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

Intanurfemi Bacandra Hismayasari, Agung Prama Warith Marhendra, Sri Rahayu, Saidin, Dedy Sutendy Supriyadi

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 determine 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 November 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 extincity 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 \([9, 11-13]\). 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 induced by estradiol 17β \([16-18]\). Vitalogenin secreted in blood and transported in oocytes causing the accumulation of yolk. This accumulation cause the changes of oocytes size and enhancement of ovary weight. 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 transformed 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. eeachamensis, M. splendidida splendidida. Cairnsichtys rhombosomoides \([3]\), M. nigran, M. Inornata \([21]\), 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° 15’45.475” 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 determined 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.

2.2 Observation of Ovary Maturation Stages

Fish ovary was taken to determined 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 formulæ [26]:

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

HSI was calculate by liver weight and body weight ratio using following formulæ [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 he 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) respectivelly, then gradually decreased on September (1.501% ± 0.18) and November (1.324% ± 0.26) (mean±standard deviation, Fig 2:).

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).

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<th>Table 1: Pearson correlation</th>
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<td><strong>HSI</strong></td>
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**Significant level 0.01

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<th>Table 2: Chi square analysis</th>
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<td><strong>Chi-Square</strong></td>
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<td>Asymp. Sig.</td>
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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. [30], and Cek et al. [30] 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 Patagomothen tesselata [1]. According to this research, GSI value has positive correlation with ovarial 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 Nototoperus 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 ovary 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 [31, 32].

5. Conclusion
The temporal analysis of M. boesemani ovarian maturation from June to November 201!3 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 (2.16%±0.18), HSI (2.76%±0.30) and mature oocytes proportion (22.66%±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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7. References
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