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REPRODUCTIVE BIOLOGY OF THE RED BIGEYE (*Priacanthus macracanthus* Cuvier, 1829) IN PALABUHANRATU BAY, INDONESIA

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ABSTRACT

The reference point of reproductive biology play an important roles in developing a baseline information for fishery management. Different waters will provide different overview of fisheries related to its biological aspects. The red bigeye (*Priacanthus macracanthus*) is one of economically important demersal fish species in Indonesia. To support the biological status of this species, a regular field observation were carried out during May 2016 to April 2017 in Palabuhanratu bay, south of West Java. The objective of this study is to estimate the spawning season and potential reproductive stages including to evaluate how the key management related to the species and its gear selectivity. Numbers of red bigeye (*Priacanthus macracanthus)* specimen was collected from bottom gillnetter and hand liner. Basic information related to length-weigth, bio-reproduction (maturity) were collected regularly to determine GSI, Fecundity and its impact of fishing (Lc, Lm) to evaluate the recent stock status. The result showed sex ratio no significant differences between males and females except in September to December. The growth pattern indicated negative allometric. The predicted of spawning seasons were around June-July and December-January. Mean of fecundity was estimated to be 230,000 ± 178,000 eggs. Management keys were obtained i.e. length at first maturity (Lm) value was to be 21.9 cm TL which is smaller than length at first capture (Lc) 22.4 cm TL for bottom gill netter and 23.1 cm TL for average fishes caught by hand liner. Therefore it is recommended to close the waters in the bay area during the spawning period. In the case of catch, it is important to apply the precautionary approach with emphasizes to the size of fish allowed to be captured more than the Lm value (above 21.9 cm TL).

Keywords: Priacanthus macracanthus; reproduction; sex ratio; fecundity; spawning season

INTRODUCTION

The Priacanthids (commonly known as red bigeyes) are the tropical fish comprising 4 genera i.e. *Cookeolus*, *Heteropriacanthus*, *Priacanthus*, and *Pristigenys*. The Genus *Priacanthus* consists of 12 species (Froese & Pauly, 2017) and *Priacanthus macracanthus* Cuvier, 1829 is an economically important demersal spesies targeted by inshore fishers in Indonesia. The trend of the Indonesian's red bigeyes annual landings (average annual production 2005-2015) generally not less than about 31,000 tonnes meanwhile the production in 2015 reached 43,000 tonnes with a value of 483 billion IDR (DGCF-MMFA, 2016).

The red bigeye, *P. macracanthus*, widely distributed in the Indo-West Pacific, East Indian region, southern and northern Japan, west coasts of Australia northward to Andaman Sea, south to Australia and New Caledonia. Typically, red bigeyes inhabits nearshore reefs (Starnes, 1999; Ramachandran & Varghese, 2009; Allen & Erdmann, 2012; White *et al.*, 2013) where it is targeted by bottom gillnet fishers in Indonesia. Especially in Palabuhanratu, the red bigeyes were found more than 50% caught by the gear (PPNP, 2015).

The benefits of harvesting this species are greatly felt by the Asian communities include Indonesia. It is used not only as marketed fresh, in dried and salted

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but also preferable species for surimi production and a potential source of natural antioxidant from fish skin gelatin hydrolysate (Starnes, 1999; Sivakami *et al.*, 2003; Phanturat *et al.*, 2010). The surimi by-products of *P. macracanthus* such as heads, internal organs, dirt and bone (Park & Morrissey, 2000) can be utilized as nutrition content in aquaculture alternative feed material (Safitri *et al.*, 2016).

Based on this benefits, it is important to study the reproductive biology of this species. Besides, the information of fish reproductive biology is one of the important parameters in understanding sustainability responses to existing fishing pressures (Lambert, 2008; Lowerre-Barbieri *et al.*, 2011a). Some aspects of biology for red bigeyes have been reported previously in several waters such as in India (Rao, 1983), demographic analysis in Tungkang Waters-Taiwan (Liu *et al.*, 1992), including feeding in Hongkong (Lester, 1968), parasitism in South China Sea (Lester & Watson, 1985), reproductive biology in East China Sea (Oki & Tabeta, 1999) and north-eastern off Taiwan (Liu *et al.*, 2001; Liu *et al.*, 2002). However, reproductive biology in Palabuhanratu Bay has never been reported yet.

The objective of this study is to provide the information regarding reproductive biology including length-weight relationship, sex ratio, sexual maturity stages, gonado somatic index (GSI), size of oocytes, oocyte development based on histological examination, fecundity, length at first maturity (Lm) and length at first captured (Lc) for red bigeyes in Palabuhanratu Bay. It is expected that the results of this study can be used as baseline information and input parameters for further assessment of the stock in this region.

MATERIALS AND METHODS

Samples of red bigeyes were obtained from the catch of local fishers including bottom gillnet (84%) and hand line (16%) in the Palabuhanratu Bay (Figure 1). Random samples were collected each month from May 2016 to April 2017 at Palabuhanratu fishing port, southern Java island, Indonesia. Whole weight (to the nearest 0.1 g) and total length (to the nearest 0.1 cm) were measured. Gonads were removed and weighed to the nearest 0.01 g, then preserved in Gilson's fluid for further examination of fecundity and oocyte diameter.

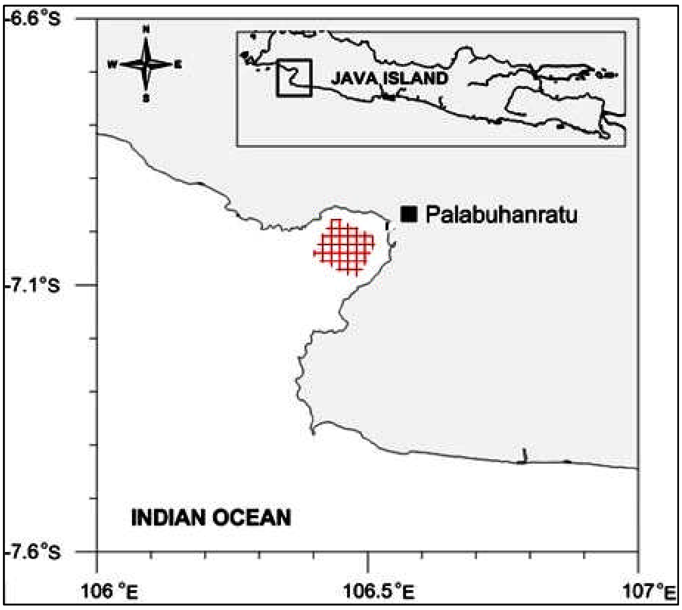


Figure 1. Fishing ground (marked red) for the red bigeyes in Palabuhanratu Bay, West Java, Indian Ocean.

Observation of biological aspects was conducted in Fishery Laboratory of Palabuhanratu National Fishing Port. Fecundity measurements was conducted in Laboratory of Fish Biology, Department of Aquatic Resources Management-Bogor Agricultural University and Fish Biology Laboratory in Research Institute for Marine Fisheries, Jakarta. Histological examination of ovaries was done in Histology Laboratory, Installation of Ornamental Fish Cultivation, Depok.

The ovaries representing sexual maturity stage III and IV were selected to confirm the oocyte diameter and to estimate fecundity. As much as 0.1 g of each ovary (the anterior, middle, and posterior) were placed in sedgewick rafter cells for enumeration of oocytes. All oocytes were measured under magnification of projector 10 x 10 to observe the oocytes diameter and 4 x 10 to enumerate eggs. Histological examination followed (Mumford, 2007) with minor modification. Ovaries were maintained in a 10%

formalin solution for a minimum of 48 hours before processing. The gonads were sectioned at 3 µm thickness and stained with haematoxylin and eosin.

Length – weight relationship was depicted by:

$$W = aL^b \quad (1)$$

where;
W : total body weight (g)
L : the total length (cm)
a and b are regression coefficients of W and L (Sparre & Venema, 1998).

T-test was performed to test the null hypothesis that 'b' was isometric.

Related to the spawning, sex ratio was described to the percentage of females where % sex ratio as a fraction (number of females/number of males) that transformed to following equation (Wu *et al.*, 2008; Tsikliras *et al.*, 2010; Wu *et al.*, 2012; Froese & Pauly, 2018):

$$\% \text{ Sex ratio} = (\sum F / \sum F+M) \times 100 \quad (2)$$

where;
F is female, M is male

Chi-square test used to identify differences between observed and expected values in sex ratio.

Gonado somatic index (GSI) was calculated by (Effendie, 1992; Oki & Tabeta, 1999; Tsikliras *et al.*, 2010):

$$GSI = (Wg/Wt) \times 10^2 \quad (3)$$

where;
Wg is gonad weight (g)
Wt is gutted body weight (g)

Fecundity (F) was estimated from the equation with modification of combined ways volumetric, gravimetric and counting (Effendie, 1992; Liu *et al.*, 2001):

$$F = (G \times X)/Q \quad (4)$$

where;
G = ovary weight preserved in Gilson's fluid
X = number of oocytes stage 3 or 4 (0.1 g ovary in 1 ml diluent water)
Q = 0.1 g ovary's sample

The relationships between fecundity and the three variables, total length (TL), total weight of fish (W) and weight of ovary (O), were expressed by the following equation (Sujatha *et al.*, 2014):

$$F = a\,L^b \quad (5)$$

where;
L = total length (cm)/fish weight (g)/ovary weight (g)
a and b are constants

Sparre & Venema (1998) described the length at first capture (Lc) was estimated from:

$$S_L est = 1 / (1 + \exp(S_1 - S_2 \times L)) \quad (6)$$

where;
$Lc = S_1 / S_2$
$S_L est$ is logistic curve;
S_1 and S_2 are constants

The Spearman-Karber method for estimating the length at first maturity (Udupa, 1986):

$$m = xk + X/2 - (X \times \sum pi) \quad (7)$$

where;
m = logarithm of length at first maturity
xk = logarithm of the middle value of the last class of 100% mature specimens
X = difference of logarithm of mean value
pi = comparison of mature gonads per length class

RESULTS AND DISCUSSION
Results

Length-Weight Relation

A total of 419 specimens were dissected, consisted of 243 males and 176 females were obtained. Total length of male red bigeyes sampled varied from 13.5-32.5 cm, and total weight varied between 42 and 532 g; and for females 15-31.5 cm TL with total weight 56-430 g. The length-weight relationships were estimated to be: $W = 6.92 \times 10^{-2} \times TL^{2.47}$ for males (Figure 2a) and $W = 5.36 \times 10^{-2} \times TL^{2.58}$ for females (Figure 2b). Their type of growth were negative allometric (each sex).

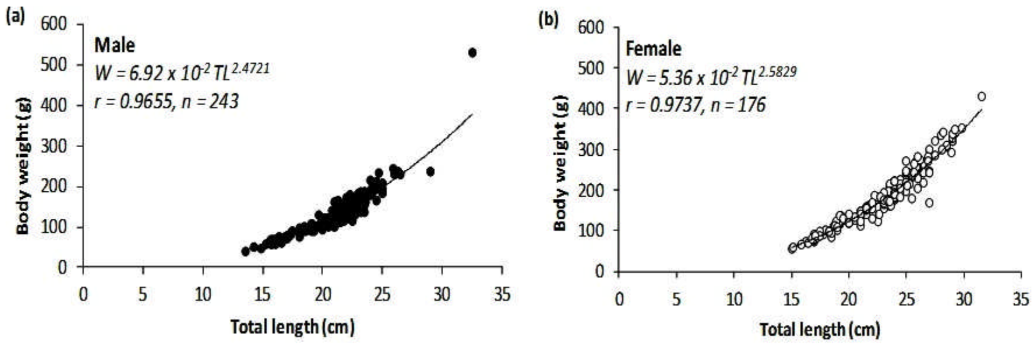


Figure 2. Length-weight relationships of red bigeye. (a) male, (b) female.

We also observed monthly length weight parameters a number of 990 specimens whether dissected or not and obtained the results as in Table 1 below. It shows that monthly length weight relationship of red bigeyes were negative allometric to isometric.

Table 1. Monthly variabilities of length weight parameters of red bigeyes in Palabuhanratu Bay during May 2016-April 2017 (pooled)

Month	n	a	b	R^2	T-test		Character of growth
					$t_{calculation}$	t_{table}	
May	67	0.0144	3.0000	0.9688	1.6808	2.000	Isometric
June	114	0.0132	3.0000	0.8559	0.4450	1.984	Isometric
July	110	0.0583	2.5102	0.7228	3.2743	1.984	Negative allometric
August	99	0.0883	2.4027	0.7804	4.6148	1.984	Negative allometric
September	92	0.0210	2.8674	0.9942	5.7334	1.984	Negative allometric
October	84	0.0124	3.0000	0.8411	0.4435	1.990	Isometric
November	73	0.0145	3.0000	0.9102	0.6071	1.990	Isometric
December	91	0.0144	3.0000	0.8951	0.1589	1.990	Isometric
January	65	0.0609	2.5233	0.9510	6.6023	2.000	Negative allometric
February	95	0.0397	2.6883	0.9646	5.8352	1.984	Negative allometric
March	50	0.0841	2.4285	0.9403	6.4713	2.021	Negative allometric
April	50	0.0131	3.0000	0.7707	1.1528	2.021	Isometric
All	990	0.0372	2.6807	0.9439	15.3507	1.962	Negative allometric

Sex Ratio

Based on sampling, the total number of males was dominant comparing to the females. The females ratio varied seasonally and from all samples was 0.42 (176 / 419). The lowest sex ratio of females was 0.22 in November 2016 and the highest in May 2016 at 0.64 (Figure 3).

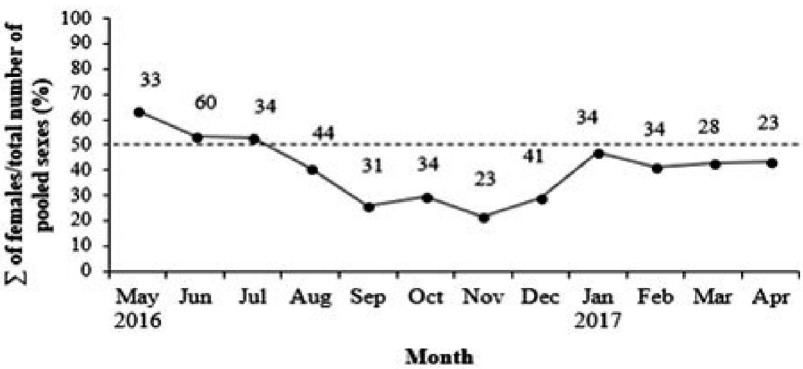


Figure 3. Monthly fluctuation in sex ratio of red bigeyes. Label numbers indicated total number of sample.

Furthermore the evaluation of the sex ratio with Chi-square showed that monthly sex ratio were no significant different between males and females except during September, October, November and December (Table 2).

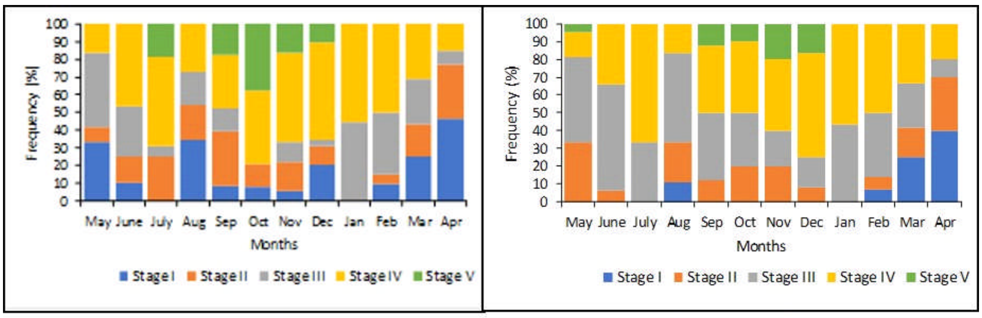
Table 2. Sex ratio of red bigeyes in Palabuhanratu Bay during May 2016-April 2017 (pooled) using Chi-square test

Month	Males	Females	Total	χ^2
May	12	21	33	2.45
June	28	32	60	0.27
July	16	18	34	0.12
August	26	18	44	1.45
September	23	8	31	7.26*
October	24	10	34	5.76*
November	18	5	23	7.35*
December	29	12	41	7.05*
January	18	16	34	0.12
February	20	14	34	1.06
March	16	12	28	0.57
April	13	10	23	0.39
Pooled	243	176	419	10.71*

* Confident level 95%

Gonad Maturity Stages

Both male and female, stage IV were found in every month. The males with the highest stage IV obtained in July (50%) and January (56%) (Figure 4a) while the female were in July (67%) and December (58%) (Figure 4b). It is estimated that the spawning season of red bigeyes occupy in May-April. The peaks of spawning occur around June/July and December/January.



(a) Male (b) Female

Figure 4. Percentage occurance of different stages of red bigeye's gonades. (a) males, (b) females.

Gonad Somatic Index (GSI)

The GSI average of males have two peaks were in July and March (2.54 and 1.56 respectively). The female also had bimodal in July and December (5.05 and 2.61 respectively) (Figure 5). Gonado Somatic Index for male and female shows that spawning season happened around July/August and December/January when GSI showed decreasingly after the peak value.

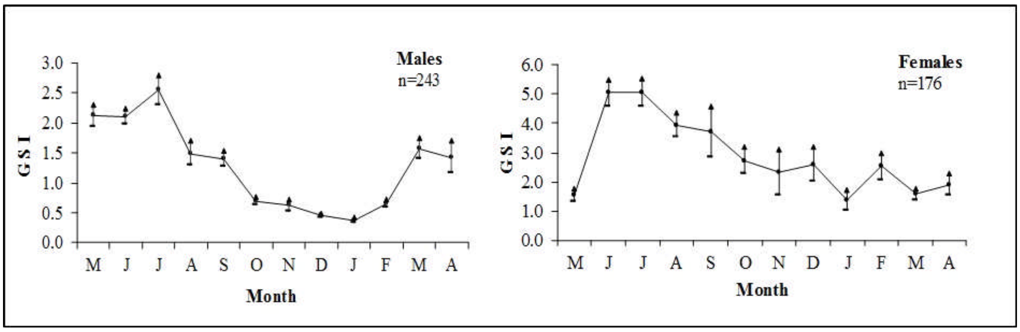


Figure 5. Monthly gonadosomatic index (GSI) of red bigeyes (data are means ± standard deviation).

Size of Oocytes

Oocytes diameter were measured of 35 ovaries in maturity stages III and IV. The oocytes frequency distribution (Figure 6) shows that each stage have one peak and two peaks pooled (both stages). This indicated that the distribution of oocytes consist of more than one group. The mode of stage III was about 0.3 mm, then shifted to 0.4 and 0.5 mm in Stage IV.

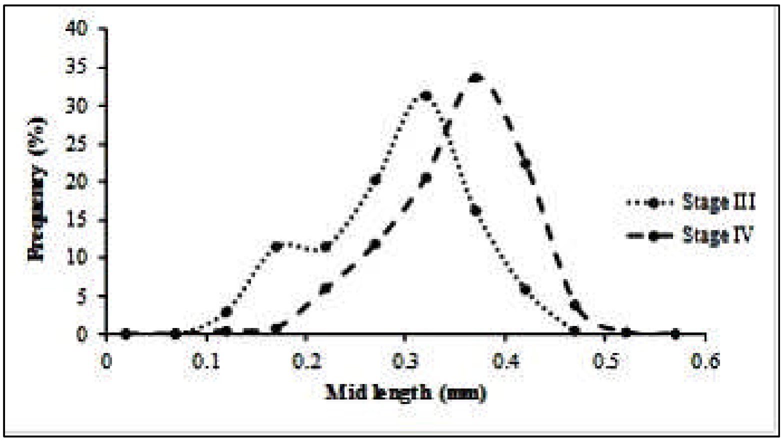


Figure 6. Oocytes diameter of red bigeyes sampled between June 2016 and February 2017

Fecundity

Fecundity was estimated based on 35 ovaries with different oocytes frequency distribution modes. Fecundity expressed as the number of oocytes with diameter of > 0.3 mm, and following equation (4) the estimation ranged from 28,700 (TL = 24.5 cm) to 806,900 (TL = 26 cm) with a mean of 230,000 ± 178,000. The relationship between fecundity (F) and variables total length (L), body weight (W) and ovary weight (O) are described in Figure 7.

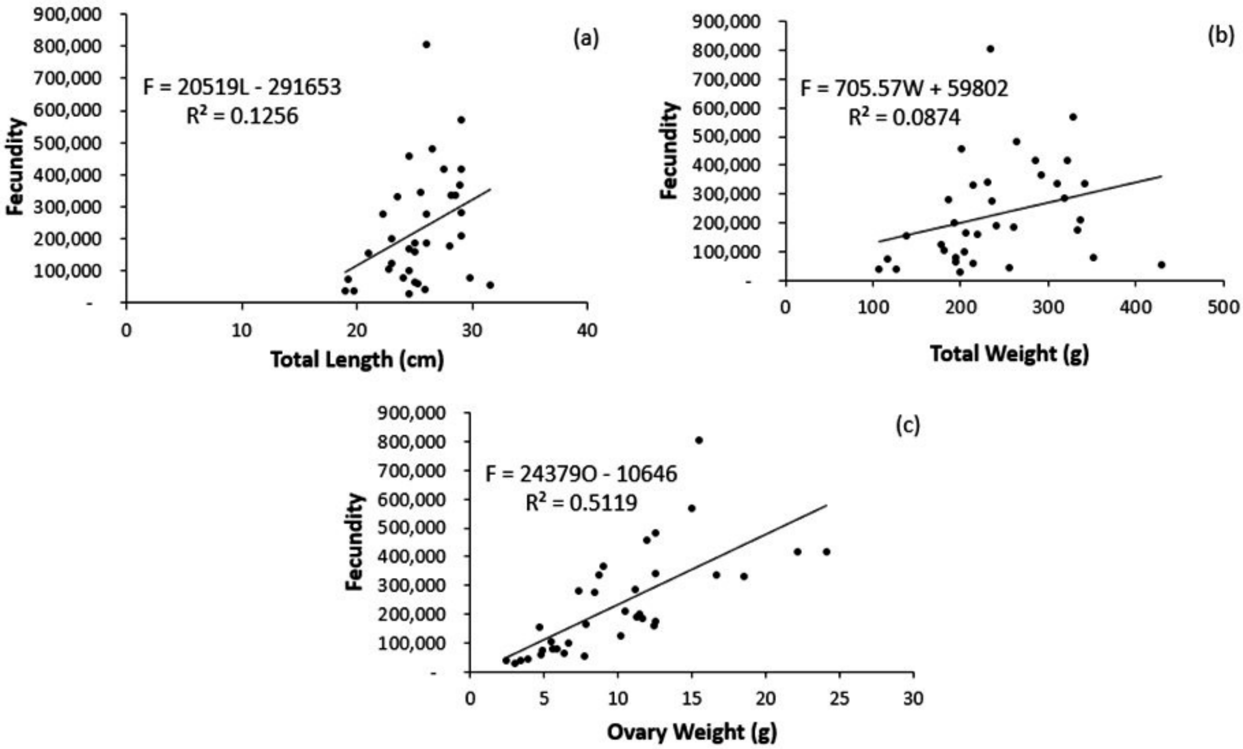


Figure 7. Relationship between (a) fecundity-total length, (b) fecundity-body weight, and (c) fecundity-ovary weight of red bigeye.

Oocyte Development

Development of oocytes for the red bigeye were determined based on histological examination of ten gonads. Through its development can be estimated at least there are five stages of sexual maturity as described below (Figure 8). The oocytes development was similar to those found by Liu (2001).

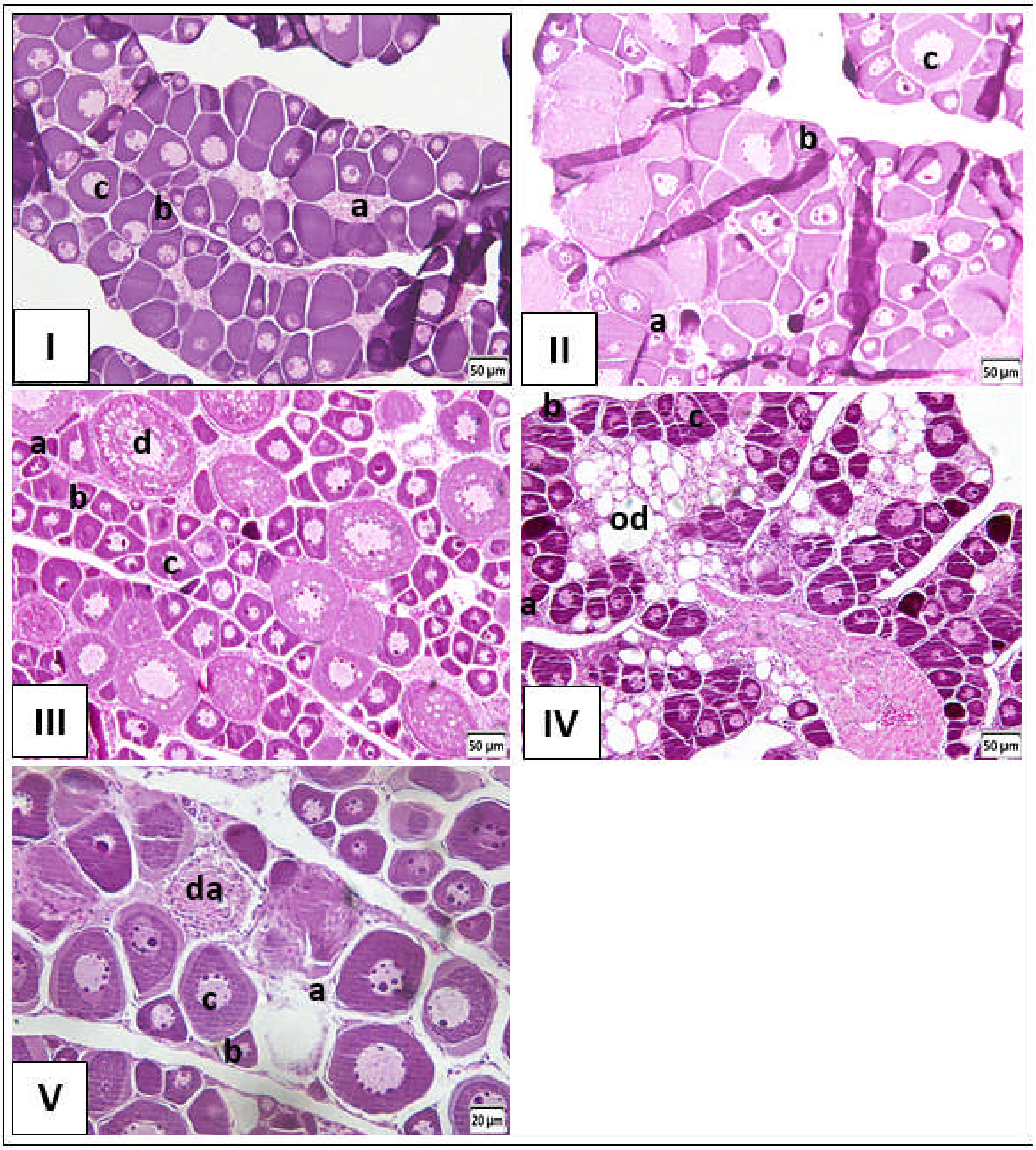


Figure 8. Histological examination of ovaries of the red bigeye. (I) & (II) Primary growth oocytes, (III) Vitellogenesis, (IV) Post ovulatory, (V) Advanced; (a) Tissue of Tunika albuginea, (b) Stage II oocyte, (c) Stage III oocyte, (d) Fully yolk oocyte, (da) Atresia of fully yolk oocyte, (od) Coalescent stage oocytes with lipid droplets combined into larger oil droplets.

Primary growth oocytes in stage I and II characterized by the development of nucleolus. Stage III described by the occurrence of egg yolk deposition process, characterized by the increasing volume of cytoplasm derived from exogenous vitelogenin that form the yolk into fully yolk oocyte. Stage IV characterized by coalescent phase of oocytes with lipid droplets combined into larger oil droplets. Stage V depicted by atresia of fully yolk oocyte.

Length at First Capture (Lc) and Length at First Maturity (Lm)

Estimation Lc value of bottom gill net was 22.4 cm TL (Figure 9) and the hook size used for hand line were number 10-11 (information of local fishers) which the average size of fish caught by hand line was 23.1 cm TL. While the Lm value was 21.9 cm TL (21.0,-22.8 cm TL).

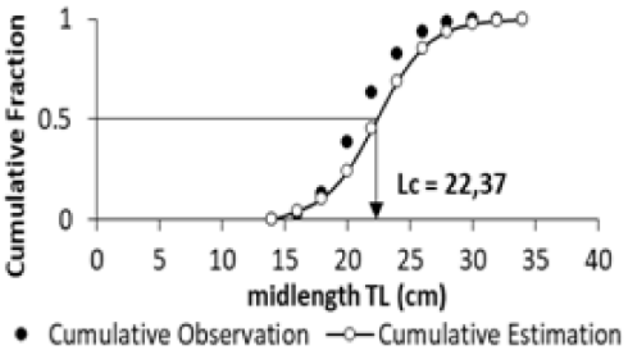


Figure 9. Length at first capture (Lc) of red bigeye from bottom gillnet fishery.

Discussion

This present study showed that length-weight relationship of males and females were negative allometric which means increasing of length is faster than its body weight. While monthly length-weight relationship were isometric in April to June and October to December where the growth of length followed by the growth of its body weight. Several studies on length-weight relationship of red bigeyes have been carried out elsewhere including: the south China Sea (Lester & Watson, 1985), Guei-Shan Island (Joung & Chen, 1992), western region (Indonesia) (Pauly *et al.*, 1996), west and east coast of Peninsular Malaysia and coast of Sarawak (Ahmad *et al.*, 2003), Beibu Gulf (Wang *et al.*, 2011) and southwestern Taiwan (Weng *et al.*, 2017). Mostly those studies showed the growth pattern of allometric negative.

The predicted spawning season of red bigeyes around June/July and December/January. It was supporting by the macroscopic feature of ovary that indicated mature ovaries appearing mostly in June/July and January/February, development of GSI values from high to low occurred in July/August and December/January and measurements of oocyte diameter revealed 1 or 2 modes from June to February, and that hydrated oocytes were also found during this period. The spawning season were also corresponds to Jabbar *et al.* (2017) which states that the recruitment of this species occurs in August-September and February-March. It shown by the appearance of small fishes in September that assumed they were 1+ month old. The growth pattern in July to September and January to March were allometric negatives which assumed due to spawning has occurred.

The monthly changes sex ratio were no significant different between males and females except during September, October, November and December. Wu *et al.* (2012) revealed a relationship between sex ratio during spawning season and fecundity. High fecundity's species to have a low sex ratio during spawning e.g. the white-tongued crevalle, *Uraspis helvolus* (Chiou & Chen, 1993). In contrast, low fecundity species have high sex ratios in their spawning season, e.g. the notchedfin bream, *Nemipterus peronii* (Wu *et al.*, 2008), the Japanese butterfish, *Psenopsis anomala* (Wu *et al.*, 2012), and as shown in the present study. The predicted spawning season in December also coincide with high ratio of females in this period where may be females were in spawning migration during their spawning season.

The fecundity of red bigeyes in the present study was predicted from 28,700-806,900 which more than that in south-western Taiwan (110,000-320,000) (Liu *et al.*, 1992), east China Sea (70,000-230,000) (Oki & Tabeta, 1999), north-eastern Taiwan (70,000-200,000) (Liu *et al.*, 2001) and that in southwestern Taiwan around 3 decades later (2,058-181,468) (Weng *et al.*, 2017). Variability of fecundity may induced by the difference of period and area (Bagenal, 1957). Furthermore Lambert (2008) said that fecundity varies in relation to parental quality (size, condition), food availability, environmental and evolutionary factors (stock biomass, fishing pressure). Besides that, when we compared the relationship between the length, body weight and weight of the ovaries with fecundity, respectively, the largest value of determination coefficient (R^2) among the variables which can explain the variability of fecundity, i.e. ovarian weight of 51.19%. The relationship between fecundity with length and fecundity with weight have small values of

R^2. It was indicated that the addition of length and weight of the red bigeyes were not related to the increase of fecundity. While the relationship between fecundity and ovarian weight indicates that the fecundity increase was quite influential by ovarian weight (Figure 7).

Related to more than one of size oocytes modes in ovarian, especially those that begin to appear at the level of sexual maturity stage III in histological examination (Figure 7; see also Size Oocyte in Result section), its assumed that the species was chategorized as multiple or batch spawners (Murua & Saborido-Rey, 2003; Lowerre-Barbieri *et al.*, 2011b).

The length at first maturity (Lm) values of the red bigeyes were vary in different region even different period as shown in Table 3. Nandikeswari (2016) listed several studies that stock density, food and water temperature may affect the growth of fish then influence the Lm value.

Table 3. Lm values of red bigeye (*P. macracanthus*) in several areas

Type of Length	***Location***	***Year of Sampling***	***Lm (cm)***	***Source***
TL	Palabuhanratu Bay	May 2016-Apr 2017	21.9	Present study
FL	South-western Taiwan	Jan 2011-Jan 2012	21.9 (M) 21.4 (F)	(Weng *et al.*, 2017)
FL		Nov 1981-Nov 1982	17	(Liu *et al.*, 1992)
FL	North-eastern Taiwan	Dec 1997-Nov 1998	18.9	(Liu *et al.*, 2001)
FL	East China Sea	May 1995-Feb 1998	19	(Oki & Tabeta, 1999)

The length at first capture (Lc) of this spesies caught by bottom gill net and hand line were 22.4 cmTL and 23.1 cmTL, respectively. Those values were higher than its length at first maturity (Lm) 21.9 cm. In terms of key management of Lc and Lm values, it means the red bigeyes were already spawned at least once before being caught by the fishing gear (Figure 10). Furthermore, size structure of the red bigeye in this research caught by bottom gill net (44%) and hand line (75%) were above the Lm value which means the red bigeyes caught by those gears were generally already mature. Nevertheless, monitoring of fish caught by bottom gillnet need to be done as the proportion of mature and immature fishes has reached 50:50.

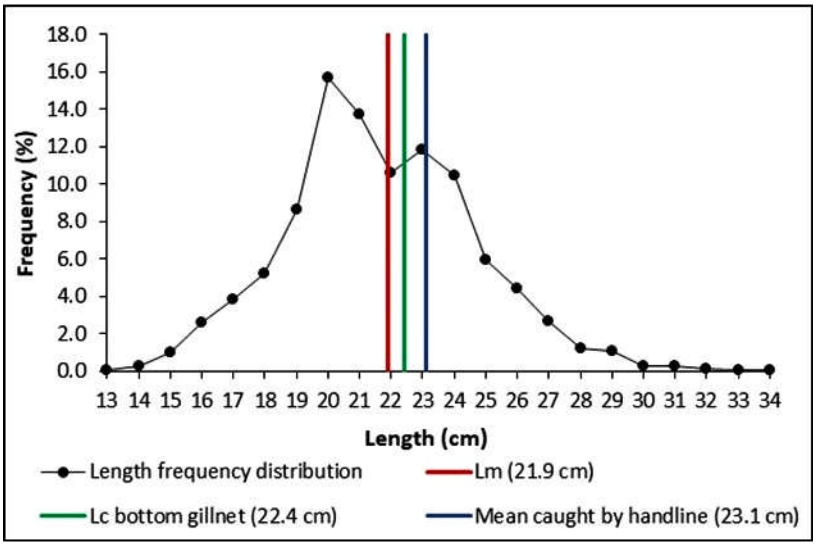


Figure 10. Lm and Lc value of red bigeye in bottom gillnet and hand line fisheries.

CONCLUSION

The red bigeyes grow in negative allometric pattern with overall sex ratio showed no significant different between males and females. They caught by bottom gillnetter and handliner mostly in mature condition. The spawning season occurred about June-July and December-January with the multiple spawner pattern. It is recommended to perform close season during the spawning season. Continuous monitoring is required in relation to the bottom gillnet's mesh size and hand line's hook size and determination of the individual size of fish caught to be above the Lm value.

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