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Reproductive biology of *Nemipterus japonicus* (Bloch, 1791) from the coastal waters of Bintulu (South China Sea), Sarawak, Malaysia

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Abstract

Samples of threadfin breams *Nemipterus japonicus* were collected from a village in Kuala Nyalau and a fish landing centre at Bintulu from April 2013 to March 2014. A total of 360 individuals of *N. japonicus* (214 male and 146 female) were used in this reproductive study. The total length (TL) of individuals were measured to the nearest 0.1 cm and body weight (BW) was recorded to the nearest 0.1 g. Month-wise distribution of the sexes was significantly higher for males in September and March, while in the month of May the number of females was significantly higher ($X^2 = 6.53$; $P < 0.05$). Males showed a preponderance in the size-class of 19.0-20.9 cm ($X^2 = 80.24$; $P < 0.001$), 21.0 -22.9 cm ($X^2 = 56.39$; $P < 0.001$) and 23.0 -24.9 cm ($X^2 = 17$; $P < 0.001$). The gonadosomatic index (GSI) values of *N. japonicus* ranged from 0.07 to 0.19 for males and 0.34 to 4.99 for females. Females had higher GSI than males throughout the study period. For males, a higher GSI (0.11-0.19) was observed during January to February, while it was found to be higher (2.73-4.99) for females during January to March, indicating the spawning season. The present study revealed that ovarian maturity based on histological analysis of *N. japonicus* was classified into seven stages namely, immature (I), immature (II), maturing (III), mature (IV), ripe (V), spawning (VI) and spent (VII). The fecundity of *N. japonicus* was estimated to be within a range of 19221 to 85923 with higher GSI (3.08-6.78) from the coastal waters of Bintulu, Sarawak.

Key words

Bintulu coast, Fecundity, Gonadosomatic index, *Nemipterus japonicus*, Reproductive biology

Introduction

Nemipterus japonicus is a species with a pinkish body that is silvery below and with 11-12 pale golden-yellow stripes along body starting from behind the head to the base. It is widespread throughout the Indo-West Pacific region, ranging from east Africa, including the Persian Gulf and Red Sea, to the Indo-Malay Archipelago. This species inhabits shallow sand or mud bottoms and occurs at depths of 5-80 m. It is an economically important species and are trawled in commercial quantities in the South China Sea (Russell, 1990; 1993).

In Malaysia, *Nemipterus* spp. are important fish used for processing “surimi” since it fits certain selection criteria including having a white flesh, low fat content and good gelling properties. Surimi refers to minced fish flesh that has undergone the process of leaching and subsequent mixing with sugar and a polyphosphate additive (Department of

Fisheries Malaysia). In Sarawak, Nemipterids occupies the second position of total landings, which was estimated to be 4162 metric tonnes in 2012. Moreover, *Nemipterus* spp. are known as an inexpensive fish that are in high demand in the market, especially for local middle class communities in the state. As demand for fish continues to increase, the task of managing and maintaining fisheries resources at sustainable levels will become more challenging.

The reproductive biology of *N. japonicus* has been studied in the Northern of Persian Gulf (Kerdgari, *et al*., 2009), off Veraval (Gopal and Vivekanandan, 1991), the Jizan Region of the Red Sea (Bakhsh, 1994), off Visakhapatnam (Rajkumar *et al*., 2003) and along Kerala Coast (Vinci, 1982). Many researchers have used GSI to determine the breeding season of various species (Zhang *et al*., 2009; Esmaeili *et al*., 2010; Mahmoud, 2009), while histological examination can be used as a complement with GSI to more accurately classify the stages of gonadal maturation during the reproductive cycle (Kopf *et al*., 2012).

Studies on the reproductive biology of *N. japonicus* is still scarce in Malaysia, especially in Bintulu coastal waters (South China Sea), Sarawak. Therefore, the present study was conducted to obtain data on the reproductive biology of *N. japonicus* namely, sex ratio, GSI, fecundity, stage of maturity and seasonal patterns of the breeding cycle. The results of this study would be helpful for fishery managers and resource users prior to make informed decisions regarding fish stock assessments and management in the South China Sea fisheries.

Materials and Methods

Sample collection : Samples of threadfin breams *N. japonicus* were collected from a commercial fish landing centre at Bintulu (3°10' 13.9" N, 113° 02' 22.9" E) and a village in Kuala Nyalau (3° 38' 26.9" N, 113° 2' 30.46" E) from April 2013 to March 2014 (Fig. 1). Thirty samples were selected randomly for the reproductive study.

Gonad collection : Total length (TL) of fish was measured from the tip of snout to the tip of caudal fin using a measuring board to the nearest 0.1 cm. Body weight (BW) was measured by an electronic balance to the nearest 0.1 g on monthly basis.

The specimens were dissected to obtain the ovaries and testes, and the sex of each fish was identified by gonad examination. The proportion of two sexes relative to one another was used to calculate the sex ratio. The sex ratio was

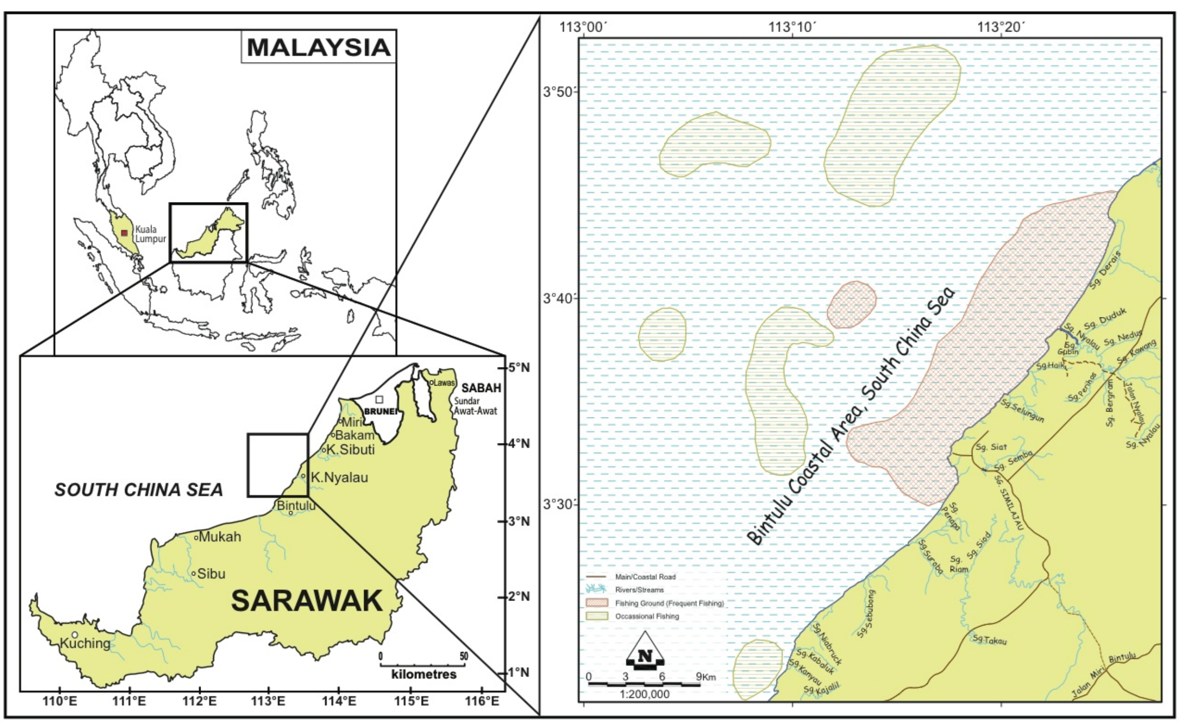

Fig.1 : Location of study area showing the Bintulu coastal waters (South China Sea), Sarawak

Table 1 : Chi square test of sex ratios of *N. japonicus* in various months during the period from April 2013 to March 2014 (*P<0.05, **P<0.01, ***P<0.001)

Month	Male	Female	Ratio (M:F)	Proportion of male (%)	X^2
April	16	14	1:0.88	53.33	0.13
May	8	22	1:2.75	26.67	6.53*
June	20	10	1:0.50	66.67	3.33
July	19	11	1:0.58	63.33	2.13
August	20	10	1:0.50	66.67	3.33
September	22	8	1:0.36	73.33	6.53*
October	16	14	1:0.88	53.33	0.13
November	17	13	1:0.76	56.67	0.53
December	17	13	1:0.76	56.67	0.53
January	16	14	1:0.88	53.33	0.13
February	20	10	1:0.50	66.67	3.33
March	23	7	1:0.30	76.67	8.53**
Total	214	146	1:0.68	59.44	12.84***

Table 2 : Chi square test of sex ratios of *N. japonicus* in different size-class (*P<0.05, **P<0.01, ***P<0.001)

Size class (cm)	Total	Male	Female	Ratio M:F	Proportion of male (%)	X^2
11.0-12.9	0	0	0	0	0	0
13.0-14.9	6	2	4	1:20	33.33	0.67
15.0-16.9	44	15	29	1:1.93	34.09	4.45*
17.0-18.9	124	55	69	1:1.25	44.35	1.58
19.0-20.9	125	89	36	1:0.40	71.20	22.47***
21.0-22.9	50	42	8	1:0.19	84.00	23.12***
23.0-24.9	10	10		1:0	100	10**
25.0-26.9	1	1		1:0	100	1

determined on monthly basis. Gonads were weighed on a digital balance to the nearest 0.0001 g, and GSI was calculated for each sample using the following formula:

$$\text{Gonadosomatic index (GSI)} = \frac{\text{Gonad weight (g)}}{\text{Body weight (g) - Gonad weight (g)}} \text{ X } 100$$

Ovaries were histologically examined to determine the matured condition and respective characteristics were noted. Ovaries were kept separately in a bottles filled with Bouin's solution for the fixation process. Ovaries were then transferred and washed in 70% alcohol and left over night for further histological process (Humason, 1972). The fixed gonads were then dehydrated with a series of alcohol from 70% aqueous alcohol to 100% ethanol. Gonads were cleared in xylene, embedded in paraffin and tissues were sectioned (5-6 µm thicknesses) and then stained with hematoxylin and eosin. Oocytes were photographed at each developmental stage and classified into developmental stages following the method of Wu *et al*. (2008).

Fecundity estimation : Fecundity was estimated gravimetrically using formalin preserved ovaries. Excess moisture was drained out, and the ovaries were weighed to the nearest milligram. Ovaries at IV, V and VI stages were used for fecundity estimation. Each ovary was divided into six portions (the anterior, middle and posterior portions of each lobe). These small samples were removed and weighed. The number of mature ova in the weighed sample was counted. Fecundity was calculated from the number of mature ova in the weighed sample counted by the following formula:

$$F = \frac{w \text{ x } n}{x}$$

Where, F = fecundity or total number of eggs in the ovaries; Where, w is the weight of the ovaries; n is the number of eggs in the small sample and x is the weight of the small sample of eggs (g)

Data analysis : Data were analysed using Statistical Analysis Software (SAS 9.2). Data were expressed as mean ± standard error. Sex-ratio was tested for any deviation from the expected ratio1:1 using Chi-Square analysis. A one-way analysis of variance (ANOVA) was used to test the homogeneity of GSI among different months. Differences were considered significantat P< 0.05.

Table 3 : Mean gonadosomatic index of male and female *N. japonicus* in the coastal area of Bintulu Sarawak

Month	GSI (%)[ns]		Unpaired T-test
	Male	Female	P value
Apr	0.15[ab]	1.94[bcd]	0.0007
May	0.18[a]	1.58[cdef]	<0.0001
Jun	0.16[ab]	1.66[cdef]	0.0028
Jul	0.07[b]	1.90[bcde]	<0.0001
Aug	0.12[ab]	1.86[bcde]	0.0011
Sep	0.11[ab]	0.92[def]	0.0089
Oct	0.10[ab]	0.52[ef]	<0.0001
Nov	0.09[ab]	0.58[def]	0.0003
Dec	0.07[b]	0.34[f]	<0.0001
Jan	0.11[ab]	2.73[bc]	<0.0001
Feb	0.19[a]	3.21[b]	<0.0001
Mar	0.17[ab]	4.99[a]	<0.0001

[ns]Index did not significantly difference throughout the study period (P<0.05)

* Mean±SE in column with dissimilar superscript letter is significantly different (p<0.05)

Table 4 : Estimated fecundity of *N. japonicus* from the coastal water of Bintulu, Sarawak

	No.	Body weight (g)	Total length (cm)	GSI (%)	Total fecundity	Relative fecundity
January	5	65.00-98.00	16.90-19.70	3.06-4.85	21926-31755	317-441
February	5	41.03-104.89	14.20-19.50	3.08-6.17	19221-81986	406-824
March	7	60.82-113.34	16.90-20.50	3.71-6.78	29070-85923	310-758

Results and Discussion

Sex ratio : A total of 360 individuals of *N. japonicus* (214 males and 146 females) were used in this study. The length and weight frequency distributions indicated that *N. japonicus* males ranged from 14.4 -26.0 cm TL with a body weight of 42.0- 217.0 g and females were 14.2 – 21.6 cm TL with a body weight of 41.0 – 152.0 g. The overall sex ratio of *N. japonicus* was significant (X^2=12.84; P < 0.001) in favour of males (Table 1). Month-wise distribution of sexes was significantly in favour of males in September and March, while in May it was significantly (X^2 = 6.53; P < 0.05) in favour of females. The sex ratios were not significant by different (P>0.05) in the month of April, June, July, August, October, November and December.

Sex ratio of 1:1 in *N. japonicus* deviated based on size-classes (Table 2). Males showed a preponderance in the size-class of 19.0-20.9 cm (X^2 = 80.24; P < 0.001), 21.0 -22.9 cm (X^2 = 56.39; P < 0.001) and 23.0 -24.9 cm (X^2 = 17; P < 0.001). There was no significant deviation of sex ratio from 1:1 in the size-class of 13.0-14.9 cm and 15.0-16.9 cm. Female *N. japonicus* were not found in larger size classes of 23.0-24.9 cm and 25.0-26.9 cm, however, they were observed in a small size class 15-16.9 cm as compared to males.

Gonadosomatic index : GSI values of male *N. japonicus* ranged from 0.07 to 0.19 and 0.34 to 4.99 for females. Females showed higher GSI than males throughout the study period (Fig. 2). GSI for both the males and females were significantly different (P <0.05, Table 3) during the study period. Highest GSI (0.11 to 0.19) for males was during January to February. GSI for females was higher (2.73 to 4.99) within January to March, which gradually decreased from April to May and then began to increase from June to July. GSI then decreased gradually from August up to December until a peak during January to March.

Maturity stage of gonad : Only female specimens were used to observe the maturity stage in the study. In *N. japonicus*, there were seven maturation stages for female gonad namely, immature (I), immature (II), maturing (III), mature (IV), ripe (V), spawning (VI) and spent (VII). The immature (I) stage was found throughout the year except in February and March. The spawning stage (VI) also was found throughout the year except in October while the highest percentage was in March (Fig. 3).

At immature stage (stage I), ovaries were small, slender and transparent. The colour was yellowish white, a bit asymmetrical and cylindrical. Oocytes were not visible to the naked eye and oviduct was long and thin (Fig. 4a). The highest percentage of eggs in this stage were observed in October. In stage II, the eggs were still immature (Figure 4b), where the colour was whitish, small transparent, a bit asymmetrical, oviduct little reduced and still restricted to the posterior part of body cavity. In this stage, oocytes were still not visible to the naked eye. This stage was observed in April to May and October to December.

At III, the maturing (Fig. 4c) oviduct was much reduced, granular in appearance, oocytes were not opaque and were just visible to naked eye. The position was up to the middle part of the body cavity. Highest percentage of ovaries during this stage was in October. At Stage IV, mature stage (Fig. 4d), ovaries are bright yellow and a bit of reddish colour. Oviducts further reduced, occupying nearly 2/3 of the body cavity and the ova still in the follicle. Highest percentage of was noted in August. At stage V, ripe stage (Fig. 4e), the ovary occupied 2/3 to 3/4 of the body cavity and blood vessels were clearly seen over the surface. At this stage, the weight of gonad increased but did not yet achieve the maximum amount. Ripe ovaries were observed during January to April, June to August and October with highest percentage in June.

At Stages VI, spawning stage (Fig. 4f), the ovary occupied almost the entire body cavity and was deep red in colour. They were asymmetrical, oviduct was reduced, and ova was larger that appeared to put considerable pressure on the abdomen. Ovary was greatly enlarged and the eggs were fully transparent. At this stage, the weight of gonad was maximum where highest GSI was recorded. Ovaries at this stage were observed during all the months, except in October. Spawning ovaries gradually increased from November to March and decreased until June. In July, the percentage increased and again decreased until September. Stage VII (Fig. 4g), spent ovaries were shrunk, collapsed/flabby, bag like with some residual ova, and contracted. Their length, width and weight were considerably reduced at this stage. The percentage of spent ovaries increased from May to July and again increased in September.

Histological analysis of ovary : Ovarian maturity of *N. japonicus*, based on histological analysis, was classified into seven stages. At stage I, the ovary consisted of oogonia clusters, and oocytes were at chromatin nucleolus stage (Fig. 5a), which occurred most by in October (Fig. 6). At stage II, which was early maturation stage (Fig. 5b), secondary growth oocytes were in the cortical alveoli (ca) stage, primary growth oocytes and oogonia were also present. The oocytes were enlarged and the ratio nucleus (nu) to oocytes area decreased. In this stage, nucleus still occupies a central position and surrounded by cytoplasm. As the ratio of nucleus decreased, the cytoplasm became less basophilic and stained pale with haematoxylin. This stage was observed during May, June and from September to December.

Stage III, which was the mid maturation stage (Fig. 5c), occurred during May and from August to December with highest percentage in September. Secondary growth of oocyte contained yolk granules (yg), and primary growth oocytes and oogonia were also present. The oocyte diameter increased dramatically during this stage. By the end of stage III, cytoplasm was half filled with yolk granules. Stage IV, the final maturation stage (Fig. 5d), was higher in August and was observed at all the months, except September. Secondary growth oocytes in the nuclear migration stage, primary growth oocytes and oogonia were present. By end of stage IV, oocytes were completely filled with yolk, cortical alveoli

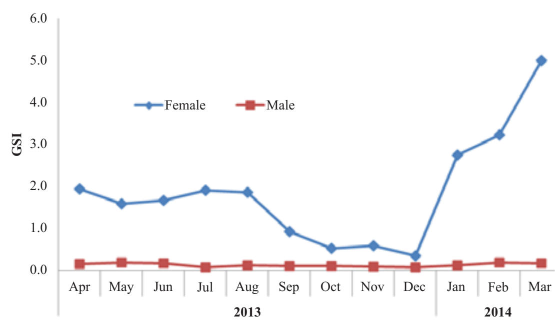

Fig. 2 : Gonadosomatic index for *N. japonicus* collected from Bintulu coastal waters, Sarawak

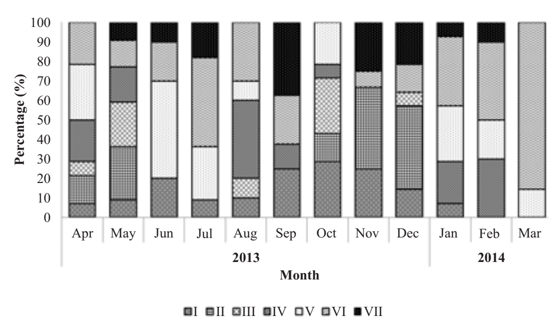

Fig. 3 : Percent composition of reproductive stages classified for female of *N. japonicus*. Immature (I); immature (II); maturing (III); mature (IV); ripe (V); spawning (VI); spent (VII)

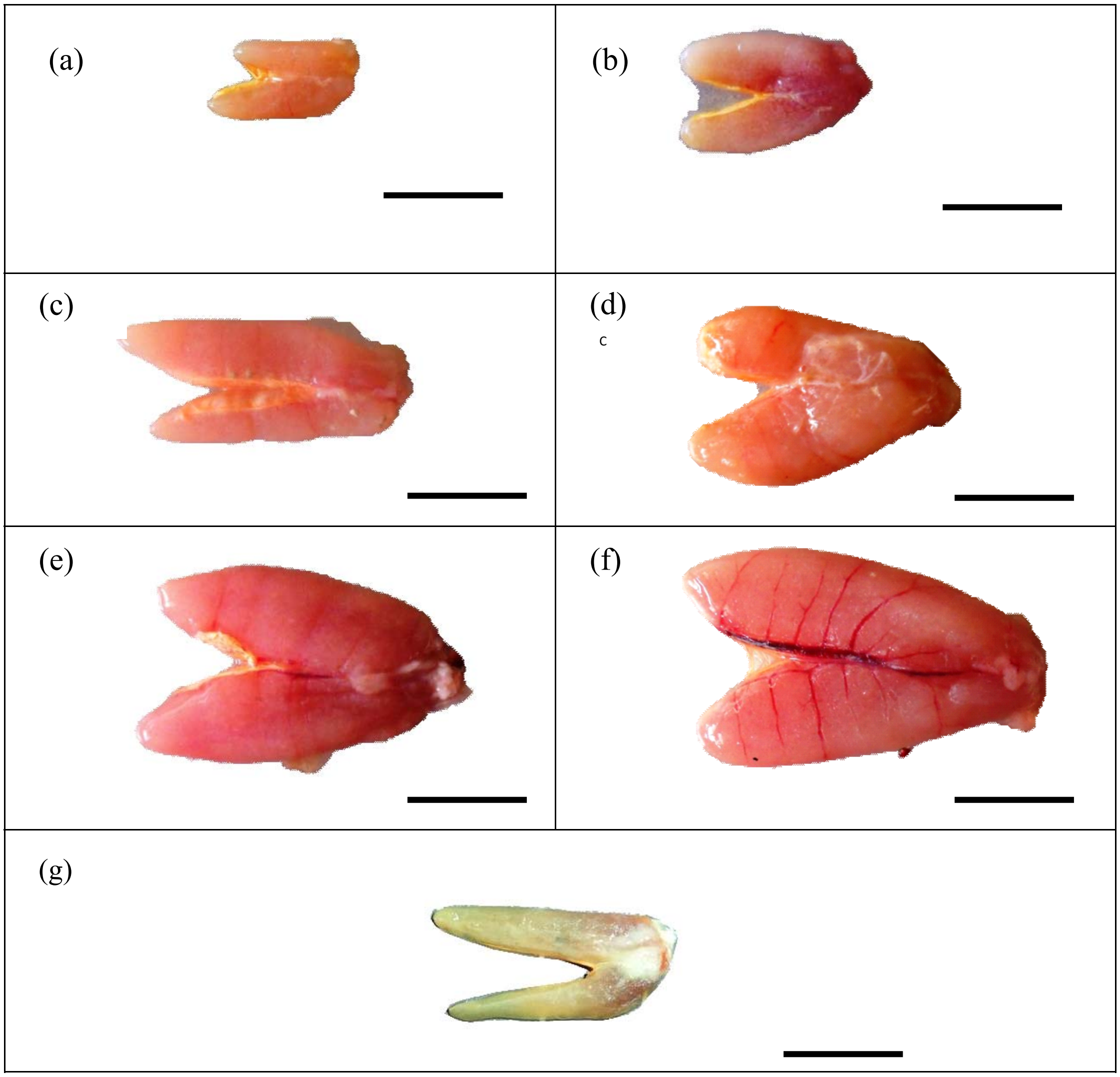

Fig. 4 : The macroscopic morphology of the ovaries of *N. japonicus*. a) immature (I); b) immature (II); c) maturing (III); d) mature (IV); e) ripe (V); f) spawn (VI); g) spent (VII). Scale bar: 1 cm

were pressed against the cell membrane (cm) and nucleus began to migrate towards the micropyle.

Stage V, spawning prepared stage (Fig. 5e), was higher in January, April, June final growth oocytes with hydrolyzed yolk granules (hyg), primary growth oocytes (po) and oogonia were present. The nucleus had disintegrated and was no longer visible. Stage VI, spawning active stage (Fig. 5f), Postovulatory follicles (pof), final growth oocytes with hydrolyzed yolk granules, primary growth oocytes (po) and oogonia were present. Ovulated eggs were present in the dorsally located oviduct. Postovulatory follicles accumulated in the peripheral tissue. This was observed in all month, except in October and November with highest percentage in March.

Stage VII, spent or rest stage (Fig. 5g), primary growth oocytes and oogonia were present. In the spent or rest stage, a great part of the completely depleted ovaries contained large number of empty nests (post spawning period), and also contained unovulated mature yolky eggs undergoing resorption (atresia). This was observed in November to February, May to July and September. Highest percentage was recorded in September.

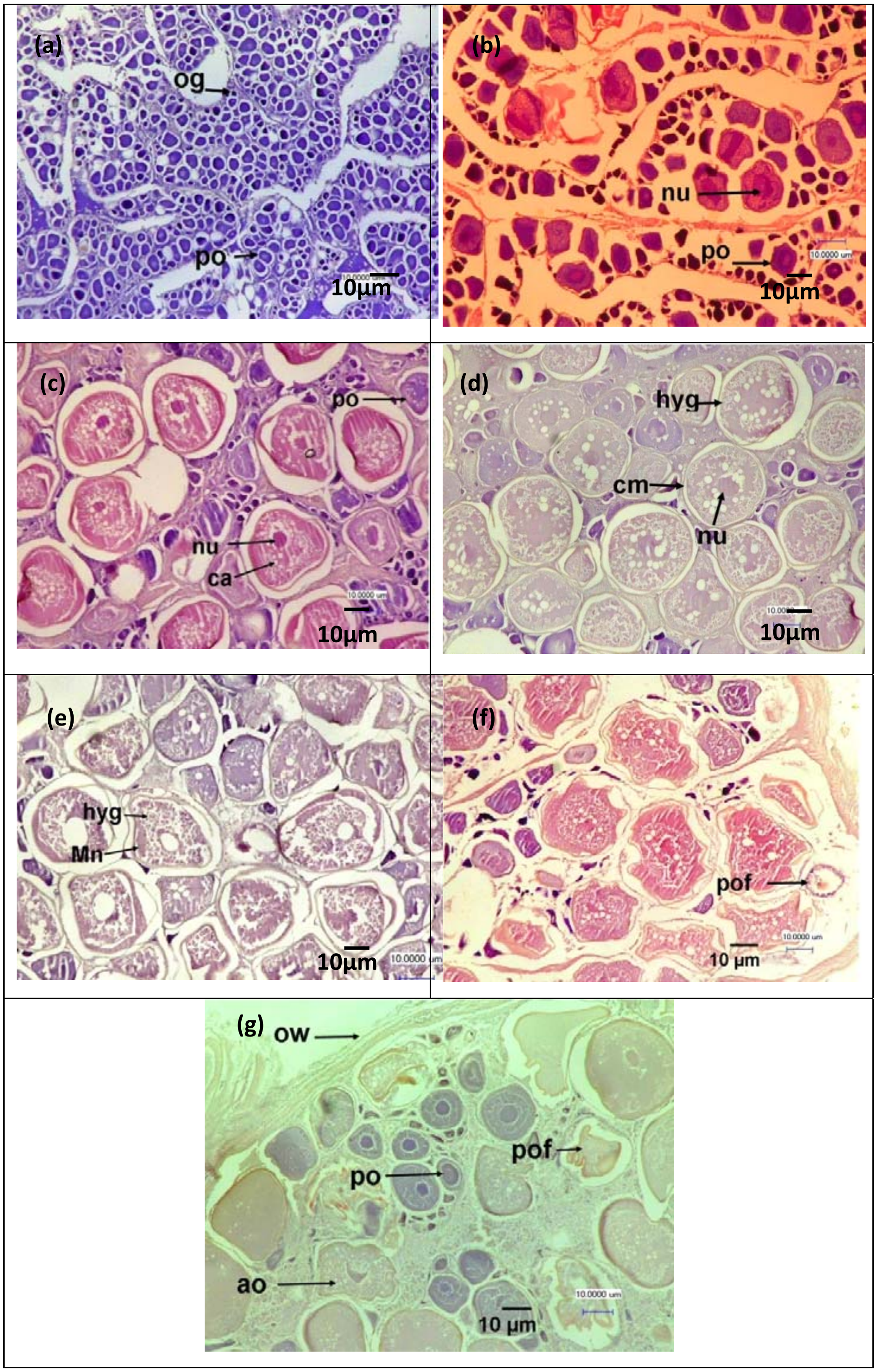

Fig. 5 : Ovarian tissue at various maturity stages of *N. japonicus*; a) immature; b) early maturation; c) mid maturation; d) final maturation; e) spawning prepared; f) spawning active; g) spent. og: oogonia, po: primary oocyte; nu: nucleus, ca: cortical alveoli, yg: yolk granule, cm: cell membrane, hyg: hydrated yolk granule, ao: atretic oocyte, ow: ovary wall, pof: post ovulatory follicle. Scale bar=10 µm

Fecundity : Fecundity was estimated when GSI higher (3.08 to 6.78) with total length and body weight of 14.2 to 20.5 cm and 41.03 to 113.34 g, respectively. The estimated total fecundity of *N. japonicus* was within the range from 19221 to 85923 (Table 4) and in significantly different (t-test, $P>0.05$) within left and right ovaries. Fecundity among anterior, middle and posterior part of each ovaries were also in significantly different. Furthermore, the fecundity of *N. japonicus* was influenced by the total length ($P< 0.05$, $r^2=0.50$) and body weight of fish ($P<0.05$, $r^2= 0.60$; Figs. 5 and 6).

The dominant specimen collected in the study were males (214 individuals) as compared to females (146 individuals) throughout the year. Similar results have also been documented for *Nemipterus* spp. in other region worldwide (Murty, 1981; Raje, 2002). Sex ratio showed preponderance of male in the present study with the male to female ratio of 1:0.68. A similar finding was also reported for *N. japonicus* in Veraval waters, India. Generally, the sex ratio during the spawning period is associated to fecundity and batch fertility. Studies in other regions have found that for those species with high fecundity such as mullet, *Mugil cephalus*, the sex ratio in the spawning season may be low. On the other hand, the sex ratio in the spawning season was high for those species with low batch fecundity such as the Indian drift fish, *Ariomma indica* (Lin and Chen, 1991) and threadfin bream in the present study. The studies in different regions in India have also been concluded that sex ratio slightly deviated from expected ratio of 1:1 and favoured male (Manojkumar, 2004). Contrastingly, in other regions, such as Jizan region of Red Sea (Bakhsh, 1994), Suez Gulf-Red Sea (Amine, 2012), Northern Persian Gulf (Kerdgari *et al*., 2009), Mediterranean Red Sea (Haweet *et al*., 2013) female *N. japonicus* were more abundant in the catch as compared to male.

An increase in the sex ratio with body size has been recognized for this species during the present study, which is probably due to the longevity of fish. In addition, differences in sex ratio, size, age, longevity, sexual dimorphism, migration and differences in growth and behaviour among sexes may also be the contributing factors (Ayub *et al*., 2011; Kerdgari, 2009; Solomon, 2011; Rao and Rao, 1991). In the present study, no sexual dimorphism was found. Therefore, the high sex ratio was probably related to different growth and longevity characteristics between the sexes.

The testis of *N. japonicus* was very small even in mature fish, making it difficult to study the process of maturation in male (Murty, 1981). Usually, spawning season of fish is based on the presence of female with ripe ovaries (Ayub *et al*., 2011). In addition, analysis related to reproduction mainly focused on females, because offspring production is limited to a greater degree by egg production, and females contributes nourishment to the developing embryo (Murua and Saborido, 2003). According to Raje (2002), female in ovarian stage III to VII are considered as mature with 13 cm length for minimum maturity. The species *N. japonicus* in the coastal waters of Bintulu has a prolonged spawning season from January to August with one peak at March. According to Bakhsh (1994), the threadfin breams

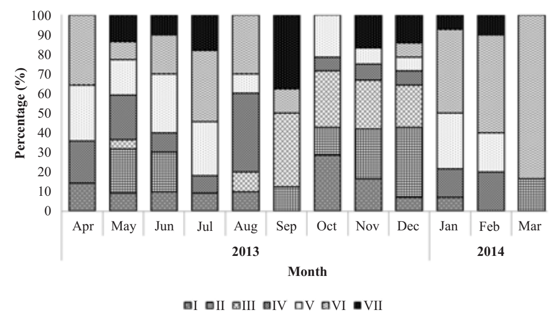

Fig. 6 : Percent composition of histological reproductive stages classified for female of *N. japonicus.* immature (I); b) early maturation (II); c) mid maturation(III); d) final maturation (IV); e) spawning prepared (V); f) spawning active (VI); g) spent (VII)

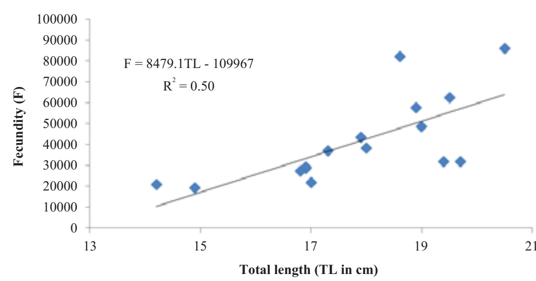

Fig. 7 : Relationship between total length of fish and fecundity of *N. japonicus*

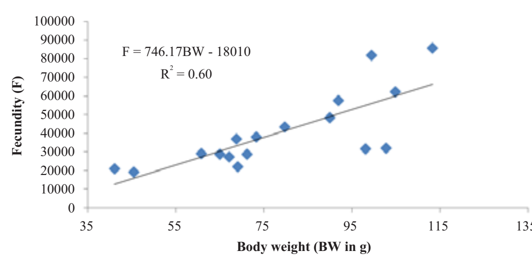

Fig. 8 : Relationship between body weight of fish and fecundity of *N. japonicus*

spawn over extended periods varied from one region to another, and was influenced by some of the environmental factors such as wind, current and temperature. *N. japonicus* in Mediterranean Sea (Haweet, 2013) also has a prolonged spawning season from April to November with one peak at warming months of the year. In Pakistan, spawning season was in spring and autumn *i.e.,* January to March (Qureshi, 1983). In Northern Persian Gulf, *N. japonicus* was spring and autumnal spawner and the main spawning occured in spring season (Kerdgari *et al*., 2009).

Histological examination showed that mature oocytes were present from January to September. The GSI in January to August was higher than other months. During present study, three methods (microscopic appearance, of the ovaries, the GSI and histological examination) were in good agreement, which suggests that the peak of spawning season for *N. japonicus* was from January to March. Similar methods were successfully applied to *N. peronii* in the waters of Southwestern Taiwan by Wu *et al*., (2008). A sharp increase in GSI was result of increased gametogenetic activities, and fully matured gonads. A sharp drop in GSI indicates that the gonads were experiencing atresia after spawning (Sikoki and Ibim, 2014), and quick egg loss without replacement (Grau *et al*., 2009). Moreover, the presence of a break in the oocytes size-frequency distributions during spawning was due to the process of separating the yolked oocyte stock from the reservoir of unyolked oocytes, which clearly indicates a cessation to the production of new oocytes (Murua and Saborido, 2003).

Generally, fecundity is the number of ripe eggs in females prior to the next spawning season (Hunter *et al*., 1989; 1992). Higher fecundity was estimated in the month of March, which indicated the peak spawning time. A positive correlation between length and weight with fecundity is in the agreement with the findings of Raje (2002). The study on the biology of *N. japonicus* off Veraval indicated that fecundity ranged from 10,260 to 184, 960, and increased with increase in fish size (both length and weight). Rao and Rao (1991) reported the average fecundity of *N. japonicus* increased from 8,259 to 27,936. Dan (1977) recorded that fecundity of *N. japonicus* varied from 10.5 to 80.8 thousand, and bred twice a year *i.e.,* December to February and June to July. Usually, a three month refinement period is necessary for the second mode to become ready for spawning. Schulz *et al*. (2007) stated that in natural water bodies like sea, the number of ripe eggs is greatly influenced by several factors such as food availability, food quality, frequency of spawning, ambient water temperature, age, size and even by genetic endowment (Okafor, 2011).

Overall, the sex ratio of *N. japonicus* was significantly in favour of males, and were the highest in September and March, while for females this was in May. Females were usually smaller in size compared to males. The spawning season of *N. japonicus* in coastal waters of Bintulu shows two peaks in August and March. The three methods (microscopic appearance, of the ovaries, the GSI and histological examination) were in good agreement, which suggests the peak of spawning for *N. japonicus* was during March and August. To ensure sustainable consumption of this threadfin bream species, adults should be protected during the major breeding seasons. The spawning season for N. japonicus was in August and March in Bintulu coastal waters. A seasonal supervision during breeding period could offer a good growth of this species, hence believed to be a good fishery management in Sarawak coastal waters, South China Sea.

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