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## Gonadosomatic index (GSI), Hepatosomatic index (HSI) and proportion of oocytes stadia as an indicator of rainbowfish *Melanotaenia boesemani* spawning season

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### Abstract

Gonadosomatic Index (GSI), Hepatosomatic Index (HSI) and proportion of oocytes stadia related with reproduction activity and already used by researcher as an indicator of spawning season. The present study, we evaluated ovary maturation estimation that indicates spawning season based on GSI, HSI and proportion of oocytes stadia. A total of 93 females rainbowfish *M. boesemani* was caught using handnet in Seta, Tiwit and Sagsiger, Maybrat Regency, West Papua. Fish samples body weight was measured then dissected to determined ovary maturation stages temporally. The highest GSI, HSI and proportion of mature oocytes analysis temporally was found in August i.e 2.163%, 2.276%, and 22.66% respectively. Based on temporally analysis from June to Nopember 2013 of GSI, HSI and proportion of oocytes stadia, we assumed that *M. boesemani* populations have experienced a series multiple spawning a year with peak of spawning season in August.

**Keywords:** *M. boesemani*, spawning season, gonadosomatic index, Hepatosomatic index. oocytes proportion

### 1. Introduction

The Rainbowfish *Melanotaenia boesemani* classified as Melanotaeniidae family [1]. *Melanotaenia boesemani* is confined to the Ayamaru Lake system, and considered to be endangered species by IUCN since 1996. To prevent the extinctness of the fish populations should be immediately initiated conservation efforts through ex situ reproduction via domestication. One of the critical success of domestication is related to the reproductive activity information of domesticated fish.

One of reproduction activity in fish is spawning season. The spawning season can be determined by ovary maturation that analyzed temporally [2, 3]. Many researcher indicated ovary maturation based on gonadosomatic index (GSI), Hepatosomatic Index (HSI) and proportion of oocytes stadia [4-13]. GSI is the percentage of gonad weight and fish weight ratio including gonads that expressed gonadal changes quantitatively [14]. HSI is defined as liver weight and fish weight ratio. HSI term usually used in fisheries science as an indicator of energy reserves in the liver [15].

HSI is related with GSI because of vitelogenesis process that synthesized vitelogenin. In fish, vitelogenin is yolk prekursor that synthesized in liver and indiced by estradiol 17 $\beta$  [16-18]. Vitelogenin secreted in blood and transported in oocytes causing the accumulation of yolk. This accumulation cause the changes of oocytes size and enhancement of ovary weighth. This vitelogenesis activity can increased HSI and GSI [15, 19, 20]. Some researchers began to make observations on the HSI in vitellogenesis and maturation stages, namely when the cells tranformed into vitellogenic oocyte [20-22].

Ovary maturation also can be determined by proportion of oocytes stadia that evolved in ovary [9, 11-13]. In teleost fish, the description of ovary maturation can be indicated by differences of oocytes stadia proportion in each maturation stages [9, 11-13]. Research about rainbowfish reproduction already done on *M. eachamensis*, *M. splendida splendida*, *Cairnsichtys rhombosomoides* [2], *M. nigran*, *M. Inornata* [23], *Telmatherina celebensis* [24], *Glossolepis incisus* [25]. The study about reproduction of *M. boesemani* was unveiled for the first time.

The aim of this study was to evaluate GSI, HSI and oocytes proportion in rainbowfish *Melanotaenia boesemani* ovary as an indicator of spawning season

**2. Materials and Methods**

**2.1 Fish samples**

Fish samples consist of 93 female *M. boesemani* that caught at three sampling sites i.e Seta (132° 15' 17. 215" E and 1° 15'64.457" S), Tiwit (132° 14'99.91" E 1° 15'45.475" S) and Sagsiger (132° 15'19.257" E and 1° 14' 97.111" S) (Fig 1:). Fish samples were caught using handnet with 2 metre opened and 1 cm mesh size. Fish samples body weight were measured then dissected to determine ovary maturation stages. Fish samples preparation were done at Research and Development of Freshwater Fish Aquaculture Installation, Sorong Fisheries Academy, then processed histologically at Pathology and Anatomy Laboratory, Faculty of Medicine, Brawijaya University. All treatment on fish samples have been approved by Animal Care and Use Committee of Brawijaya University No. 135-KEP-UB. The fish sampling were done once a month for 5 months (June to November 2013) excluding Oktober.



**Fig 1:** Sampling sites (Google earth, 2013)

**2.2 Observation of Ovary Maturation Stages**

Fish ovary was taken to determine its maturation stage, ovary weight, and oocytes proportion. Determination of ovary maturation stage was categorized based on gonadal development and maturity by Pusey *et al.*, 2001. Ovarian weight was measured to calculate the value of GSI by using following formulae [26]:

$$GSI = \frac{\text{ovary weight (gram)}}{\text{body weight (gram)}} \times 100 \%$$

HSI was calculated by liver weight and body weight ratio using following formulae [27]:

$$HSI = \frac{\text{liver weight (gram)}}{\text{body weight (gram)}} \times 100\%$$

The proportion of oocytes stadia was measured using ovary histology. Fresh ovaries were fixed in 10% Neutral Buffered Formalin (NBF), dehydrated in ascending grades of alcohol start on 70% until absolute, dealcoholized in xylol and embedded in paraffin. Section of about 5-6 μm thickness were cut and stained with Hematoxylin eosin (HE). Ovarian histology slices were observed using Olyvia version 2.2 software. The proportion of the oocytes were counted for determined oocytes cell type distribution in each maturation stages temporally. The proportion of the oocytes determined by

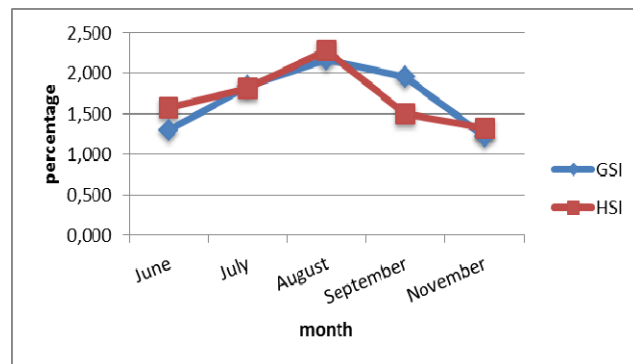
comparing the number of oocytes cells with the total number of oocytes were found in ovarian histology slices in the form of percentage [12]. Oocytes development stage classified as oogonia, early perinuclear oocytes, late perinuclear oocytes, cortical alveoli oocytes, early vitellogenic oocytes, mid-vitellogenic oocytes, mature oocytes, and atresia oocytes [28, 29].

**2.3 Data Analysis**

The data obtained in this research was tabulated in graphs and analyzed descriptively to estimate ovary maturation that indicates spawning season from June to November 2013.

**3. Results**

Ovary maturation can be indicated by GSI, HSI, and proportion of oocytes stadia that evolved in the ovary temporally. The GSI gradually increased from June to August i.e 1.290%±0.14, 1.839%±0.19, 2.163%±0.18 respectively, then gradually decreased on September (1.948%± 0.27) and November (1.220%± 0.13) (mean±standard deviation, Fig.2). The HSI value, increased gradually from June to August i.e (1.570%±0.11), (1.816%±0.15), (2.276%±0.30) respectively, then gradually decreased on September (1.501% ± 0.18) and November (1.324% ± 0.26) (mean±standard deviation, Fig 2:).



**Fig 2:** Mean of GSI and HSI values

The analysis results showed that GSI values increased along with the increase of HSI. Based on Pearson correlation (Table 1) and Chi square (Table 2) analysis showed that GSI and HSI values were positively significant correlated (r = 0.838, p value = 1.000).

**Table 1:** Pearson correlation

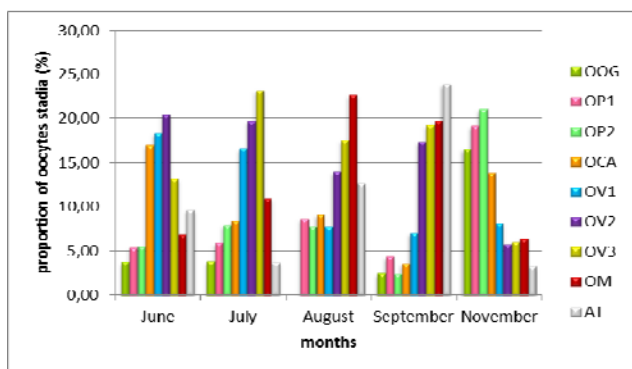
		HSI	GSI
HSI	Pearson Correlation	1	.838**
	Sig. (2-tailed)		.000
	N	81	81
GSI	Pearson Correlation	.838**	1
	Sig. (2-tailed)	.000	
	N	81	81

\*\*Significant level 0.01

**Table 2:** Chi square analysis

	GSI	HSI
Chi-Square	7.531 <sup>a</sup>	.975 <sup>b</sup>
df	70	79
Asymp. Sig.	1.000	1.000

Ovary maturation can be also indicated by the proportion of vitellogenic oocytes that evolved in the ovary. In June, oocytes with high proportion were cortical alveoli oocytes (17.04%), early vitellogenic oocytes (18.34%), mid-vitellogenic oocytes (21.41%) and late vitellogenic oocytes (13.21%). In July, oocytes with high proportion were early vitellogenic oocytes (16.61%), mid-vitellogenic oocytes (19.72%), late vitellogenic oocytes (23.14%). In August, oocytes with high proportion were mid-vitellogenic oocytes (14.00%), late vitellogenic oocytes (17.55%), mature oocytes (22.66%). In September, oocytes with high proportion were mid-vitellogenic oocytes (17.37%), late vitellogenic oocytes (19.27%), mature oocytes (19.70%). In November, oocytes with high proportion were oogonia (15.81%), early perinuclear oocytes (18.28%), late perinuclear oocytes (26.40%) and cortical alveoli oocytes (11.46%). The ovary maturation that indicated from atresia follicles showed that the lowest was found in November (3.03%) and the highest was found in September (23.86%) (Fig 3:)



**Fig 3:** The proportion of oocytes stadia that evolved in the ovary of *M. boesemani*

(OOG = oogonia; OP1 = early perinuclear oocytes; OP2 = late perinuclear oocytes; OCA = cortical alveoli oocytes; OV1 = early vitellogenic oocytes; OV2 = mid-vitellogenic oocytes; OV3 = late vitellogenic oocytes; OM = mature oocytes; AT = atresia follicles)

#### 4. Discussion

The present study, showed that GSI and HSI values increased as the increasing of oocytes development and decreasing as also, temporally. The highest value of GSI and HSI were on August. Many research about rainbowfishes [2, 25, 30] stated that the higher level of ovary maturation, higher value of GSI and HSI. The result of this study also appropriate with research was conducted by Kingdom dan Allison [31], Oso *et al.* [6], and Cek *et al.* [20] which is stated that the increasing of GSI and HSI concomitant with the increase of ovary maturation level and gametogenesis. Its was also related with the accumulation of vitellogenin in the oocytes as precursor of yolk during vitellogenesis which is implicates with enhancement of liver and gonads weight [21, 22].

The increasing of GSI value that concomitant with ovary maturation also found in *Patagonotothen tessellata* [7]. According to this research, GSI value has positive correlation with ovarian development. GSI value can also used for indicator of ovary maturation. The present study, showed that HSI of *M. boesemani* increased along with the increase of ovarian maturation. Contrast with this finding, in *Notopterus notopterus* fish research that conducted by Sudarshan and Kulkarni [10] stated that HSI was gradually decreased along

with ovarian maturation. This decreasing HSI was caused by the used of the energy stored in the liver for the development of the ovaries [10].

The results of GSI, HSI and proportion of oocytes stadia in *M. boesemani* rainbowfish that analyzed temporally from June to November showed that an increase in ovarian maturation from June, July and the peak maturation was in August. The high proportion of atresia follicles in September indicates post ovulation. According to the rainbowfishes research [2, 25], GSI and HSI associated with ovary maturation can be used for indication to determined spawning season in fish. The result of GSI, HSI, and mature oocytes proportion analysis that evolved in *M. boesemani* ovary have the highest value in August. From this finding we can assumed that spawning season of *M. boesemani* happened in August during observation. This spawning time perhaps related with rainfall. The high rainfall condition related with supply of foods and survival of larvae [32, 33].

#### 5. Conclusion

The temporal analysis of *M. boesemani* ovarian maturation from June to November 2013 that associated with GSI, HSI and oocytes proportion showed that the peak of maturation and spawning season was happened in August. This estimation was based on the highest value of GSI ( $2.163\% \pm 0.18$ ), HSI ( $2.276\% \pm 0.30$ ) and mature oocytes proportion ( $22.66\% \pm 1.83$ ). To better understand their annual spawning system, we will investigate more than 10 populations inhabit all corners of the lake.

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