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# SPAWNING SEASON OF MILKFISH *Chanos chanos* (FORSSKÅL, 1775) IN THE NATURE

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## ABSTRACT

The spawning season of the milkfish *Chanos chanos* (Forsskål, 1775) of the family Chanidae in the Central Coast of Vietnam was determined. A total of 220 specimens (3.20 to 4.50 g body weight, BW) was collected from seven provinces (Nghe An, Ha Tinh, Quang Binh, Quang Tri, Binh Dinh, Khanh Hoa, Ninh Thuan) from January to December 2021. Results showed that the Gonado Somatic Index (GSI) ranged from 2.03 to 16.02 in females and 1.15 to 9.87 in males and had two peaks, one in April–May (13.47–16.02 for females and 8.03–9.87 for males) and another in August-September (11.45–12.32 for females and 6.75–7.98 for males). Conditional factors in females and males ranged from $3.68\times10^{-2}$ to $4.11\times10^{-2}$ and $3.02\times10^{-2}$ to $3.43\times10^{-2}$, respectively with the highest values found in May ($4.11\times10^{-2}$ for females and $3.46\times10^{-2}$ for males) and September ($3.92\times10^{-2}$ for females and $3.34\times10^{-2}$ for males). The Fulton's and Clark's indexes were highest in November, 1.82% and 1.67%, respectively and these were then decreased in the following months; the lowest values were recorded between August to September and April to June (0.66–0.74% Fulton and 0.49–0.72 Clark). The gonadal development stages III and IV in females and males were observed from April to June and August to September of the year. Our findings suggest that the main spawning seasons of milkfish take place from April to June and August to September.

**Keywords:** Milkfish, Gonado Somatic Index, spawning season, *Chanos chanos*.

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## INTRODUCTION

The milkfish *Chanos chanos* (Forsskål, 1775), also known as the marine milkfish, is the only extant species in the family Chanidae. This species is one of the important farmed fish in the Southeast Asia. Milkfish meat has high nutritional value, in which protein content and unsaturated fatty acids account for 24.18% and 32.11%, respectively (Malle et al., 2019). Essential amino acids account for 49.49% of the total amino acids, of which glutamic acid is the predominant amino acid (18%), followed by leucine (8%), lysine (7.3%), phenylalanine (6.7%), and histidine (6.1%) (Murthy et al., 2016).

In Vietnam, *C. chanos* is distributed along the coast from Nghe An to Binh Thuan province, highly abundant in Binh Dinh and Khanh Hoa provinces. This species has been included in the Red Book of Vietnam under the endangered classification - VU A2d (Vietnam Red Book, 2007) and in the list of rare genetic resources in the national database of aquatic resources, belonging to the species group that needs to be preserved and developed (Decision No. 188/QD-TTg, 2012).

The *C. chanos* has a high reproductive capacity, however, survival rates of its embryonic and juvenile stages are considerably low, which is due to the highly intensive fishing in the coastal lagoons and the increasing pollution in nearshore waters. *C. chanos* has been known as a new crop for Vietnamese fish farmers. They have been cultivated in monoculture systems such as cages, and ponds, or polyculture with shrimp and crabs in brackish water areas, contributing to additional income and solving the water pollution in intensive shrimp farming areas (Nguyen Thi My Dung et al., 2020). However, the artificial seed production of milkfish is still only on the laboratory scale and the culture industry relies on fry captured from the wild.

Currently, little data are available regarding the reproductive biology of milkfish in Vietnam, whereas most of the studies have focused on distribution, taxonomy, and morphology (Nguyen Thi My Dung et al., 2020). Therefore, it is necessary to study the spawning season as a basis for artificial spawning, developing seed production technology and subsequently providing this species to the aquaculture industry. Together, this help contributes to diversifying culture species, maintaining, developing resources, protecting genetic resources, and conserving biodiversity in the Central region and the entire country in general.

## MATERIALS AND METHODS

### Sample collection

Two hundred twenty specimens (135 specimens of females and 85 specimens of males) with (3.20 g BW to 4.50 g BW) were collected from 7 provinces in Central Coast Vietnam, namely Nghe An, Ha Tinh, Quang Binh, Quang Tri, Binh Dinh, Khanh Hoa, Ninh Thuan (Fig. 1). 15–20 individuals were randomly collected each month by using a trawl net, bottom net, and racket. After collection, samples were washed, refrigerated and transferred to the laboratory at Institute for Agriculture and Natural Resources, Vinh University. Sampling time occurred from January to December 2021.

### Data collection and analysis

Total length (TL, cm) and standard length (SL, cm) were measured using a Palme ruler (cm); body weight (W, g), gonad weight (Wtsd, g) and body weight without internal organs (Wo, g) were determined using an electrical balance with two odd numbers.

Gonad development was categorized into the following six stages: inactive (In), early active (Ea), late active (La), ripe (R), spent (Sp) and degenerative stage (D) (Xakun & Buskaia, 1968).

In addition, the determination of gonadal was also based on the results of findings on the change of oocytes corresponding to each stage of gonadal development. Gonadal histology specimens were made by paraffin-moulded excision and stained with Haematoxyline and Eosin by Drury & Wallington (1967).

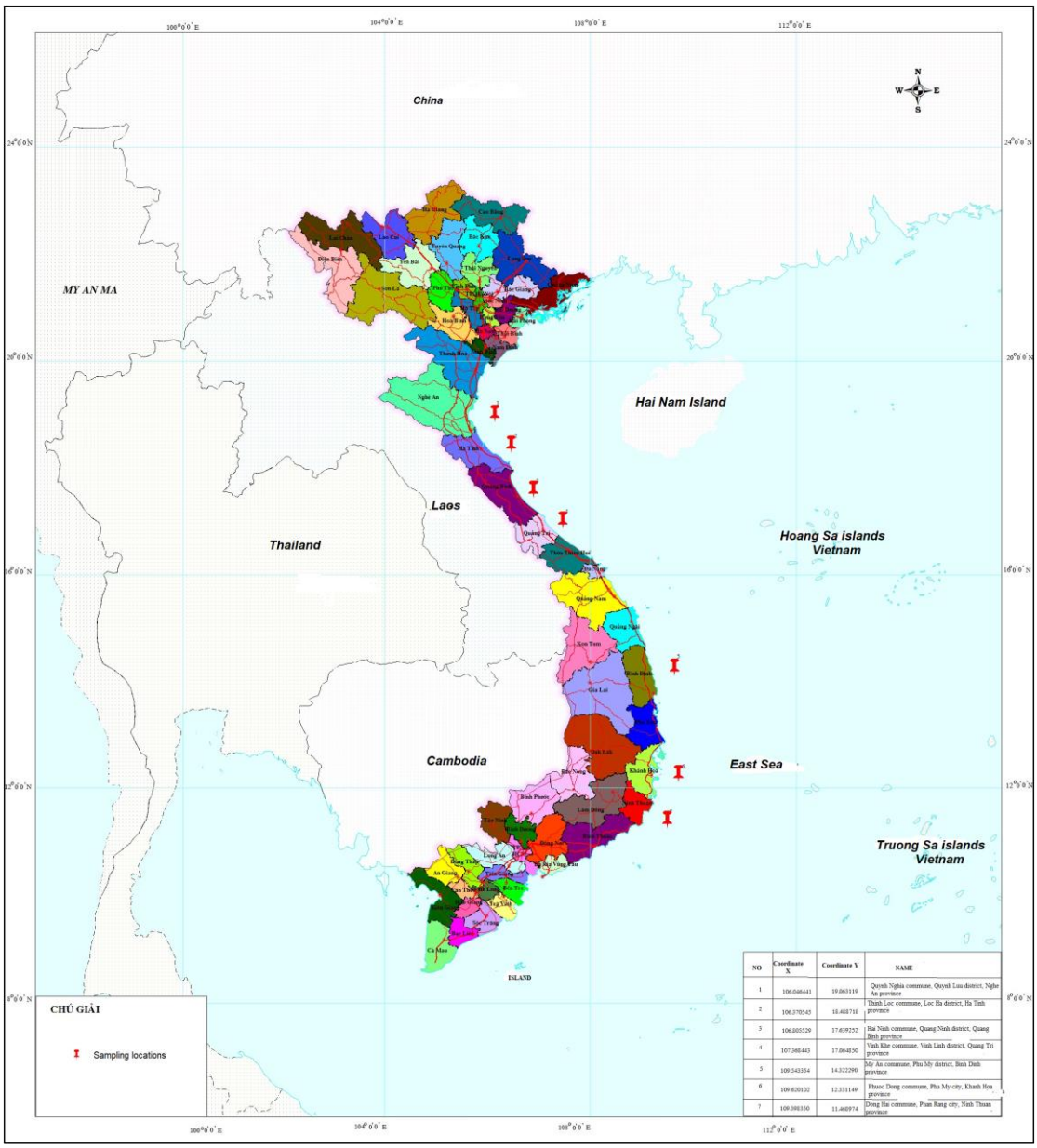


*Figure 1*. Map of sample collection locations (Vu Van Luong, 2021)

The spawning season was predicted by results of Fulton and Clark, GSI, CF, gonadal development stages obtained from collected fish samples over a period of 1 year (study time).

(i) ***Gonadosomaticindex (GSI)*** is a factor to predict spawning seasons of fish and it was calculated monthly following the equation of Biswas (1993):

$$GSI(\%) = 100 * W_{tsd} / W$$

*Where:* $W_{tsd}$: Gonad weight (g); W: Body weight (g).

***(ii) Fulton and Clark's factors*** were calculated with the following equations:

Fulton: $F = W \times 100 / SL^3$

Clark: $C = W_o \times 100 / SL^3$

***(iii) Conditional factor*** **(CF)** of a fish reflects physical and biological circumstances and fluctuations by interaction among feeding conditions, parasitic infections and physiological factors. The CF was calculated with the following equation: $CF = W/TL^b$, *Where:* W: Body weight with internal organs; $W_o$: Body weight without internal organs; TL: Total length; b: growth rate (the correlation between length and weight).

## RESULTS AND DISCUSSION

### Sex discrimination of milkfish

It is difficult to distinguish immature males and immature females when observing external morphological characteristics. However, mature males have a darker or dark olive-green back, while females have their back in light silver. In the females, there are 3 main openings in the anal region instead of 2 as found in the males. When pressed to the abdomen of a ripe male, it is easy to see vaginal discharge. The female's abdomen is enlarged and has yellow fatty fluid. Anatomically, the eggs and sperm are developed symmetrically. Figure 2 distinguishes some sexual characteristics of milkfish.

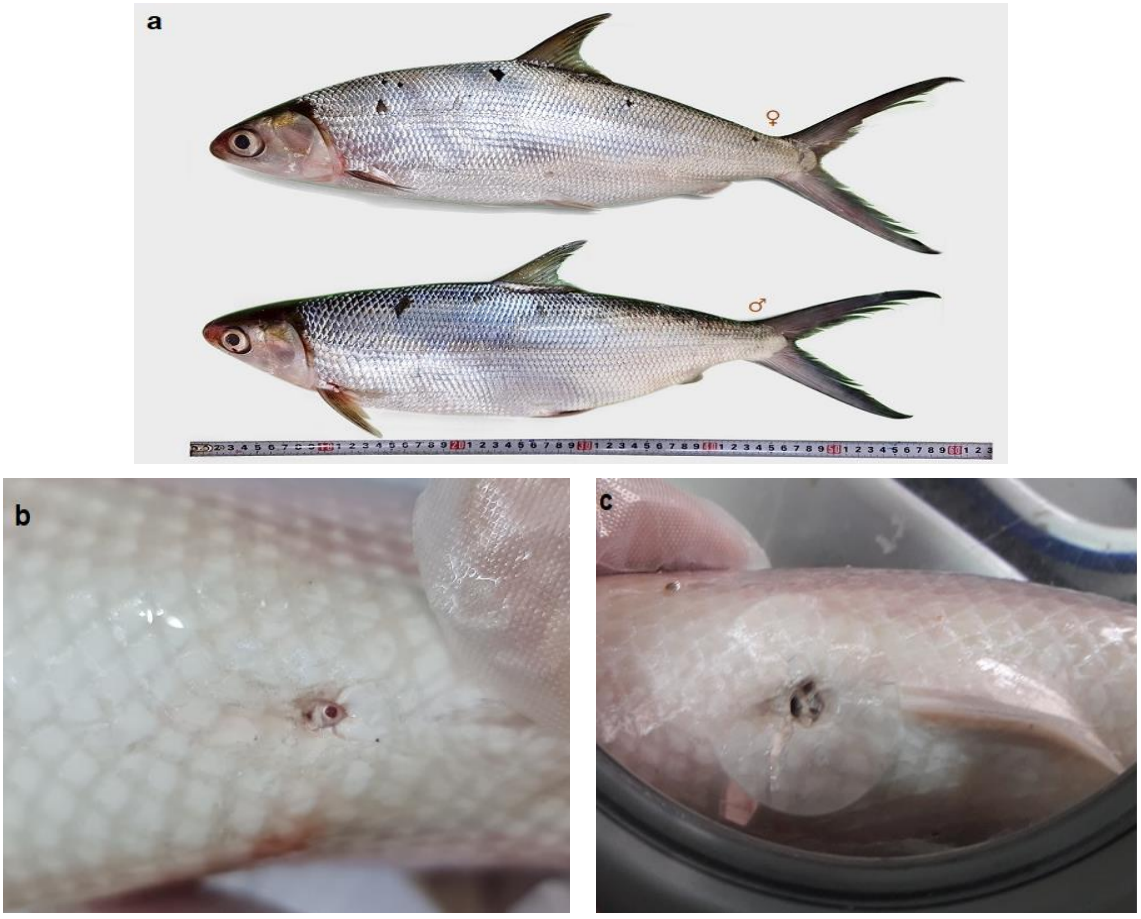


*Figure 2.* Distinguishes some sexual characteristics of milkfish. a. Milk fish male and female, b. Male genital papilla; c. Female genital papilla

When analyzing the number of 220 milkfish samples in each age group, the majority of fish were in the age group from 0+ to 4+. The sex composition by age group was

presented in Figure 3. The results indicated that It was unable to identify sexes by external morphology in the age group 0+ to 1+ accounted for (100%) and the age group 1+ accounted for (6.77%). The age group 2+ began to distinguish male and female by external morphology (males accounted for 37.7–47.37%; females accounted for 52.5–61.29%).

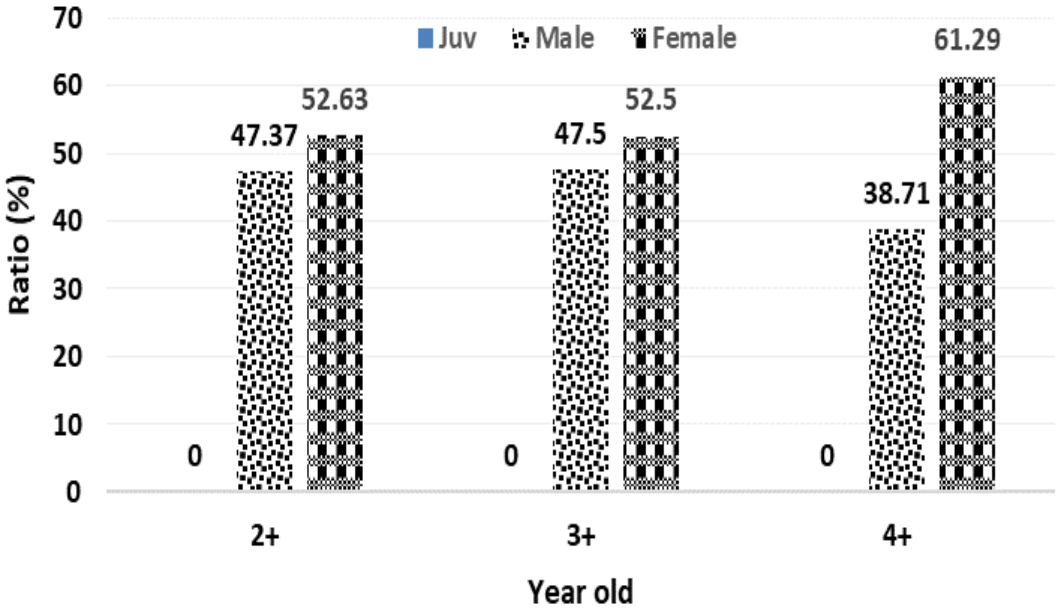


*Figure 3.* Sex composition of milkfish by age groups

***Gonadosomatic index (GSI)***

The GSI is a good indicator of fish reproductive activity, so it has been used to determine the stages of gonadal maturation (Le Cren, 1951; Hojo et al., 2004). Table 1 shows that the GSI in males and females changed continuously over 12 months. The GSI in females were higher in April to May and August to September 2021, with the highest mean in April to May 2021 (13.47–16.02% and 8.03–9.87% for females and males, respectively) and sharply declined between December and January, 2.03–4.15% for females, and 1.15–1.65% for males.

The variation of GSI indicates that after spawning, the majority of the reproductive products (eggs and sperms) are released into the external environment, so the gonads will decrease rapidly in size and weight along with GSI. Therefore, the GSI is one of the crucial conditions to predict the maturity level of the fish's reproductive products.

Thus, the main spawning seasons of milkfish were predicted in April–May and August–September every year. Our findings are consistent with the survey results of fishermen in the coastal area of Khanh Hoa, which reported that the spawning seasons of milkfish usually occur around May–July and September–December of the lunar calendar every year.

Table 1 shows that the GSI in females was higher than that in males, which is due to the fact that the female ovary is much bigger at the same development stages. Pham Minh Thanh & Nguyen Van Kiem (2009) suggested that the increase in the gonad weight was determined by the intrinsic nutrient metabolism from the muscle and liver in the fish body.

According to Marte & Lacanilao (1986), the GSI of milkfish caught in Panay Island (Philippines) had a GSI of 5–20 (BW: 5–14 kg), with a mean value of 10. These values are similar to fish caught in Indonesia; while fish caught in Hawaii have lower GSI (> 5; observed in July–August).

*Table 1.* Monthly variation in GSI of *Chanos chanos*

| Month/year | Males (n = 85) | | | Females (n = 135) | | |
|---|---|---|---|---|---|---|
| | W(g) | $W_{tsd}$ (g) | GSI (%) | W (g) | $W_{tsd}$ (g) | GSI (%) |
| 1/2021 | 3,476.43 ± 705.77 | 29.36 ± 3.56 | 1.65 ± 0.54 | 3,579.08 ± 563.56 | 48.53 ± 4.76 | 4.15 ± 1.35 |
| 2/2021 | 3,584.85 ± 295.34 | 16.45 ± 2.54 | 1.24 ± 0.43 | 3,687.54 ± 347.43 | 83.16 ± 21.45 | 3.34 ± 1.15 |
| 3/2021 | 3,752.65 ± 753.23 | 158.88 ± 48.43 | 4.98 ± 1.32 | 3,855.36 ± 237.54 | 138.25 ± 32.43 | 6.18 ± 2.34 |
| 4/2021 | 4,326.54 ± 615.43 | 319.42 ± 125.43 | 8.03 ± 1.76 | 4,429.19 ± 575.34 | 396.61 ± 104.57 | 13.47 ± 3.56 |
| 5/2021 | 4,263.83 ± 453.83 | 392.84 ± 154.32 | 9.87 ± 2.32 | 4,366.48 ± 478.43 | 499.51 ± 123.43 | 16.02 ± 5.68 |
| 6/2021 | 3,831.56 ± 285.54 | 178.52 ± 65.54 | 5.39 ± 1.14 | 3,934.21 ± 375.54 | 402.46 ± 98.91 | 10.23 ± 2.76 |
| 7/2021 | 3,931.54 ± 532.65 | 158.74 ± 76.32 | 4.75 ± 0.85 | 4,034.19 ± 276.54 | 381.23 ± 112.87 | 9.45 ± 2.32 |
| 8/2021 | 4,263.25 ± 715.79 | 259.76 ± 102.43 | 6.75 ± 1.12 | 4,365.9 ± 465.45 | 465.89 ± 98.54 | 11.45 ± 3.65 |
| 9/2021 | 4,194.65 ± 932.54 | 306.73 ± 132.76 | 7.98 ± 0.98 | 4,297.36 ± 276.68 | 329.42 ± 89.65 | 12.32 ± 3.83 |
| 10/2021 | 3,463.26 ± 375.65 | 72.173 ± 32.67 | 1.16 ± 0.32 | 3,565.91 ± 189.43 | 115.17 ± 23.73 | 3.23 ± 1.54 |
| 11/2021 | 4,258.54 ± 672.21 | 59.30 ± 27.21 | 2.05 ± 0.87 | 4,361.19 ± 543.54 | 179.68 ± 54.53 | 4.12 ± 1.32 |
| 12/2021 | 4,021.76 ± 423.63 | 18.25 ± 8.34 | 1.15 ± 0.43 | 4,124.41 ± 432.76 | 83.72 ± 18.76 | 2.03 ± 0.98 |

*Note:* The data are expressed as mean ± standard deviation; W(g) is body weight; Wtsd (g) is gonadal weight; GSI (%) is Gonado Somatic Index.

**Condition factor (CF)**

The condition factor of the milkfish was estimated from the relationship between standard length and body weight following the equations, $W = 192.01 \times L^{2.1058}$ and $W = 21.235 \times L^{2.2275}$ for females (n = 135) and males (n = 85), respectively (Fig. 4).

The CF of females and males varied from $3.68 \times 10^{-2}$ to $4.11 \times 10^{-2}$ and $3.02 \times 10^{-2}$ to $3.43 \times 10^{-2}$, respectively and peaked in May and September with females ($4.11 \times 10^{-2}$ and $3.92 \times 10^{-2}$) and males ($3.46 \times 10^{-2}$ and $3.34 \times 10^{-2}$), respectively (Fig. 5). Thus, the CF reached its maximum when the GSI reached the highest level.

According to King (1995), the CF refers to the status or development conditions of fish at each time of the survey. The CF evaluates the increase in fish weight relative to length and the rapid increase in fish weight over a certain period is mainly due to the increase in gonad weight (e.g., during their sexual maturation), especially in females.

We found that the GSI of milkfish peaked in May and September (Table 1). The field survey results showed that the majority of specimens collected during this time had gonads developed in stages III and IV when the gonad weight reached its maximum and was about to complete the

growth stage. According to Nguyen Van Kiem (2004), this is the period of nutrient accumulation and therefore, the gonad weight is constantly increasing. This means that total body weight is at its maximum, leading to an increase in CF, which is consistent with the normal development of the species.

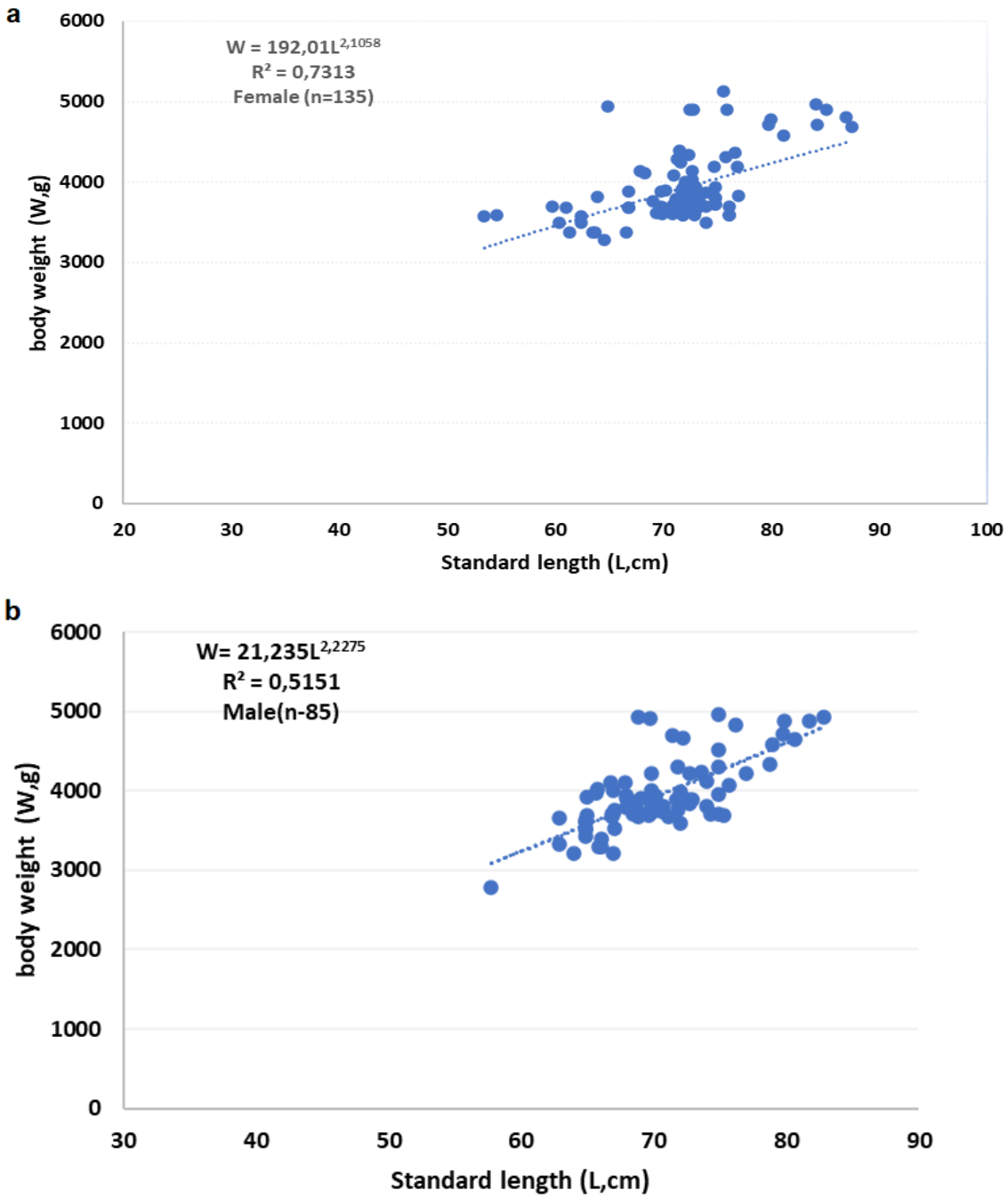


*Figure 4.* Relationship between standard length (SL) and total weight (W): a. females; b. Males

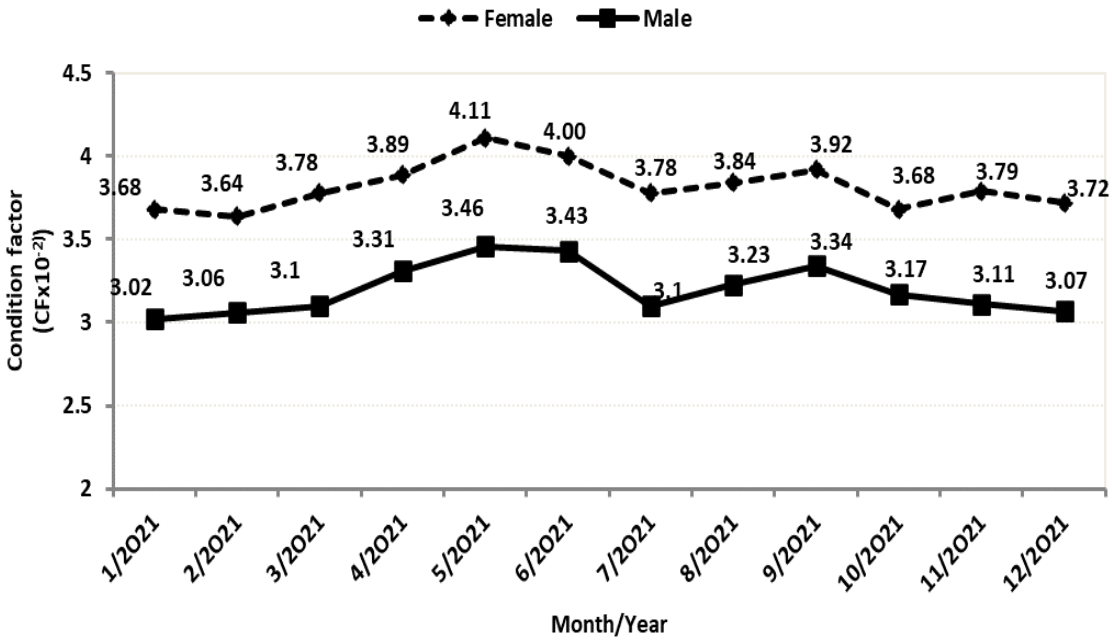


*Figure 5.* Monthly variation in the CF of *Chanos chanos*

***Fulton and Clark indexes***

The Fulton and Clark indexes varied significantly with maturity and time (Fig. 6) and ranged from 0.66% to 1.82% in Fulton and 0.49 % to 1.67% in Clark, respectively.

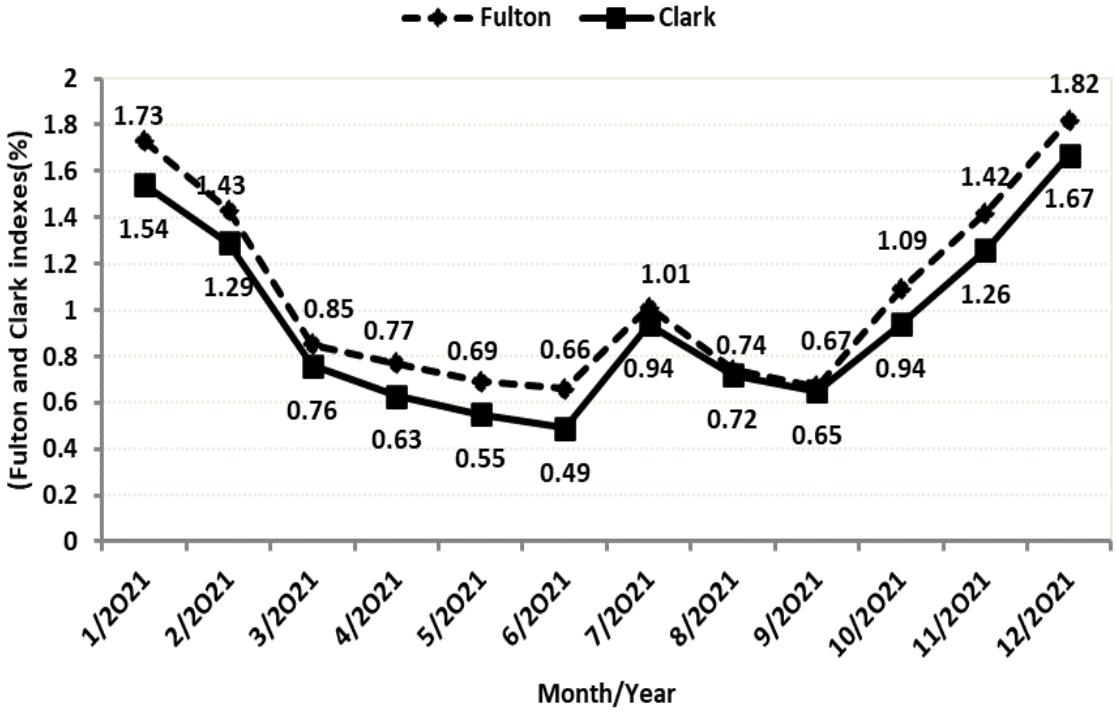


*Figure 6.* Variation in Fulton and Clark indexes of the studied *Chanos chanos*

Fulton and Clark indexes were highest in December 2021, reaching 1.82% and 1.67%, respectively. These factors gradually decreased in the following months, dropping to the lowest values of 0.66–0.74% (Fulton) and 0.49–0.72% (Clark) in August–September 2021 and April–June 2021. This was attributed to the conversion of nutrients accumulated in other organs to gonads to prepare for the spawning season. During sexual maturation, the accumulation and transfer of nutrients to produce eggs and sperm occur simultaneously. In the early stages of egg production, the level of accumulation of nutrients in the body is often higher than the metabolism to produce eggs (Chung Lan, 1969 according to Nguyen Van Kiem, 2004).

Therefore, in the rearing period, it is essential to ensure foods contain essential nutrients (proteins, lipids, vitamins, minerals) so that the fish can accumulate enough nutrients to perform the best maturity. From April, the Fulton and Clark indexes decreased drastically, and this could be due to the fastest gonadal growth rate in this time to peaked in May (Fig. 5). Following that period, the factors began to increase gradually, on the contrary, the GSI began to decrease. The finding indicates that after spawning time, fish are able to increase nutrient accumulation to prepare for the next spawning season.

### *Spawning season*

The spawning season is based on the variation of GSI and the rate of gonadal development stages over time (months). The spawning season of milkfish was determined based on Figure 7 and Table 2.

The Fulton and Clark indexes varied significantly over the months and both factors peaked in January (Fig. 7). This variation is related to the GSI of fish due to the fact that before sexual maturity, many fish species normally accumulate lipids in a number of different organs (Tran Thi Thanh Hien & Nguyen Anh Tuan, 2009; Lai Van Hung, 2004). GSI and Fulton/Clark are always two opposite indicators at all times.

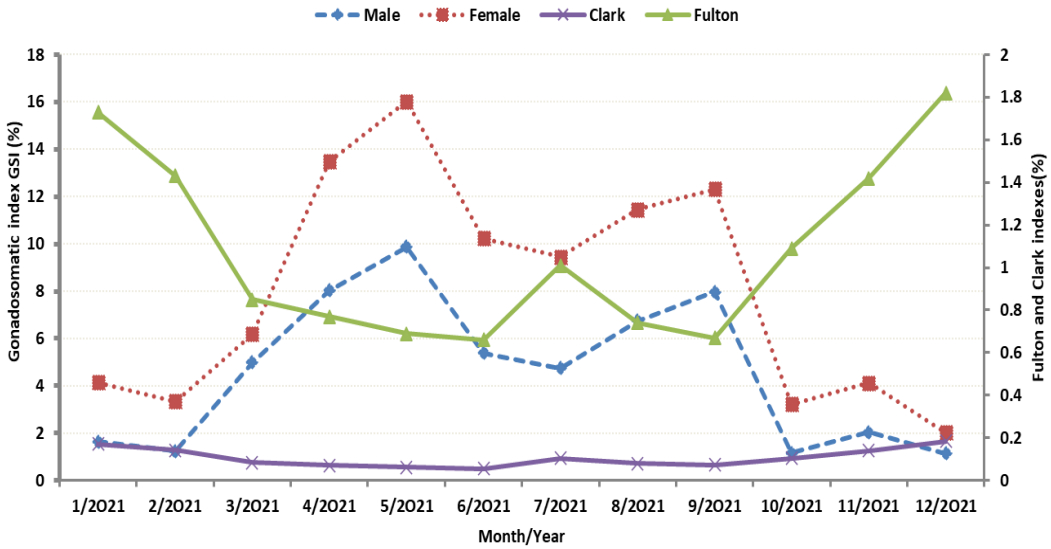


*Figure 7.* Variation in GSI, Fulton and Clark factors of *Chanos chanos*

Figure 8 and Table 2 show that the majority of female fish (60.0% to 86.67%) had gonads at stages I–II in the months of October, November, December and January 2021. The proportion gradually decreased and reached the lowest value in June and July 2021 (6.67%). No fish with gonads in stages I–II were observed between May and August

2021. The proportion of female fish with gonads in stage III reached the lowest in October 2021, accounting for 13.33% and the highest in March and July 2021 (53.33%). Fish with gonads at stage IV did not appear in October and December 2021, but reached the highest in April, June and August 2021, ranging from 40.0% to 46.67%. While fish with gonads at stage V did not appear in February, October and January 2021, but peaked in May 2021 (53.33%).

Thus, the percentage of females and males with mature gonads increased from April to June and August to September. During this time, gonadal stages of III, IV and V accounted for over 70% (Fig. 9).

*Table 2.* Monthly variation in the percentage of gonadal development stages of the studied milkfish female (n = 135)

| Month/Year | Percentage of gonadal development stages of *C. chanos* (%) | | | |
|---|---|---|---|---|
| | I–II | III | IV | V |
| 1/2021 | 73.33 | 20.00 | 6.67 | - |
| 2/2021 | 46.67 | 40.00 | 13.33 | - |
| 3/2021 | 20.00 | 53.33 | 20.00 | 6.67 |
| 4/2021 | 6.67 | 33.33 | 40.00 | 20.00 |
| 5/2021 | 0.00 | 20.00 | 26.67 | 53.33 |
| 6/2021 | 6.67 | 26.67 | 46.67 | 20.00 |
| 7/2021 | 6.67 | 53.33 | 26.67 | 13.33 |
| 8/2021 | - | 26.67 | 40.00 | 33.33 |
| 9/2021 | 6.67 | 20.00 | 33.33 | 40.00 |
| 10/2021 | 86.67 | 13.33 | - | - |
| 11/2021 | 60.00 | 26.67 | 13.33 | - |
| 12/2021 | 80.00 | 20.00 | - | - |

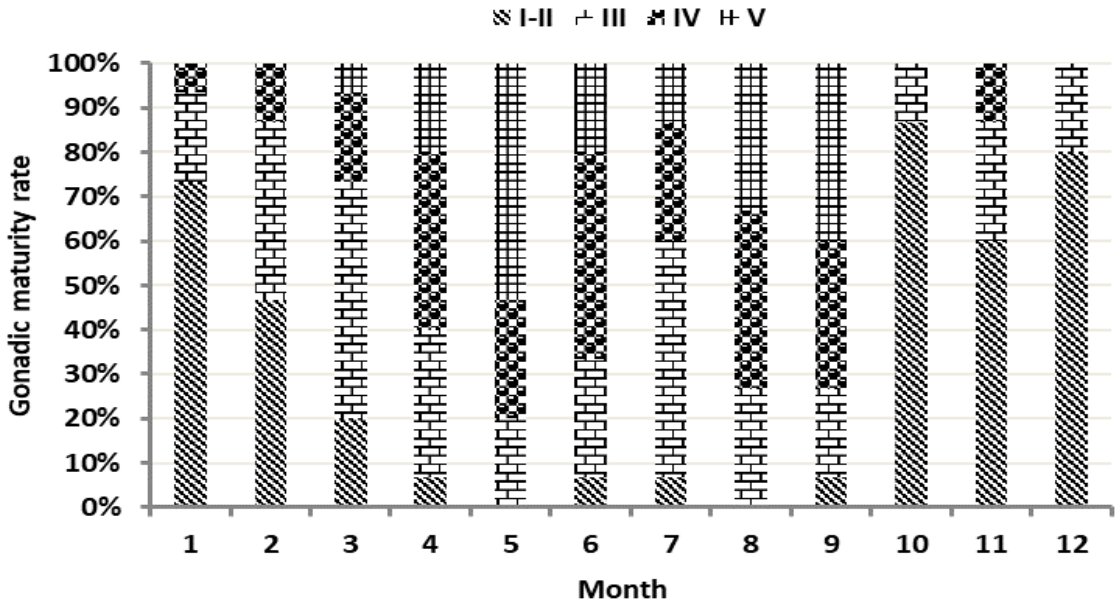


*Figure 8.* Monthly variation in gonadal development stages

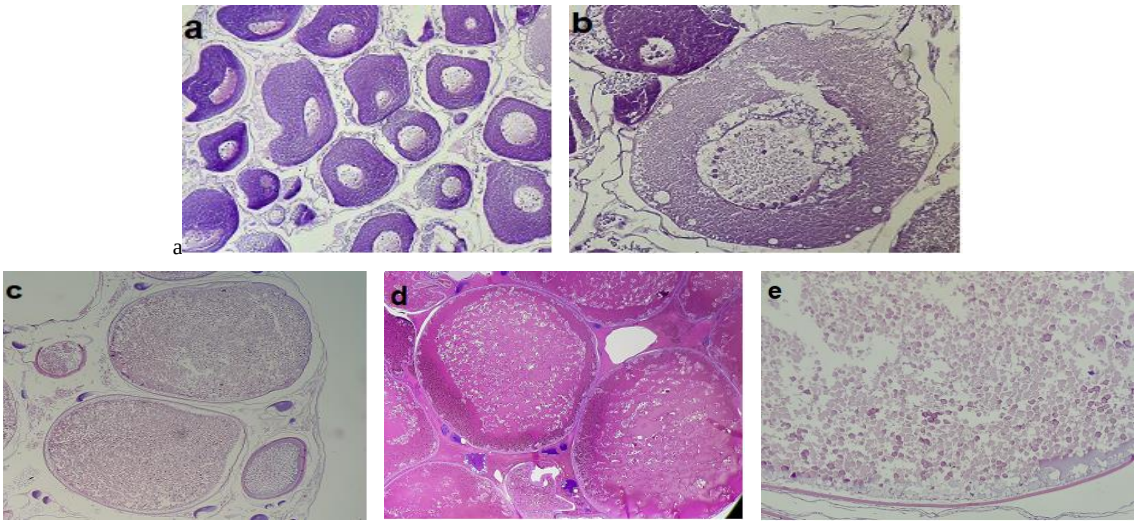


*Figure 9.* Gonadal female histology structures: a. Oocyte histology Stage I; b. Oocyte histology Stage II; c. Oocyte histology Stage III; d. Oocyte histology Stage IV; e. Oocyte histology Stage V

The results of GSI, Fulton and Clark factors, and gonadal development stages showed that the sexual maturity cycle and spawning season of milkfish were determined through the percentage of mature fish at stages III, IV and gonadal index. It can be clearly seen that the percentage of mature fish in stages III, IV and V appeared mainly from April to June 2021 and August to September 2021. Therefore, it can be inferred that the spawning season of milkfish is from April to June and August to September (Fig. 10). In artificial spawning, it is necessary to rely on the spawning season to create suitable conditions such as environmental factors, spawning grounds, flow regimes, etc. to stimulate fish reproduction and to obtain the highest spawning rate.

| Month | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Spawning season | | | | | Season 1 | | | | Season 2 | | | |

*Figure 10.* Graphical diagram showing the spawning season of milkfish

## CONCLUSION

Milkfish spawns sporadically from March –October, but concentrate in April–June and August–September. Therefore, it is possible to judge that the spawning seasons of milkfish in Central Coast Vietnam are two times a year, April–June and August–September.

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