Sex ratio, size structure and fecundity in *Hampala bimaculata* (Cyprinidae) from Betung Kerihun National Park, West Kalimantan Province, Indonesia

[1,2]Widadi Padmarsari Soetignya, [1]Bambang Suryobroto, [3]Mohammad Mukhlis Kamal, [4]Arief Boediono

[1] Department of Biology, Faculty of Mathematics and Natural Sciences, Bogor Agricultural University, Bogor, West Java, Indonesia; [2] Faculty of Agriculture, Tanjungpura University, Pontianak, Indonesia; [3] Department of Aquatic Resources Management, Faculty of Fisheries and Marine Sciences, Bogor Agricultural University, Bogor, West Java, Indonesia; [4] Department of Anatomy, Physiology and Pharmacology, Faculty of Veterinary Medicine, Bogor Agricultural University, IPB Dramaga, Bogor, West Java, Indonesia. Corresponding author: W. P. Soetignya, wdpadmasr@yahoo.co.id

Abstract. *Hampala bimaculata* is an endemic species in Borneo and a low density of the fish is noted at the surveys. However, little is known about important aspects of its reproduction in order to assist the fisheries management planning. The main objective of this study was to determine some aspects of the reproductive biology of *H. bimaculata* with regards to sex ratio, size structure, and fecundity. A total of 181 individuals specimens were collected between February and October 2013 and continued between July and November 2014 using gill nets and anglings in Betung Kerihun National Park, West Kalimantan, Indonesia. The overall sex ratio was 1:2.23 (males:females) which shows significant deviation from the expected 1:1 ($p<0.05$). Dominance of females over the males was observed. A significant difference in sex ratio of size structure departed for fish measuring between 350 and 549 mm total length. The length-weight relations showed allometric negative growth for males ($b=2.73$) and isometric for females ($b=2.98$). The number of eggs produced by each female varied from 27,890 to 168,530. Relative fecundity, expressed as eggs g^{-1} of body weight of the females, ranged between 23.77 eggs g^{-1} and 94.94 eggs g^{-1}. The relationships between fecundity and the parameters under study showed the fecundity is significantly correlated with total length, total body weight, and gonade weight. This study presents new original data on basic traits of the natural history of *H. bimaculata*. The results may be used to assist the management strategies and conservation of the species and its habitat.

Key Words: reproductive biology, length-weight relations, allometric, isometric.

Introduction. The genus *Hampala* belongs to the family Cyprinidae and consists of six species all occurring in Southeast Asia. They are characterized by 25-30 lateral line scales, last simple dorsal ray finely serrated behind, but distinguishable each other by their color patterns (Doi & Taki 1994). Three species occurs in Indonesian waters i.e. *H. ampalong, H. bimaculata* and *H. macrolepidota.* Aspects of the biology and ecology of *H. macrolepidota* have been investigated (Abidin 1986; Zakaria et al 2000; Intan et al 2013; Makmur et al 2014). In contrast, poor biological information is available for *H. bimaculata* and *H. ampalong.*

H. bimaculata is an endemic species in Borneo (Ryan & Esa 2006) locally known as Dungan, Arungan and Barb Hampala var. bimaculata. It characterized by two vertical blotches on the side, one under the dorsal and one on the anterior part of the caudal peduncle (Kottelat et al 1993*).* It is a food fish as well, being popular in sport fishing, which is very sensitive to poor water quality and mainly in clear rivers or streams with running water and sandy to muddy bottoms (Intan et al 2013). A few surveys on the fish populations in West Kalimantan and its low representation in ichthyologic collections suggest that it is a rare species with small populations (Rachmatika & Haryono 1999; Rachmatika 2001; Septiani & Sidabutar 2015).

Knowledge of the reproductive biology and the factors affecting are important issues in fish and fisheries biology for culture and conservation. Some aspects of reproductive biology are important relation to its reproductive potential and growth such as sex ratio, size structure, and fecundity. At present, no specific management arrangements exist for *H. bimaculata*, no assessment of the status of its populations has been made and little is known of its reproductive biology. Therefore, the main objective of this study was to determine on some aspects of reproductive biology in *H. bimaculata* with regards to sex ratio, size structure, and fecundity.

Material and Method

Fish collection. Fish samplings were performed between February 2013 and October 2013 and continued between July and November 2014 in Embaloh (1°24'31.6"N-1°19'18.3"S and 112°23'44"-112°29'36.8"E) and Sibau (1°20'33.6"N-1°02'39.8"S and 112°53'23"- 113°15'08.1" E) watersheds, Betung Kerihun National Park, West Kalimantan Province, Indonesia. Figure 1 shows the location of the sampling locations in Embaloh and Sibau Watersheds. Embaloh Watershed consists of few rivers and its tributaries such as Embaloh, and Tekelan rivers. The Embaloh river flows to south as long as ±130 km. Sibau Watershed consist of few rivers and its tributaries as Sibau, Menjakan, and Apeang. Sibau river flows to south as long as 105 km. A total of 181 individuals specimens were collected using gill nets (with 20 and 30 mm mesh sizes each) and anglings. The total body length (L) was measured to the nearest 1 mm, and the total body weight (W) of specimen was weighed to the nearest 1.0 gram using digital balance. Gonads were weighed to an accuracy of 0.01 g.

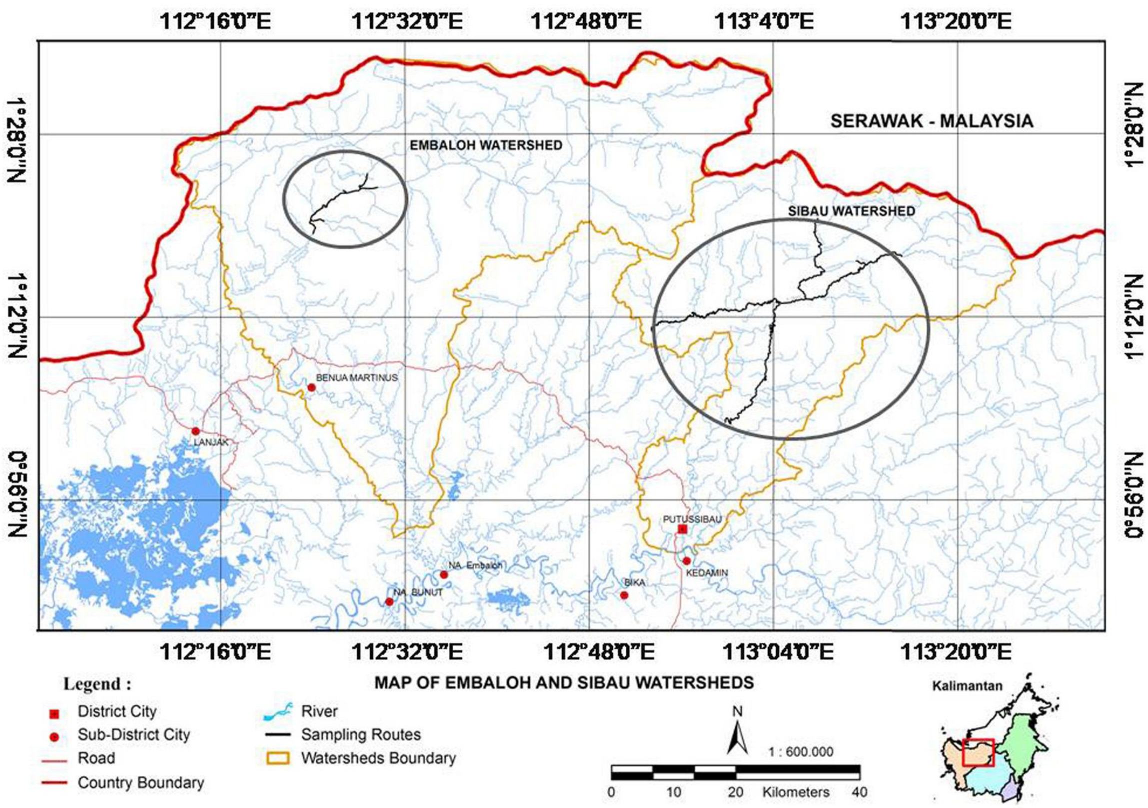

Figure 1. Study sampling area (in black circles).

A total 181 individuals (56 males and 125 females) of *H. bimaculata* were captured in Embaloh and Sibau watersheds.

The specimens was dissected laterally and identified for sexes macroscopically. The ovaries of 15 mature females were immersed in Gilson's solution to estimate fecundity of the species. To obtaine samples accurately, three sub-samples from the posterior, middle and anterior sections of two lobes of the each ovaries were taken and weighed (Weng et al 2005). The samples were weighed and the number of ripe eggs was counted. The total number of ripe eggs in the ovary is estimated by multiplying the number of ripe eggs in the sample by the ratio of the ovary weight to the sample weight, F = (gonad weight x number of eggs in the sub-sample) / sub-sample weight (Yeldan & Avsar 2000; Hossain et al 2012). The relative fecundity (Fr) was expressed by dividing the absolute fecundity (Fa) by the fish body weight. The result was the number of eggs per gram of body weight.

Data analysis. The sex ratio was expressed as (the number of males)/(number of both sexes combined). The sex ratio was analyzed by Chi-square test, in order to verify whether the proportion of males and females differed from the expected ratio 1:1 (Lanes et al 2012). The length-weight relationship was calculated using the expression: $W = aL^b$, where the W is the body weight in g and *L* is the total length. Parameter a and b were estimated by linier regression analysis based on natural logarithms: ln(W) = ln (a)+b ln (L) (Hossain et al 2012). Values of the exponent b provide information on fish growth. When b = 3 increasing in weight is isometric and the value of b is other than 3, weight increase is allometric, (positive allometric if b>3, negative allometric if b<3). The null hypothesis is of the isometric growth (Ho, b = 3) was tested by t-test, using statistic; $t_s = (b-3)xS_b^{-1}$, where S_b is the standard error of the slope, for $\alpha = 0.05$ for testing significant differences among slope (b) between two regressions for the same species (Morey et al 2003; Sangun et al 2007). Linier regression analysis was used to determine relationship between fecundity and total length, total body weight, gonade weight (Hossain et al 2012). The all analysis of statistics and diagrams were performed using R program.

Results and Discussion. Sex ratio of *H. bimaculata* samples during the study period is presented in Table 1. For *H. bimaculata*, the overall sex ratio was 1:2.23 males to females, which shows significant deviation from the expected 1:1 (p<0.05), however, the monthly sex ratio is equal except April, June and November. This means that females predominate over males.

Table 1

Sex ratio of *Hampala bimaculata* samples during the study period

Months	*No of fish examined*		*Sex ratio*	*p value*
	Male	*Female*		
February	3	7	1:2.33	0.21
March	5	12	1:1.71	0.09
April	7	22	1:3.14	0.01*
May	2	5	1:2.50	0.26
June	2	9	1:4.50	0.04*
July	15	20	1:1.33	0.40
August	5	12	1:2.40	0.09
September	9	16	1:1.78	0.16
October	5	8	1:1.60	0.41
November	3	14	1:4.67	0.01*
Total	56	125	1:2.23	0.00*

*significant at 5% level.

The data of size structure of sample showed that males ranged between 200 mm to 475 mm in total length and 105 g to 800 g in total weight. Females were 220 mm to 620 mm in total length and 110 g to 3050 g in total weight. The sexes comparisons in size structure of *H. bimaculata* samples are presented in Table 2. Size structure ranged from

200 to 620 mm total length (TL) with significant difference in sex ratio which departed from (1:1) for fish measuring between 350 and 549 mm TL.

Table 2
Chi-square test for *Hampala bimaculata* sex ratio comparisons by size class in Embaloh and Sibau watersheds

Range of total length (mm)	*No of fish examined*		*Total*	*p-value*
	Male	*Female*		
200-249	6	14	20	0.07
250-299	14	18	32	0.47
300-349	19	27	46	0.24
350-399	12	29	41	0.01*
400-449	4	15	19	0.01*
450-499	1	10	11	0.01*
500-549	0	9	9	0.00*
550-599	0	2	2	0.16
600-650	0	1	1	0.31
Total	56	125	181	-

*Significant at level 0.05%.

The length-weight relations of *H. bimaculata* samples are shown in Figure 2. The relationships between total body weight and total length were to be:

lnW = 2.73 ln L - 10.10 (n = 51, r^2 = 0.90; p<0.0001) for males and

lnW = 2.98 ln L -11.36 (n = 115, r^2 = 0.89; p<0.0001) for females.

In this study, the b values for the male and females of *H. bimaculata* were 2.73 and 2.98, respectively. These values characterize negative allometric growth for male indicating that this species increases more in length than predicted by its increase in weight, and the b value of female *H. bimaculata* was close to 3 (b ~ 2.98), indicative of isometric growth.

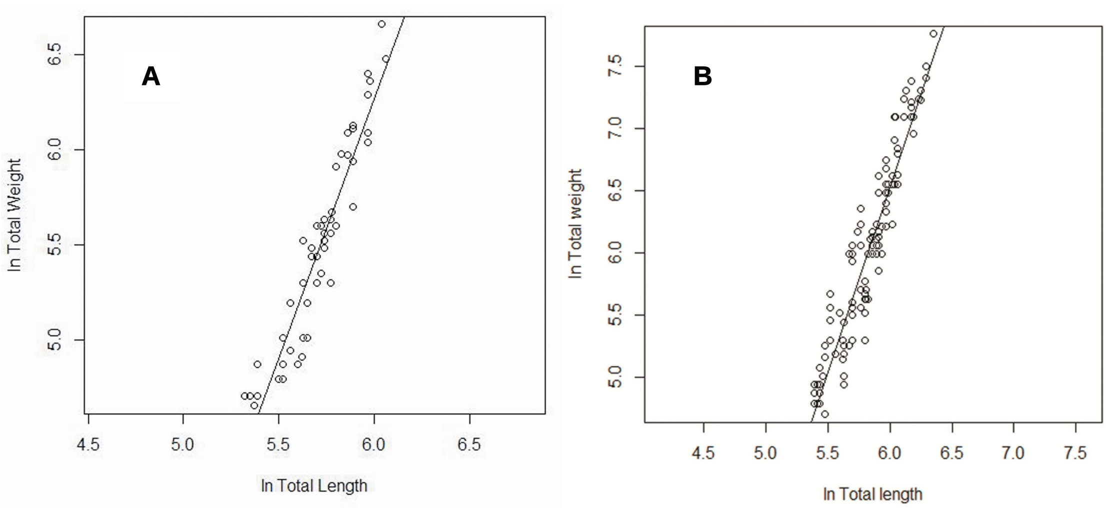

Figure 2. The length-weight relationship of *Hampala bimaculata* described with linear function. A: Male (ln W = 2.73 ln L - 10.10; n = 51, r^2 = 0.90; p<0.0001). B: Female (ln W = 2.98 ln L -11.36; n = 115, r^2 = 0.89; p<0.0001).

The absolute fecundity varied from a minimum of 30,640 to a maximum of 168,530 with a mean of 66,400 eggs, corresponding to fish total length 420 to 620 mm. Relative fecundity, expressed as eggs g^{-1} of body weight of the females, displayed an interval, from a minimum of 25.53 eggs g^{-1} to a maximum of 94.94 eggs g^{-1}. The relationships between absolute fecundity and the parameters under study are illustrated in Figure 3a-c. The results showed that absolute fecundity significantly correlated to total length, total body weight, and gonade weight. As well, analysis of regression showed that there were significant relationship between relative fecundity (F_r) and total body length, total body weight and gonade weight which these are illustrated in Figure 3d-3f.

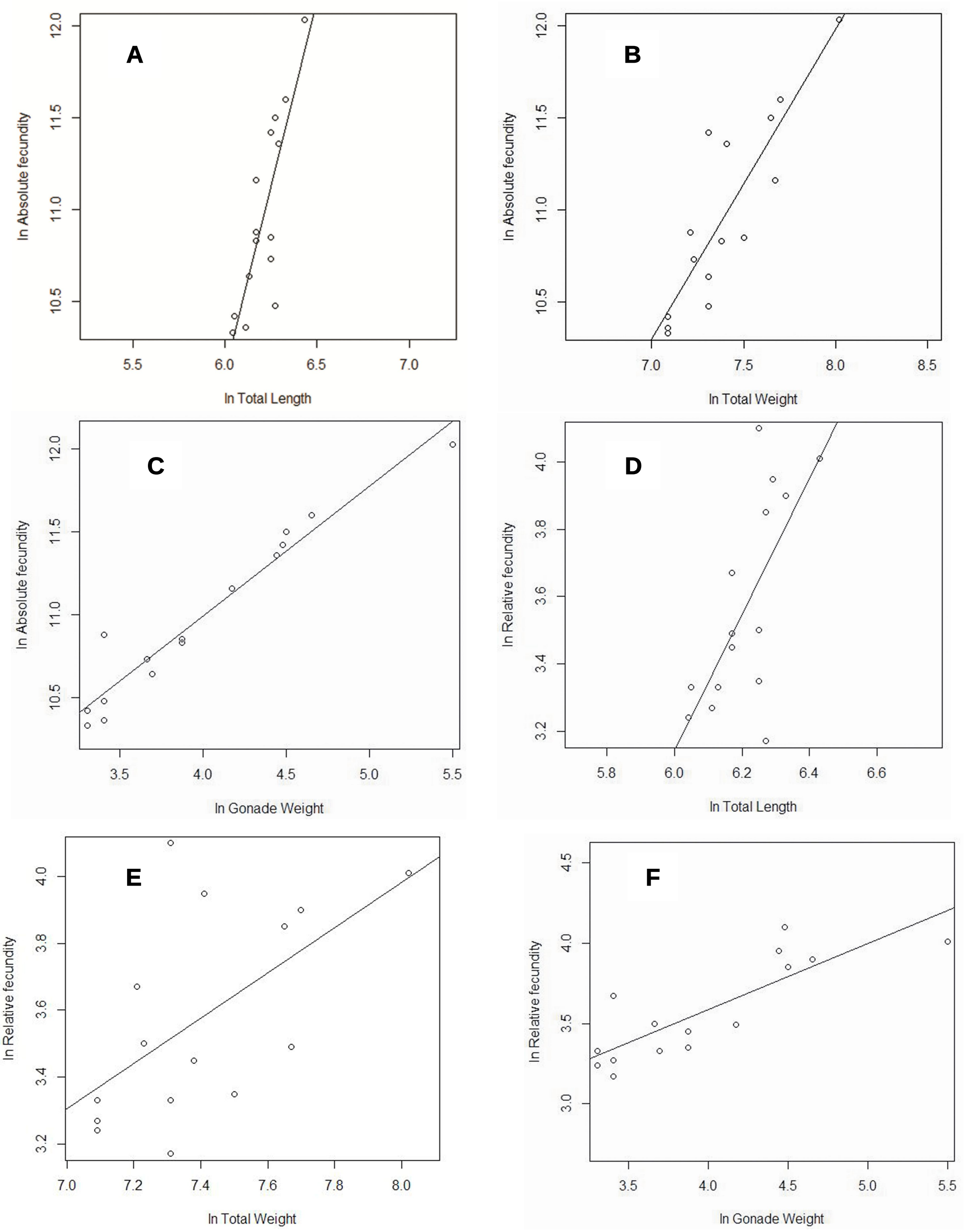

Figure 3. Linear function of *Hampala bimaculata* for: A: Absolute fecundity-total length relations (ln Fa = 4.03ln TL - 14.05, r^2 = 0.68 p<0.001); B: Absolute fecundity-total weight relations (ln Fa = 1.68ln W - 1.44, r^2 = 0.76 p<0.001); C: Absolute fecundity-gonade weight relations (ln Fa = 0.78ln W_G + 0.75, r^2 = 0.94 p<0.001); D: Relative fecundity-total length relations (ln Fr = 2.02ln L - 8.984, r^2 = 0.47 p<0.001); E: Relative fecundity-total weight relations (ln Fr = 0.68ln W - 1.45, r^2 = 0.34 p<0.05); F: Relative fecundity-gonade weight relations (ln ln Fr = 0.45ln W_G + 1.94, r^2 = 0.70 p<0.001).

In this study, the overall males, females sex ratio was 1:2.23, which shows significant deviation from the expected 1:1 ($p<0.05$). This ratio is similar to that observed in other Cyprinids: *Labeo cylindricus* (1:1.63) (Weyl & Booth 1999), *Gymnocephalus cernuus* L. (1:5.63) (Lorenzoni et al 2009), *Labeobarbus batesii* (1:1.42) (Tiogue et al 2013). However, this is found in different numbers with population of *H. macrolepidota* with being not significantly different from the expected ratio 1:1 (Abidin 1986).

Sex ratio varies considerably from species to species, but in the majority of species it is close to one. When this trend is not followed, should be take into account that some factor is modifying the equilibrium. The sex ratio may be influenced by a number of factors including a sex difference in longevity, a sex difference in growth, and the sampling methodology (Liao & Chang 2011; Ma et al 2012). The sex ratio might also be affected by differential fishing factors related to seasons and schooling in feeding and spawning grounds. Skewed ratios may also occur as a result of the differences in natural and fishing mortality between sexes (Guoping et al 2008) and food availability.

In this study, there is a significant difference in sex ratio in size structure which departed from 1:1 for fish measuring between 350 and 549 mm TL. In this case, that may be related to growth rate. This is similar to that observed in other species, *Micropogonias furnieri* (Vicentini & Araujo 2003). An unbalance in sex ratio can be caused by the growth rate differentiation between sexes. The gender with faster growth rate will go through the most vulnerable smaller size phase quickly, and therefore is reduced the proportion of predation. Meanwhile, the gender displaying slower growth rate will be probably to encounter predation, with its abundance reduced out of proportion in the next development phases (Vicentini & Araujo 2003).

We also observed significantly length-weight relations in *H. bimaculata*, the b values for the males and females of *H. bimaculata.* These values characterize negative allometric growth for male and the b value of female *H. bimaculata* was close to 3 (b ~ 2.98), indicate an isometric growth. According Bagenal & Tesch (1978), the b value can vary among species in the same genus as well as stocks of the same species. The value of b differs not only between species, but sometimes also between the stock of the same species due to sexes, maturity, seasons and even time of day because of changes in stomach fullness.

Females had higher values for b. The greatest slope found in females suggested a higher growth rate, and it was probably related to the greater weight of the gonads of females compared to males. The b values on females were higher than of males. This was agreeing with few researches as upon *Gymnocephalus cernuus* (Lorenzoni et al 2009) and *Trachinotus marginatus* (Lemos et al 2011). This difference might be related to the faster relative development of the ovaries which contribute to the increase of fish size, with characterized body shape changes of the females during the sexual cycle.

No report on the fecundity of *H. bimaculata* from other places was available for comparison with that of the present study. The absolute fecundity of *H. bimaculata* can be considered to be high compared with other species, *H. macrolepidota* which it was 7,132 to 62,031 with a mean of 29,445 eggs with total lengths ranging from 205 to 375 mm (Abidin 1986). This result was probably related to the size of the fish sampled from the study areas, where *H. bimaculata* mature samples (420 to 620 mm) were caught with bigger size than *H. macrolepidota.* According to Lambert (2008), potential fecundity is strongly influenced by female size, trade-off between egg size and egg number, reproductive strategy and spawning pattern of the species.

The fecundity of *H. bimaculata* increases with fish size (total length, total body weight, and gonade weight). Several studies also reported that there is a linier relationship between fecundity with total length, total body weight and gonad weight (Weng et al 2005; Alam & Pathak 2010; Abedi et al 2011; Khaironizam & Zakaria-Ismail 2013). According to our observations, the regression coefficient for the relationships between fecundity and ovary weight was higher than length or body weight. Similar correlations have been reported for other Cyprinids: *Labeo rohita* (Alam & Pathak 2010), *Garra rufa* (Abedi et al 2011; Khaironizam & Zakaria-Ismail 2013). This suggests that the eggs number in the ovaries may describe the ovary weight increases of the fish (Jons & Miranda 1997). Jonsson & Jonsson (1999) suggested that increases in body size will increase fecundity because the increases of body size will be in accordance with the amount of energy available for eggs production and body cavity accommodating eggs. In addition, the fecundity variation among the fishes is generally depending upon the various factors such as condition and age of the fish, space and also depends upon the food intake by the fish (Alam & Pathak 2010). Fecundity variation between populations may close from environmental factors, of which temperature is estimated as the most likely selective factor (Jonsson & Jonsson 1999).

Conclusions. The population of *H. bimaculata* in Embaloh and Sibau watersheds indicated a significantly higher proportion of females against males. A significant difference in sex ratio of size structure departed for fish measuring between 350 and 549 mm total length. The length-weight relations indicated negative allometric growth for males and isometric growth for females, which females displayed high absolute fecundity. There was a significant relationship between fecundity and fish size (total length, total body weight), and also between fecundity and gonade weight. This study presents new original data on basic traits of the natural history of *H. bimaculata*. The observed results may be used to assist the development of management strategies and conservation of the species and its habitat.

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Authors:
Widadi Padmarsari Soetignya, Bogor Agricultural University, Faculty of Mathematics and Natural Sciences, Department of Biology, Indonesia, West Java, Bogor, IPB Dramaga, Agathis Street, Postal Code: 16680; Tanjungpura University, Faculty of Agriculture, Indonesia, Pontianak, Prof. Hadari Street, Postal Code: 78124, e-mail: wdpadmasr@yahoo.co.id
Bambang Suryobroto, Bogor Agricultural University, Faculty of Mathematics and Natural Sciences, Department of Biology, Indonesia, West Java, Bogor, IPB Dramaga, Agathis Street, Postal Code: 16680, e-mail: suryobroto@ipb.ac.id
Muhammad Mukhlis Kamal, Bogor Agricultural University, Faculty of Fisheries and Marine Sciences, Department of Aquatic Resources Management, Indonesia, West Java, Bogor, IPB Dramaga, Agathis street, IPB Dramaga, Postal Code: 16680, e-mail: m_mukhliskamal@yahoo.com
Arief Boediono, Bogor Agricultural University, Faculty of Veterinary Medicine, Physiology and Pharmacology, Department of Anatomy, Indonesia, West Java, Bogor, IPB Dramaga, Agathis street, Postal Code:16680, e-mail: aboedi@yahoo.com

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