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REPRODUCTIVE CHARACTERISTICS OF MALABAR BLOOD SNAPPER *Lutjanus malabaricus* (Bloch &Schneider, 1801) IN COASTAL MARINE ZONE OF NGHE AN AND HA TINH PROVINCES, VIETNAM

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ABSTRACT

Malabar blood snapper *Lutjanus malabaricus* (Bloch & Schneider, 1801) inhabits in coastal marine waters of Nghe An and Ha Tinh provinces, Vietnam. An investigation of the reproductive characteristics of *Lutjanus malabaricus* in the coastal marine waters of Nghe An and Ha Tinh provinces was undertaken between June 2020 and May 2021. The spawning season of *L. malabaricus* occurred throughout the year, fish were in the ripe or spawning condition in most months; but spawning peaks from late March/April and August. Females matured at a smaller size than males, with total length (TL) at first maturity being 280–330 mm for males and 230–280 mm for females. The sex ratios of 365 individuals were approximately 1/1, and the total ratio G/W was approximately 0.08−5.65% with a mean of 0.32%. The highest GSI mean value in males was 0.77 (stage IV in July) and the lowest mean value 0.03−0.11 was recorded during stages I and II in November and December. Females had the highest mean value of 1.22−1.41 (stage IV in May) and the lowest mean value of 0.07 during stages I and II in January, and 0.20 at stages I and II in October. These show that fish (*L. malabaricus*) were ripe or spawning from March and August in the studied waters. The first maturity size was 280−330 mm for males and 230–280 mm for females. Absolute fecundity (Fa) ranged from 24.000 to 769.000 (the mean value 112.000) ova per ovaries for *L. malabaricus*. In the main spawning period, high gonadal index values were found around the time of the last quarter to the first quarter moon for each month from late March or early April to August. It is suggested that *L. malabaricus* is a lunar-synchronized spanner in the studied waters.

Keywords: Lutjanidae, *Lutjanus malabaricus*, Nghe An, Ha Tinh, reproduction.

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INTRODUCTION

Snappers (family Lutjanidae) are one of the important fisheries resources in tropical and subtropical waters such as the Caribbean and Indo-Pacific waters (Allen, 1985). Available information on the reproductive biology of over 40 species of the family Lutjanidae found throughout the world's oceans is reviewed. The snappers are apparently gonochoristic, i.e. males and females are separate, and do not change sex. Sexual maturity occurs at approximately 40−50% of maximum body length. Analysis of length at maturity revealed that populations and species associated with islands mature at a significantly higher ($0.005 > p > 0.001$) proportion of maximum length (511 mm) than continental species and populations (431 mm) (Grimes, 1987). Deep (> 91 m) dwelling species and populations mature at a significantly higher ($0.005 > P > 0.001$) proportion of maximum size (49%) than shallow (< 91 m) species (43%). Lutjanids are highly fecund, with large females producing 5 7×10^6 ova. Spawning appears to take place at night, sometimes timed to coincide with spring tides at new and full moons. Many features of the reproductive biology of the snappers (e.g., spawning site preference, spawning seasonality, lunar periodicity, and spawning behavior) appear to be a strategy to introduce gametes into an environment where predation is relatively less intense (Allen, 1987; Grimes, 1987; Emata et al., 1999; Pinon & Ducan, 2009; Atsushi et al., 2010; Pradeep, 2017). In Vietnam, the family Lutjanidae has identified 10 genera, 25 species. Most of them are distributed in the Gulf of Tonkin (18 species of 4 genera), but the most notable species are *Lutjanus argentimaculatus*, *Lutjanus erythropterus*, *Lutjanus vitta*, and *Lutjanus russelli* (Le Trong Phan et al., 1999; Nguyen Dich Thanh, 2013).

Malabar blood snapper *Lutjanus malabaricus* (Bloch & Schneider, 1801) is found widespread in the Indo-West Pacific from the Fiji Islands to the South China Sea, to the Arabian Sea and the Persian Gulf, and from Australia to southern Japan. It is an important market species and a major recreational and aquaculture species in northern Australia and parts of Asia and the Pacific (Allen, 1985; Emata et al., 1999; Le Trong Phan et al., 1999; Anderson et al., 2001; Andamari et al., 2004). Several previous studies have clarified the spawning, settlement, feeding habits, age and growth of Malabar blood snapper *L. malabaricus* in Indonesia, Australia (Andamari et al., 2004; Fry et al., 2009; Newman &Williams, 2000; Newman, 2002; Ernawati & Budiart, 2019). According to Fry et al. (2009), spawning in red snappers *L. erythropterus* and *L. malabaricus* occurred throughout the year in northern Australia and eastern Indonesia; at least 10 – 30% of females and 40–80% of males were in the ripe or spawning condition in most months, the spawning peak from September to March in northern Australia and two peaks from January to March and October in eastern Indonesia. Size at first maturity was 240 mm for males and 250–300 mm for females in eastern Indonesia. L_{50} estimates were similar between species in northern Australia: 270–280 mm (males) and 350–370 mm (females). Maximum batch fecundity was 997,000 oocytes for *L. malabaricus*. Length at maturity at 50% level (L_{50}) was 413,5 mm and 95% level (L_{95}) was 532 mm for *L. malabaricus* in Western South Sulawesi, Indonesia (Ernawati et al., 2019). The size of the first-time maturity was 290 mm (males) and 370 mm (females) for *L. malabaricus* in Pinrang waters, Makassar Strait, south Sulawesi, Indonesia and the highest spawning of red snappers in Pinrang waters occurred in June (Nuraeni et al., 2020).

In Vietnam, research on Malabar blood snapper *L. malabaricus* is still very limited in the Gulf of Tokin and has not been carried out synchronously and systematically (Le Trong Phan et al., 1999). The objective of this study was to provide information on the reproductive biology of *L. malabaricus* resident in northern central Viet Nam that can be used for future management strategies for this species.

MATERIALS AND METHODS

Sample collection

Biological data for this study were collected during a collaborative research project of Vietnam-Russia mixed tropical science and technology research centre "Research on the structural and functional organization of coastal marine ecosystems for conservation, restoration and sustainable use". Malabar blood snapper specimens were obtained from a number of sources including the catches of selected local commercial fishers at fish landing sites across Nghe An and Ha Tinh provinces between June 2020 and May 2021. Samples were obtained every week in order to be consistent with lunar cycles (new moon, first quarter moon, full moon and last quarter moon) except in bad weather conditions. Fish samples including 184 males and 181 females were caught using drift nets, bottom drop lines, or fish traps (Fig. 1).

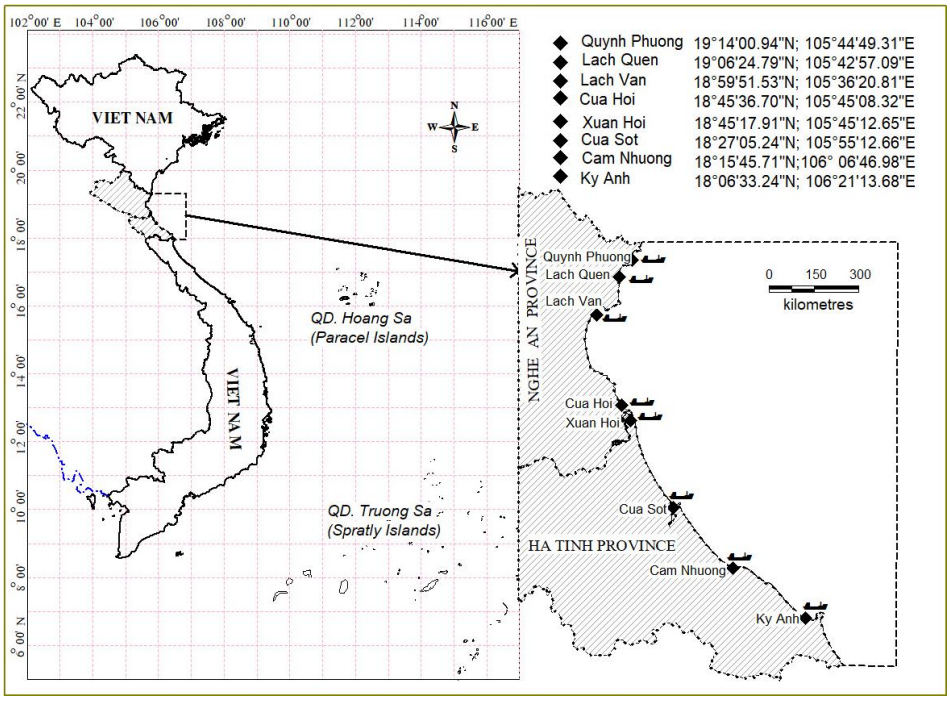

Figure 1. Sites sampled for the Malabar blood snapper *Lutjanus malabaricus* in studied waters

A total of 365 fish specimens were measured for standard body length (SL, nearest 5mm), whole body weight (g) and gonads (ovaries/ testes) weight (nearest 0.01g). The color, shape and size of the gonads were observed before preserving in 10% formalin in sea water solution. Lateral sections of the preserved ovaries/ testes were taken at 0.4 microns thickness and photos were taken at 10x on a trinocular microscope fitted with camera. Five stages of the maturity scale for ovaries were followed in estimating the maturity stages (Nicolsky, 1963; Pravdin, 1966; Pradeep, 2017).

Reproductive characteristics

Gonadal stages, spawning season and length-at first maturity were estimated for female Malabar blood snappers through histological analyses and relative gonadosomatic (GSI).

GSI was calculated for each fish for both sexes to determine the spawning seasons using the equation: GSI = (gonad weight (g)/[whole weight (g) – gonad weight (g)]) × 100. For histological examination of gonads, a subsample of gonadal tissue was removed from each fish. These gonad samples were

weighed (±0.001 g), placed in tissue cassettes, dehydrated and impregnated with wax. Histological sections were cut at 5–8 µm from each block using a tissue microtome, mounted on glass microscope slides and stained with Harris's haematoxylin and eosin counterstain. Each histological section was scored by estimating the percentage that each of the gonad maturity stages occupied within the total area of the section (Nicolsky, 1963; Pinon & Ducan, 2009; Pavlov et al., 2016; Mokhtar, 2017; Pradeep, 2017).

Size at first maturity

Stages I &II were treated as immature and stage III, IV&V was considered as mature for calculating the size at first maturity. Gonads were classed as ripe when the majority of the gonad was in maturity stages IV and V for both females and males. Fish were in spawning condition when the greatest proportion of their gonad was in stages IV & V for both females and males. To estimate the size of fish in the population where 50% of fish in a length class were mature (L_{50}), logistic curves with 95% confidence intervals were applied to the proportion of mature-at-length data.

Fecundity

The fecundity was estimated by using 25 females with matured oocytes (see ''Results''). For each ovary, three small pieces of the ovary (at 3 positions at the beginning, middle and end of the ovary) were extracted. The oocytes of ≥ 0.4 mm in diameter were regarded as matured oocytes and counted under a Nikon profile projector at 10x magnification. Then, the fecundity was estimated in accordance with absolute/batch fecundity. (Fa) was determined according to the method of Nicolsky (1963), Oven (1976) as Fa = nG/g (G- Ovarian mass; g- The weight of 1 egg sample removed for counting; and n- Number of eggs in 1 sample above. Relative fecundity (Fr) = absolute fecundity (Fa)/body weight (W)).

RESULTS

Reproductive characteristics

The total number of fish captured during the study period was 365 fish which consisted of 184 males and 181 females' fish. The total length (TL) of fish ranged from 125 mm to 840 mm. The dominant size of fish captured was 403 mm and a size of 840 mm was rarely captured during the study period (Table 1).

Table 1. Size statistics of 365 *Lutjanus malabaricus* during the study period

Note	TL	SL	W	Age	GIS
n	365	365	365	365	365
Min	125	100	35.8	1	0.02
Max	840	740	10885.2	17	5.99
Average	381	325	1031.9	5	0.32
Stdev	237	209	1799.2	4	1.65
Median	403	343	1465.3	5	1.19

Note: TL- Total length (mm); SL- Standard length (mm); W- Whole body weight (g); Age- Year; GSI- Gonadosomatic index.

The relationship between fish length and weight was described by the power relationship: for males $W = 0.073 \times TL^{2.549}$ ($r = 0.8$) and for females $W = 0.034 \times TL^{2.739}$ ($r = 0.89$), *where:* W- The whole body weight (g); and TL - Total length (mm).

Gonads from 365 individuals (including 184 males and 181 females) of *L. malabaricus* were examined to determine some reproductive characteristics (eg. sex ratio, gonad maturity stage and size at maturity).

The total sex ratios of 365 gonads were approximately 1/1 (total males 50.5%/females 49.4%), the total ratio G/WW was approximately 0.08−5.65%, with a mean of

0.32%. However, the sex and G/W ratios differed by month and gonad stages (Fig. 2).

The highest GSI mean value in males was 0.77 (stage IV in July) and the lowest mean value 0.03−0.11 were recorded during stages I and II in November and December. Females had the highest mean value of 1.22−1.41 (stage IV in May) and the lowest mean value of 0.07 during stage I and II in January, and 0.20 (stages I and II in October. These above results show that fish *L. malabaricus* were in the ripe or spawning period from late March/April and August in the studied waters. The mean monthly GSI for *L. malabaricus* suggested that most spawning takes place between the months of late March to August. There was a second peak of GSI for both males (in October) and females (in November). However, this may be caused by low sample numbers in these months (Fig. 3).

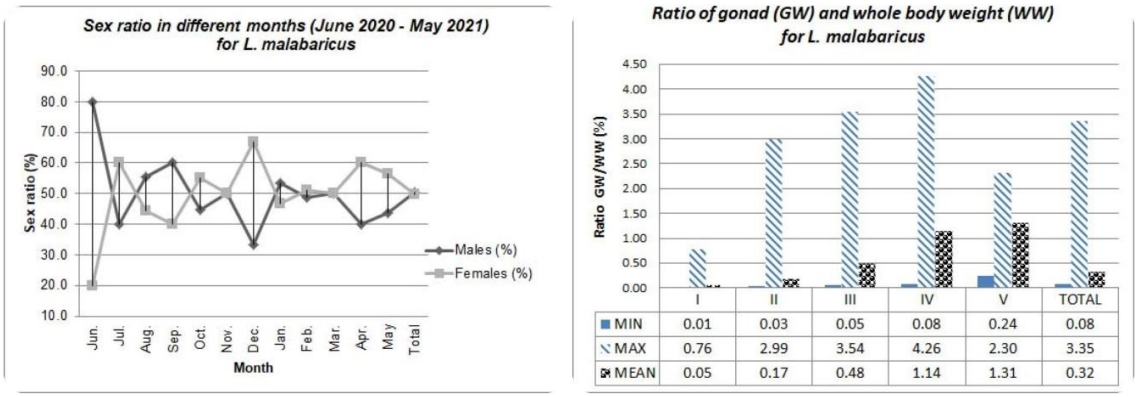

Figure 2. Fluctuation of sex and G/WW ratios by month and gonad stages

The histological examination of gonads supported the reproductive pattern shown from the GSI data; months with the highest GSI values recorded corresponded to months where most gonads were staged as the ripe and spawning condition. The majority of oocytes in female gonads of *L. malabarius* were classed as stage IV and V in the months of March to August. Furthermore, it was in these months that female gonads had the highest proportion of spent gonad tissue (stage V). The males in the sample had gonads with mature spermatozoa (stages IV and V) throughout most of the year. Histological examination of gonads has also shown there was a proportion of fish that were in ripe or spawning condition, at least 20–35% of females and 45–75% of males, in most months of the year (Figs. 3, 4).

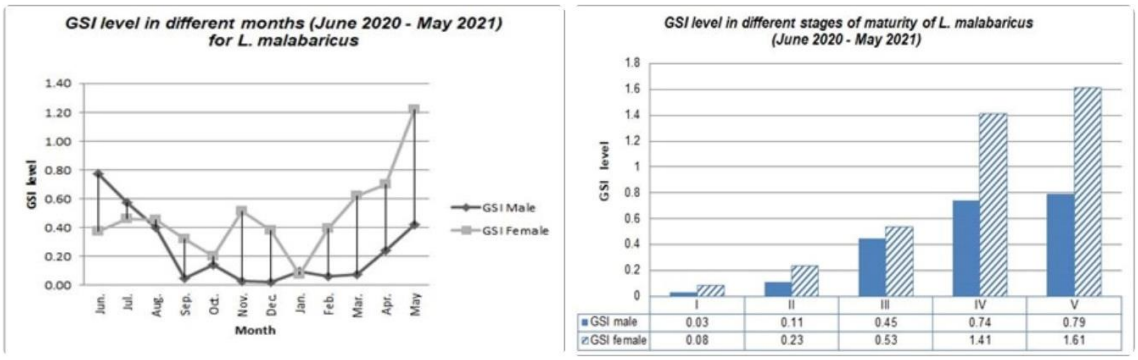

Figure 3. The values GSI of male & female (left) and gonadal stages for 365 gonads

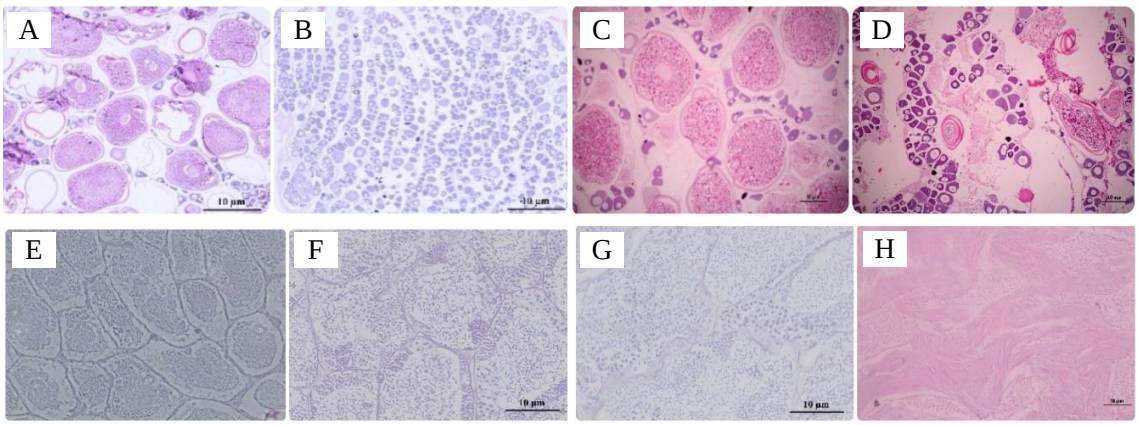

Figure 4. Histological appearance of *Lutjanus malabaricus* gonads, following haematoxylin and eosin staining: Female gonads at stages II/ III- Developing/Maturing (A); III/IV- Maturing/Mature (B); IV–V - Ripe/ Spent (C, D). Male gonads at stages III - Developing (E); IV - Maturing (F); IV–V- Mature (G, H)

Stages of ovarian development

The ovaries of *L. malabaricus* had two lobes of approximately equal size, oval sac-shaped located on both sides of the abdominal cavity, and are suspended from the body cavity wall by the mesentery of the ovary. In stage IV, the ovary has one major blood vessel running down the middle and numerous branching vessels that hug the ovary. The ovary extends from the tip of the balloon to the genital opening. The posterior part of the ovary is a short fallopian tube that connects to the outside through the genital opening. At this stage, the size of the ovary reaches the limit, round and soft.

The size of the ovary depends not only on the degree and Gonadosomatic Index (GSI) but also on the size of the fish. Ovaries are considered an important indicator to assess the maturity level of fish. However, ovarian mass is not the best indicator, it is necessary to determine accurately based on egg morphology, egg diameter and maturation index.

There are many studies on the reproductive characteristics on many different fish. The ladder to determine the degree of sexual maturity has also been proposed by many authors Nikolsky (1963), Oven (1976), Nuraeni et al. (2000). However, many ladders are difficult to apply and the degree of accuracy has not been determined. In this study, the ladder of Nikolsky (1963) was used as the standard. Due to the short research time, and not many fish catches, the collection of research samples was difficult, so during the implementation of the study, only samples with gonads stage II, III, IV and V were collected.

Stage I (Imamature): The individual is young, the gonads have not yet developed, lying close to the inside wall of the body (on the sides and under the airbags) are long, narrow cylindrical strings, cannot distinguish male and female by the naked eye. At this stage, the sex cells are oocysts, ova transparent and devoid of yolk deposition. Fish measures are 155–600 mm TL and 60–2,100 g W (whole body weight).

Stage II (Developing): The gonads grow thicker, the egg seeds are small, the eyes can barely see the egg seeds. At this stage, it is possible to distinguish males from females. The gonads are small, occupying a very small part of the body cavity. The ovaries are pink. Observation of histological specimens showed that the majority of oocytes were in phase 2 and phase 3 (cytoplasmic growth) (Nikolsky, 1963; Andamari et al., 2004; Nguyen Dich Thanh, 2013; Pradeep, 2017). The basophilic

cytoplasm picks up the purple hematoxyline dye very strongly and surrounds the nucleus. The large, round nucleus in the centre occupies most of the oocyte. Many subnuclei of different shapes are distributed around the periphery of the nucleus, forming a circle around the nuclear membrane. At this stage, the ovule and vacuole have not yet formed. The oocyte is angular in shape. Fish measures are 210–560 mm TL, 130–2,297 g W.

Stage III (Maturing): The gonads develop strongly, and the egg seeds are small but the egg seeds can be seen with the naked eye. Stage III is the process of egg production or trophoplasmatic growth. Ovarian follicles are formed around the oocyte. Oocyte size increased rapidly. The ovaries are pale yellow. Observation of histological specimens, cytoplasm showed a lighter hematoxyline dye stain. Small yolk sac granules are formed near the nucleus and then develop centrifugation. The large nucleus is still located in the middle of the oocyte and has many nuclei of different sizes and shapes, distributed around the nuclear membrane, the vacuoles appear interspersed with the yolk granules or vacuoles. Fish measures are 230–720 mm TL, 185−3,900 g W.

Stage IV (Mature): The yolk sac process ends, the ovary has the largest size and volume. The ovaries are yellow, round, and soft. Eggs in clumps stick together loosely. This phase lasts during the migration of the germ sac from the centre to the periphery, creating polarization of the oocyte.

Observation of the histological specimen showed that the eggs were large, the yolk sacs were visible, the oocytes were round, and the nucleus was eccentric. At the end of stage IV, the ovaries are at their maximum, and they are occupying 2/3 of the body's cavity. Egg seeds are bright yellow, most eggs are 0.45−0.52 mm in diameter.

The number of yolk sacs surrounding the nucleus increases sharply and makes up 1/2 to 2/3 of the oocyte. In the ovary, there are oocytes in stage II and stage III. Therefore, like some other tropical marine fishes, *L. malabaricus* is a scattered spawning species all year round (Nikolsky, 1963; Andamari et al., 2004; Pradeep, 2017). Fish measures are 450−840 mm TL and 1,132–9,000 g W.

Stage V (Spent): Yellowish, occupying 2/3rd to 3/4th of the body cavity, blood vessels ramify over the surface. Fish measures are 540–670 mm TL and 2,095–4,800 g W.

Testicular stages

Stage I (Immature): The individual is young, and the gonads have not yet developed. Fish measures are 125–760 mm TL and 36–10,885 g W.

Stage II (Developing): The striatum is shaped like a thin, white tape. This stage is characterized by the existence of sex cells at the beginning of spermatogenesis, which is spermatozoa in a reproductive state. Owing to the process of reproduction of this cell, the spermatozoa have increased in size. Fish measures are 215–580 mm TL and 38–2,612 g W.

Stage III (Developing): The sperms are larger in size, milky white in color. Small pink streaks appear on the surface, which is a sign of vascular growth. On histological specimens, mainly secondary spermatozoa are in the process of dividing into spermatozoa. Fish measures 280–600 mm TL and 280–2,588 g W.

Stage IV (Maturing): The sperm cell is much larger in size than in the previous stages, has a milky white color, and has strong blood vessels. Observed on histological specimens, the cell composition is mostly sperm that pour into the common cavity of the vas deferens. Fish measures 330–750 mm TL and 618–7,521 g W.

Stage V (Mature): Pale white, viscous fluid oozes out from the cut surface. Fish measures are 460–50 mm TL and 1,301–5,400 g W.

Size at maturity

Sexual maturity occurs at about 30–57% of the maximum total body length for males

and 43–64% for females. There were differences in body length at first maturity between the sexes, with males first reaching sexual maturity at around 280–330 mm TL, whereas females began maturing between 230–280 mm TL (Fig. 5).

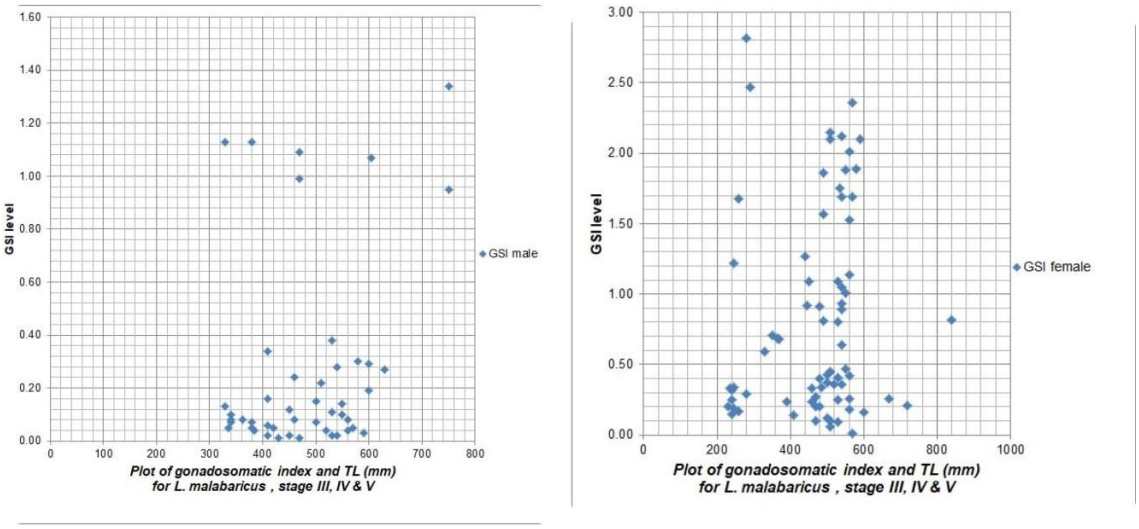

Figure 5. The gonadsomatic index (GSI) and total length (TL) for males (left) and females (right) of *Lutjanus malabaricus*

The logistic curves fitted to data are shown that the L_{50} estimates for the populations in studied waters were 502 mm for males and 429 mm for female. The logistic functions showed a poor fit to these data (r^2 = 0.29 and 0.38) (Fig. 6**)**. This is likely due to smaller sample numbers available for these populations and therefore L_{50} was inaccurate to be estimated for these populations.

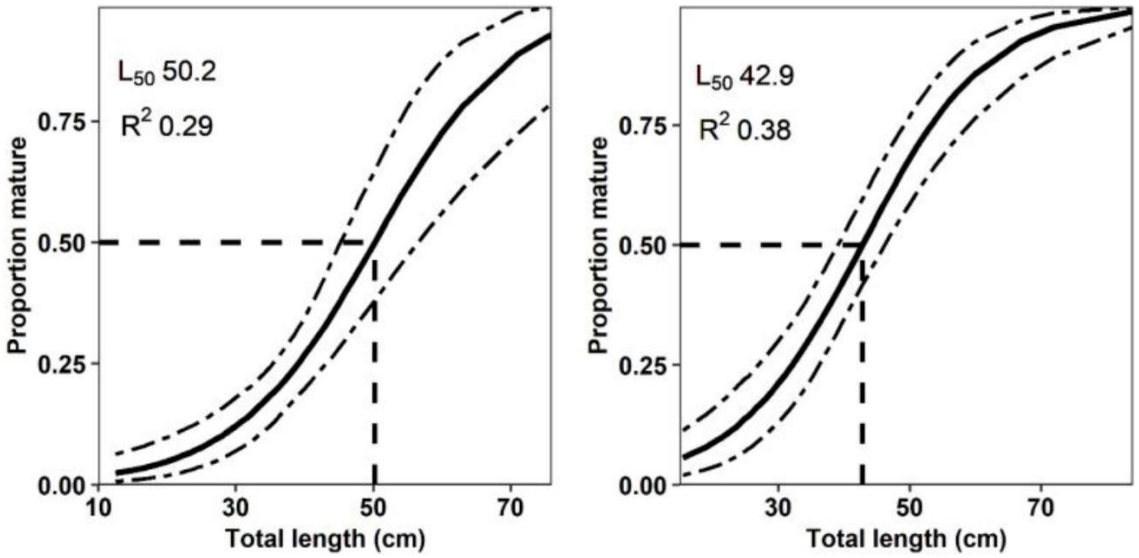

Figure 6. The proportion of mature fish in each total length class for males (left) and females (right) of *Lutjanus malabaricus*

Fecundity

Gonads from 25 females of *L. malabaricus* were counted to determine the fecundity in accordance with absolute fecundity (Fa) and relative fecundity (Fr). The gonadosomatic index (GSI) for female was approximately 0,46−4,26% and the mean was 1.55 ± 0.85% of whole body weight (W); Fa = 23,887–769,257 and the mean 112,478 ± 155,195 eggs/female; Fe = 12–244 and the mean 49 ± 47 eggs/g W (Fig. 7, Table 2).

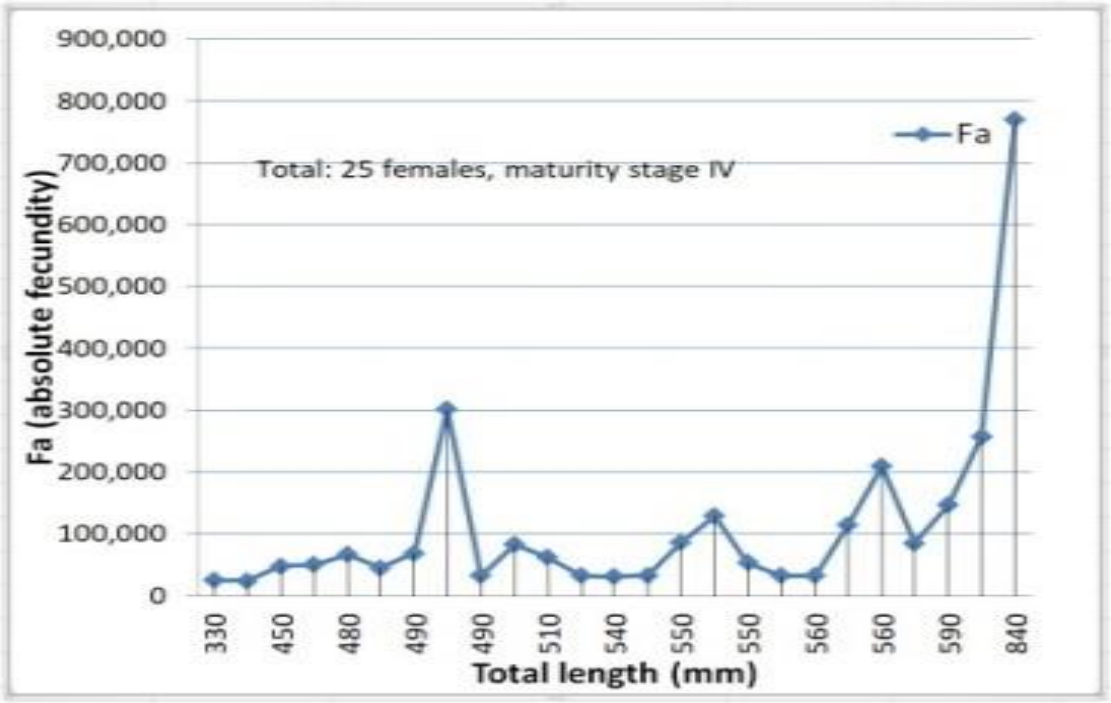

Figure 7. Mean Fa and TL of 25 females *Lutjanus malabaricus* with gonadal stages IV

Table 2. Fecundity of 25 females with gonadal stages IV

Notes	TL	W	G	G/W	Fa	Fr
Total	25	25	25	25	25	25
Mean	530	2.2	28.6	1.6	112	49
Stdev	90	1.6	14.9	0.9	155	47
Min	330	618	9.3	0.5	24	12
Max	840	9.000	72.9	4.3	769	244

Note: TL- Total length (mm); W- Whole body weight (g); G- Gonad weight (g); G/W (%); Fa- Absolute fecundity (egg); Fr- Relative fecundity (egg).

Lunar periodicity

There were 47 female fish (gonad stages IV–V) caught over lunar periodicity between March and May 2021 in the studied on waters. Samples were obtained every week in order to be consistent with lunar cycles (new moon (NM), first quarter moon (FQ), full moon (FM) and last quarter moon (LQ)), except in bad weather conditions. Although ripe female fish were caught over the entire lunar cycle, 68% of the females were caught after the full moon (FM) and up to the last quarter (LQ) **(**Fig. 8**)**.

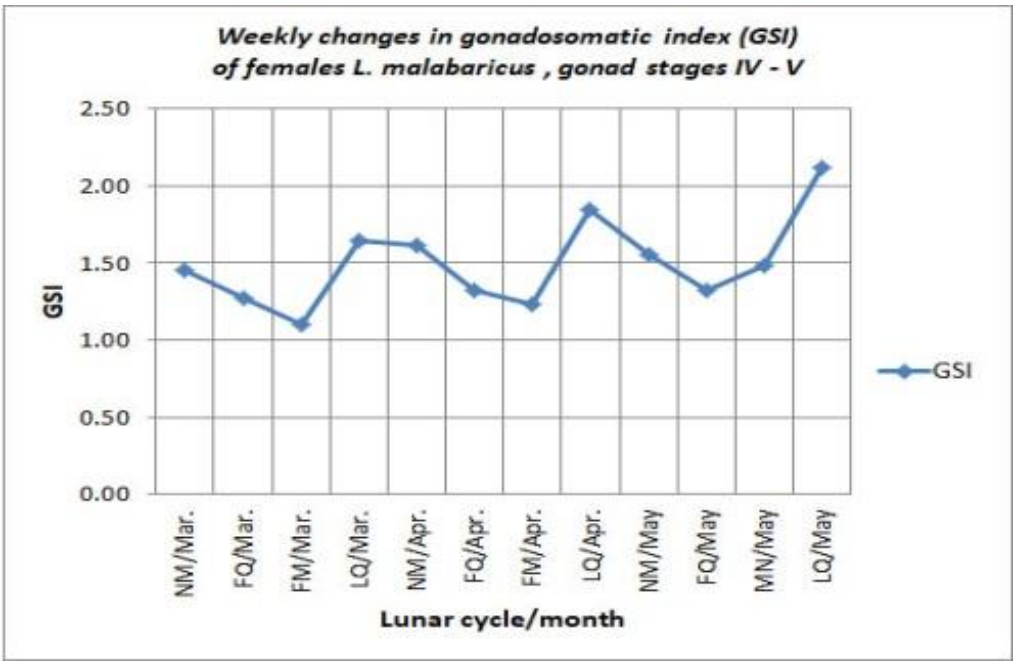

Figure 8. Lunar cycle changes in GSI of females *Lutjanus malabaricus* with gonadal stages IV–V. *Note:* NM- New moon, FQ- First quarter moon, FM- Full moon, LQ- Last quarter moon

Clear lunar-synchronized GSI fluctuations of females were found during the main spawning season (March-August) showing the highest GSI values around the last quarter moon of each month between March and June (Fig. 8).

DISCUSSION

Previous studies (Grimes, 1987; Newman, 2002; Andamari et al., 2004; Fry et al., 2009; Ernawati & Budiarti, 2019) observed that there were inconsistent variations in reproductive characteristics of most tropical snappers, each species is having a different size at first gonad maturity. Even in the same species, the size of fish at the first maturity level might be also different. Some researchers claimed that "at geographically dispersed at latitudes of more than five degrees, there will be differences in size and age when fish reaches first gonad maturity level. In addition, the difference in size also occurs due to differences in ecological conditions of the waters" (Rapi et al., 2020).

In their study of maturation of *L. malabaricus*, some studies suggested females can be preponderant at larger sizes and that the size (both length and weight) at first maturity for males was significantly lower than females, and that males matured earlier at 4 years than females at 5 years. This was in contrast with our results, males first reaching sexual maturity were larger than females (the males around 280–330 mm TL at 4.6 years and females 230–280 mm TL at 5.7 years on average.

The length of the fish that first gonad maturity *L. malabaricus* is used to analyze the number of fish which allow to be exploited for commercial fishing. For example, Nuraeni et al. (2020) found female fish reaches their first gonads maturity at a length of 290 mm and males at 370 mm in Pinrang waters, Indonesia.

CONCLUSION

The malabar blood snapper in the mature gonad stage is mostly caught between late March and August in the coastal marine zone of Nghe An and Ha Tinh provinces. The malabar blood snapper sex ratio is considered a balanced condition. There were differences

in body length at first maturity between the sexes, with males first reaching sexual maturity at around 280–330 mm TL, whereas females began maturing between 230−280 mm. Fecundity ranges Fa = 23,887–769,257 and the mean is 112,478 ± 155,195 eggs/female; Fe = 12–244 and the mean is 49 ± 47 eggs/g WW. The highest GSI values are around the last quarter moon of each month between March and June during the main spawning season (March–August).

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