537 mm in length. But, the existing data show that the ratio of male fish was increased as total length increased; hence, we suspect

that all fish have changed sex to male at sizes above 600 mm. For comparison, Renones *et al.*<sup>24</sup> reported that the female dusky grouper *E. marginatus* transforms its sex initially from female to a male at a size of 680 mm and all males were detected at size 800 mm. In addition, Tan and Tan<sup>25</sup> reported that *E. tauvina* begins to transform their sex from female to male at the size of 650 mm, while at the size of 700 mm all fish are recognized as male. According to Burhanuddin and Fami<sup>26</sup> the occurrence of sex transformation in hermaphroditic fish is species dependent and strongly influenced by environmental factors.

Dataset 1.The total length, body weight and sexes of the 56 individual fish sampled

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### **Conclusions**

The white spot grouper *Epinephelus coeruleopunctatus* is a protogynous hermaphroditism. Sex differentiation was detected at the total length of 183 mm and at this size the fish are female. The sex transformation began to occur at 302 mm total length.

### Data availability

Dataset 1: The total length, body weight and sexes of the 56 individual fish sampled. doi, 10.5256/f1000research.11090.d155119<sup>27</sup>

### Author contributions

UB was responsible for developing research proposal and study design and approved the final draft of the paper. MM was responsible for sample collection and processing, and data analysis. ZAM is responsible for manuscript preparation and proofreading of the draft

### Competing interests

No competing interests were disclosed.

### **Grant information**

This study was supported by the Ministry of Research, Technology and Higher Education of the Republic of Indonesia through the Fundamental Research Scheme (contract number, 014/SP/HATTA-1/LPPM/II/2013).

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

### Acknowledgements

The authors thank the Ministry of Research, Technology and Higher Education for providing the financial support to this study. Appreciation goes to our colleagues who helped the authors during field sampling and laboratory analysis.

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# **Open Peer Review**

# **Current Referee Status:**





# Version 1

Referee Report 18 April 2017

doi:10.5256/f1000research.11962.r21903

# Rudy Agung Nugroho

Department of Biology, Faculty of Mathematics and Natural Sciences, Mulawarman University, Samarinda, Indonesia

Review report for article entitle "Hermaphroditism in the white spot grouper *Epinephelus coeruleopunctatus* (Pisces: Serranidae) harvested from Padang City waters, Indonesia". Overall, this article has displayed originality in the work and the outcome of this work adds benefit to the area of the research. This article is presented well with cohesiveness. However, editorial suggestions should be addressed by the author. Below, the basis for suggestion followed by some special editorial concern by section.

### 1. Title:

The title is short, informative and well constructed.

### 2. Abstract:

Suggestion: Information regarding on how sex differentiation was performed should be written.

### 3. Introduction:

The introduction has provided quality relevant information particularly with regard to hermaphroditism. Suggestion: The introduction required to include relevant information specific to sex differentiation by using gonad identification followed by relevant references.

### 4. Methods:

- a) Please provide specific instrument that used to measure the total length and body weight.
- b) Gonad measurement was performed but there is no gonad weight data in the result section.
- c) Microscopic observations of the gonad is not clearly describe.
- d) "The data were analyzed descriptively" the statement is not clear which data referring to in this article. Please be specific

### 5. Results:

- a) Length and weight frequency distribution class in the table 1 and 2 is not clearly defined.
- b) Set data or the number of the sample is too small.
- c) It is stated that the occurrence of sex transformation in hermaphroditic fish is species dependent and strongly influenced by environmental factors. The author should include the environment report in the study area. This environment report will give another perspective on this study.

### 6. Conclusion:

To conclude the finding that the female differentiated at 183mm and sex changed to male occurred at 302 mm is too "early" because is based on the length and weight. It is better to include histological, endocrinological or event molecular study in this article.

### 7. References:

Suggestion: delete ref #27



Competing Interests: No competing interests were disclosed.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Referee Report 11 April 2017

doi:10.5256/f1000research.11962.r21782



## **Bradley J. Pusey**

Centre of Excellence in Natural Resource Management, The University of Western Australia, Perth, WA, Australia

Title and abstract both appropriate.

Article content - design, methods and analysis all appropriate

Conclusions are sensible and balanced. The authors might consider expressing maturation in terms of the length at which 50% of the sample are one sex or another, in addition as say minimum length.

Perhaps more needs to be said of the fact that not all fish in the very largest size class had changed sex into males.

Competing Interests: No competing interests were disclosed.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Referee Report 29 March 2017

doi:10.5256/f1000research.11962.r21264

# ?

### **Ambok Bolong Abol-Munafi**

Institute of Tropical Aquaculture, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia

1. Title

The title is suitable. Only the word 'harvested' should be deleted.

2. Abstract

Suggestion: A total of 56 white spot groupers were sampled; of these 22 were male, 28 were female and 6 were undifferentiated.

3. Introduction

Accepted but the English Language needs to be revised.

### 4. Methods

- i) Please provide the instrument used to measure the total length and body weight.
- ii) Method for gonad measurement can be removed since the data is not tabled in this article.
- iii) Must have explanation on gonad structure/conditions of functional male and functional female based



on microscopic observations that were used for sex determination. I cannot find the explanation in Muchlisin *et al.* (2010)<sup>1</sup>. Please elaborate on "The data were analyzed descriptively" or the statement is not relevant in this article.

iv) Please explain on what basis the length class and weight class were decided. Why the differences are not equal. Please recalculate.

### 5. Results

- i) The first sentence need to be rephrased.
- ii) Regarding my comment on what basis the length class was decided, the article suggested that the female differentiated at 183mm. If you refer to your Dataset 1, the smallest female is 22.3cm (or 223 mm). Based on the length class, the article suggested that the sex change from female to male occurred at 302 mm. Your Dataset 1 showed that the smallest male caught was 35.0cm (or 350 mm). Please elaborate your data.

### 6. Conclusions

- The sample size is too small and the duration of the study is too short to conclude the finding that the female differentiated at 183mm and sex changed to male occurred at 302 mm.

### 7. References

- No 27 is considered as one of the references?

### General remark:

- The English language must be revised. Standard terminology should be used, eg: sex change instead of sex transform; differentiated instead of recognized.

### References

1. Muchlisin ZA, Musman M, Azizah MN: Spawning seasons of Rasbora tawarensis (Pisces: Cyprinidae) in Lake Laut Tawar, Aceh province, Indonesia. *Reprod Biol Endocrinol*. 2010; **8**: 49 PubMed Abstract | Publisher Full Text

Competing Interests: No competing interests were disclosed.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.