Morphoanatomical indexes of female Siamese fighting fish *Betta splendens* under different photoperiod regimes

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Abstract. Photoperiod represents light manipulation to advance or delay reproduction in Siamese fighting fish (*Betta splendens* Regan, 1910). This study aimed to evaluate different photoperiod regimes against ovarian maturity of fish by examining morphoanatomical indexes. The female Siamese fighting fish underwent different photoperiod regimes, for 45 days, i.e. P1 (10h light, 14h dark), P2 (14h light, 10h dark), and P3 (ambient light as control). Morphoanatomical indexes were accessed through gonadosomatic index (GSI), hepatosomatic index (HSI), and viscerosomatic index (VSI), being corelated with vitellogenesis. Vitellogenesis was analyzed through ovarian histology preparations. Results showed that GSI, HSI, and VSI were statistically significantly different in P2 (38.25%, 24.61%, 34.93%). P1 (25.48%, 18.23%, 22.53%) and P3 (15.92%, 12.55%, 15.71%) were not different. These photoperiod regimes affected female morphoanatomical indexes. GSI, HSI, and VSI increased comparably with vitellogenesis. HSI and VSI promoted GSI at a high-level.
Key Words: gonadosomatic index, hepatosomatic index, viscerosomatic index, vitellogenesis.

Introduction. Ornamental fish present interest for culturing, being appreciated due to their beauty and aesthetics (Anjur et al 2021). One of the most appreciated ornamental fish in Indonesia is the Siamese fighting fish *Betta splendens* Regan, 1910. People adore the fish due to the color and amazing tails (Amiri & Shaheen 2012; Syaifudin et al 2017; Ayuningthias et al 2021). Demand on this fish species increases year to year (Dewantoro 2001). This enhances some aqua culturists to adopt technologies in Siamese fighting fish production. The adopted technologies include, one of them, photoperiod regimes to succeed in reproduction or spawning of fish (Abdollahpour et al 2020; Patmadevi et al 2022).

Photoperiod regimes involve environmental manipulation to advance, or to delay, gonadal maturation (Aragón-Flores et al 2017; Türker & Yildirim 2011; Veras et al 2013). Also known as lighting periods, the regimes determined reproduction effective in terms of onset or ceasing gonadal development (Mustapha et al 2014; Yun et al 2015). Light is provided as environmental cues that would be received by brain photoreceptors. These signals would be transmitted to the hypothalamus, pituitary, gonad, and liver (Wijayanti et al 2009). Light intensity, generally, endured for triggering optimal growth, however, short periods of light stimulated gonadal maturation, e.g. in Mexican cichlid *Cichlasoma beani* (Aragón-Flores et al 2017; Good et al 2016).

In Siamese fighting fish, photoperiod evenly presented an important environmental signal in the development of gonads and reproduction (Lee et al 2017). As in other many fish, Siamese fighting fish reproduction rely on internal reproductive organs, i.e. gonad, liver, and viscera. To evaluate reproductive performance of fish, morphoanatomical indexes were formulated as gonadosomatic index (GSI), hepatosomatic index (HIS), viscerosomatic index (VSI) (Pratiwi et al 2020; Sulistyo et al

2000). The current study dealt with evaluation of different photoperiod regimes in ovarian maturation of fighting fish, based on morphoanatomical indexes.

Material and Method

Location and research period. This research was conducted for 2 months, calculated from May-July 2018. The research took place at the Aquatic Fisheries and Marine Science Laboratory, Faculty of Jenderal Soedirman University, Purwokerto.

Experimental design and sample preparation. Female Siamese fighting fish, of halfmoon strain, ageing about 7 months, were collected from local culturists in Beji Village, Banyumas District, Central Java Province, Indonesia. A completely randomized design tested 3 treatments, i.e. P1 (10h light, 14h dark, 10L:14D), P2 (14L:10D), and P3 (ambient light as control). Each treatment was run, for 45 days, in quintuplicates.

Morphoanatomical indexes

Gonadosomatic index (GSI). This index represents quantitative performance of gonad (testis or ovary) in fish. In general, an individual fish presented GSI ≥ 19% to be considered as sexually mature and apt to spawn. GSI was formulated as follows (Korkut et al 2007):

$$Gonadosomatic\ index\ (\%) = \frac{gonads\ weight\ (g)}{body\ weight\ (g)}\ x\ 100$$

Hepatosomatic index (HSI). This index represents energy reserve in liver, properly reproduction support for fish (Tresnati et al 2018). Formulation of HSI was done as follows (Déniel 1981):

$$Hepatosomatic\ index\ (\%) = \frac{liver\ weight\ (g)}{body\ weight\ (g)}\ x\ 100$$

Viscerosomatic index (VSI). Visceral organs are found in the abdominal cavity, excluding gonads and liver, storing energy reserves, generally in form of fat around the intestine. The index maximized (4-6%) in pre-spawning season and, contrary, depleted in post-spawning (Sulistyo et al 2000). VSI was formulated as follows (Korkut et al 2007):

$$Viscerosomatic\ index\ (\%) = \frac{viscera\ weight\ (g)}{body\ weight\ (g)}\ x\ 100$$

Data analysis. GSI, HSI, and VSI data were arcsine transformed before ANOVA test (0.05 level), followed with Tukey's honest significant difference (HSD). GSI, HSI, and VSI were analyzed in correlation with vitellogenesis stages. Vitellogenesis stages derived from ovarian histological analysis.

Results

Gonadosomatic index (GSI). GSI, presented in Figure 1, was statistically significantly different (0.05) for all treatments. Highest GSI was in P2 (38.25%), whilst P1 and P3 were 25.48% and 15.92%, consecutively.

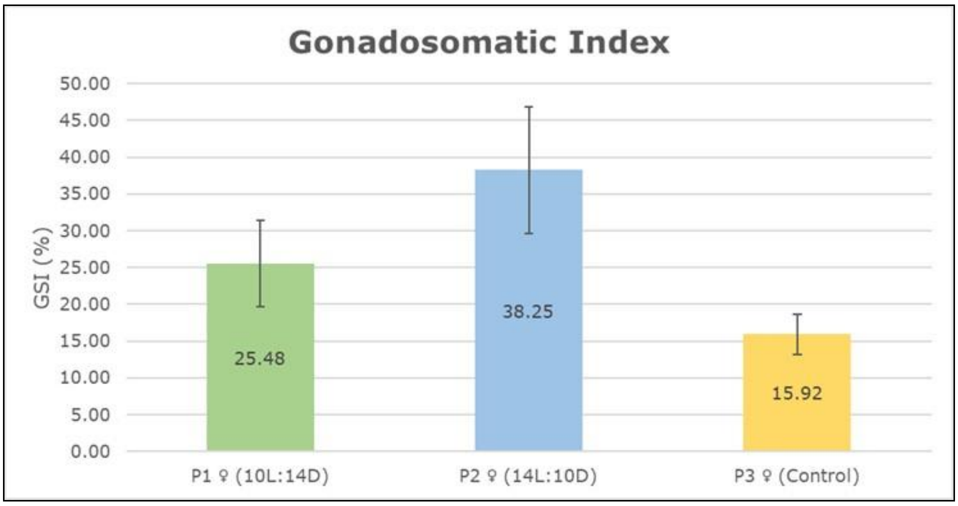

Figure 1. Gonadosomatic index (GSI) of Siamese fighting fish according to treatments.

Hepatosomatic index (HSI). Hepatosomatic index from all treatments showed that P1 (34.93%) was significantly different, from P2, and P3 being 22.53%, and 15.71%, consecutively. According to data, P1 was highest, followed with P2 and P3, as seen in Figure 2.

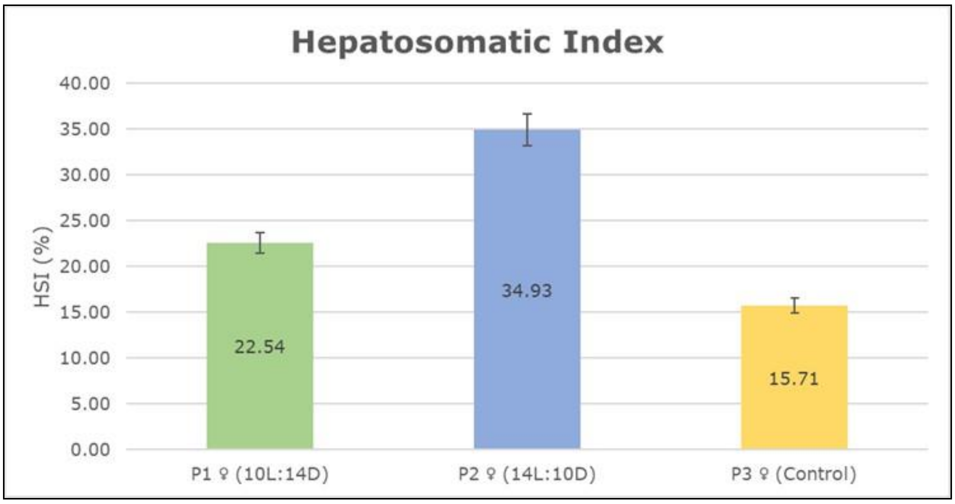

Figure 2. Hepatosomatic index (HSI) of Siamese fighting fish according to treatments.

Viscerosomatic index (VSI). VSI, displayed in Figure 3, presented not statistically significant differences from all treatments. P1, P2, and P3 were consecutively 18.23%, 24.61%, and 12.55%. P2 tended to be higher than P1 and P3.

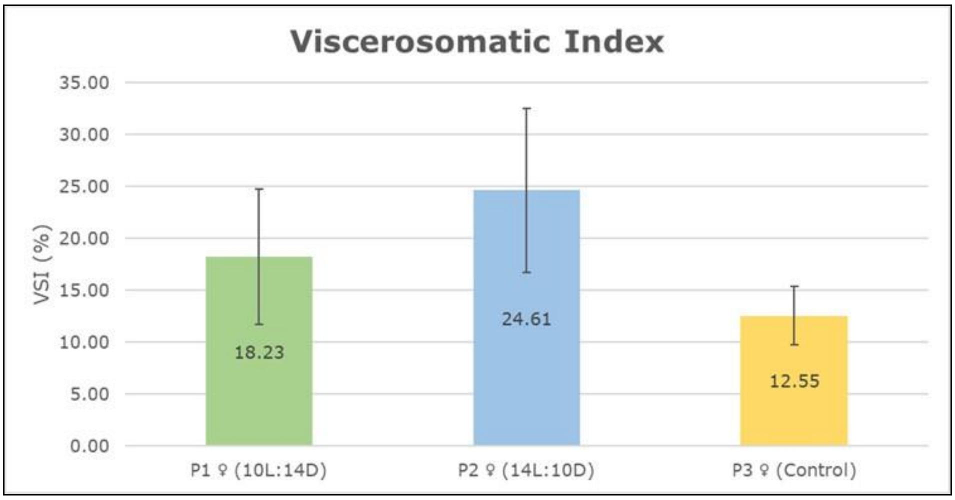

Figure 3. Viscerosomatic index (VSI) of Siamese fighting fish according to treatments.

Morphoanatomical indexes correlation with vitellogenesis

Gonadosomatic index (GSI) with vitellogenesis. A significant correlation between GSI with vitellogenesis stage proportions (y = 3.7091x – 55.382) was observed. Regression coefficient was positive (R^2 = 1). This indicated that increasing GSI synchronized with increasing proportions of vitellogenesis stages, as showed in Figure 4.

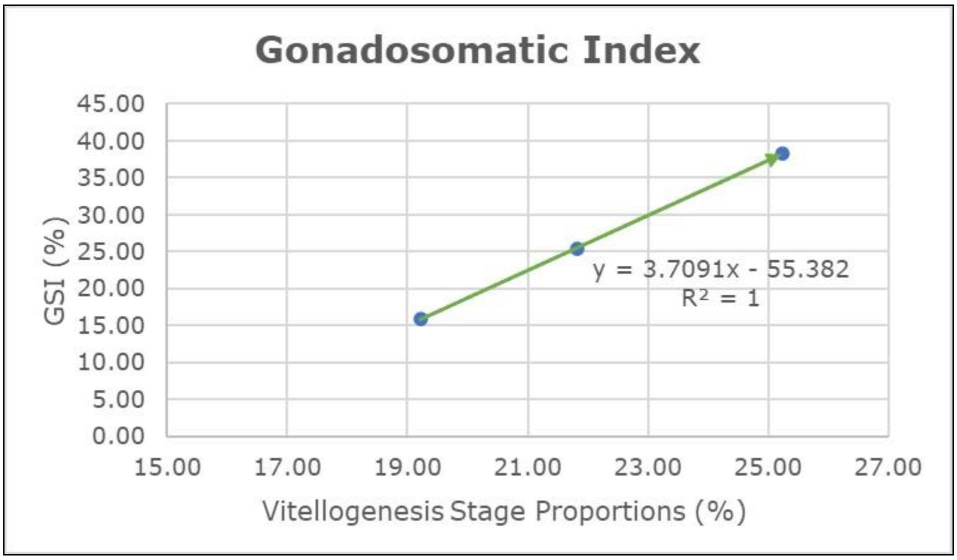

Figure 4. Correlation between gonadosomatic index (GSI) with vitellogenesis stages.

Hepatosomatic index (HIS) with vitellogenesis. A correlation between HSI with vitellogenesis was detected (y = 3.2147x – 46.616), with R^2 = 0.98. Two possibilities on this, firstly, increasing in proportions of vitellogenesis stages caused increasing in HSI. Secondly, increasing in proportions of vitellogenesis stages was a subsequent of more energy required from the liver to support vitellogenesis stages. This correlation was shown in Figure 5.

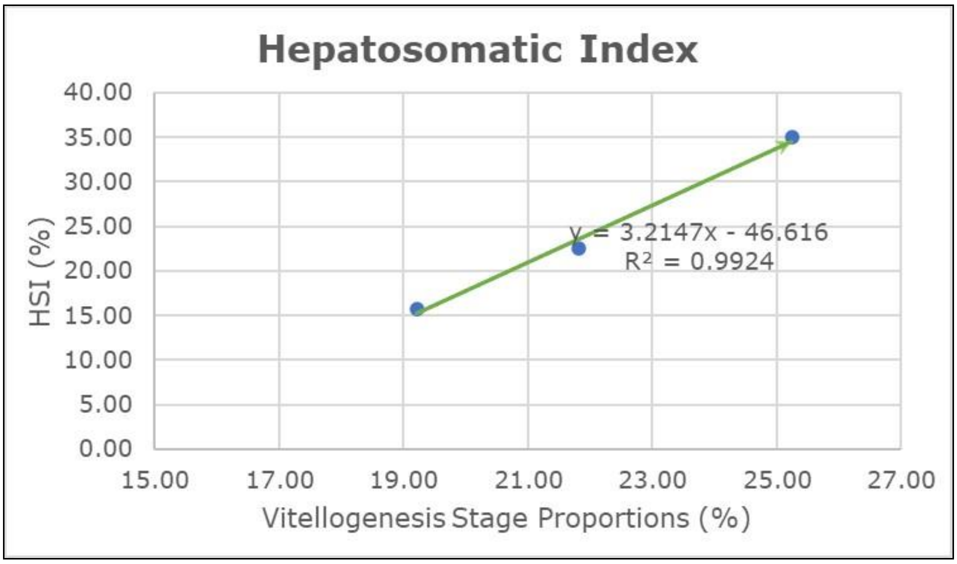

Figure 5. Correlation between hepatosomatic index with vitellogenesis stages.

Viscerosomatic index (VSI) with vitellogenesis. VSI correlated positively with vitellogenesis (y = 1.9958x – 25.625), with R^2 = 0.998. This indicated that high VSI clearly favored the increase of proportions of vitellogenesis stages. Correlation was shown in Figure 6.

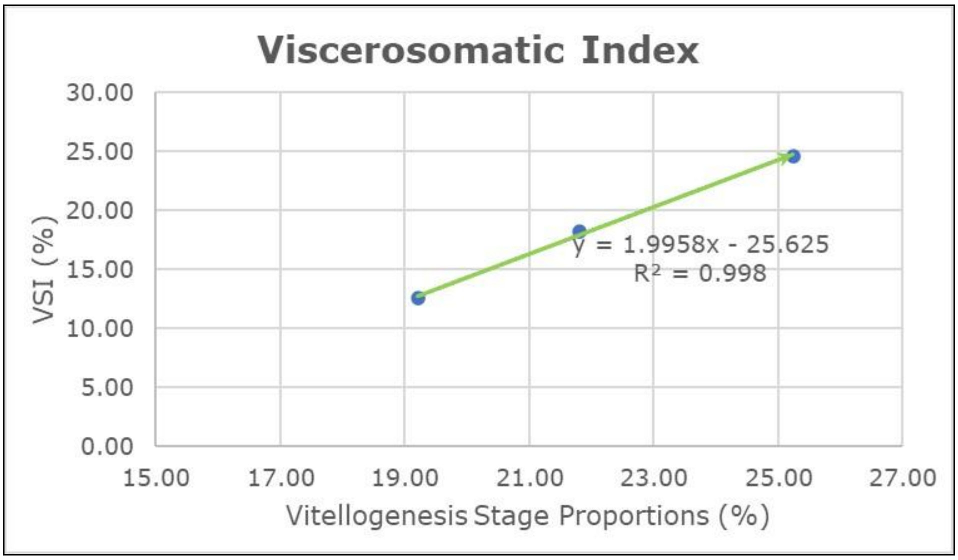

Figure 6. Correlation between viscerosomatic index with vitellogenesis stages.

Correlation between gonadosomatic index (GSI) and viscerosomatic index (VSI). GSI correlated positively with VSI (y = 0.538x + 4.1781) and R^2 = 0.997. GSI and VSI grew together since ovarian development required more energy deriving from visceral organs. This correlation was displayed in Figure 7.

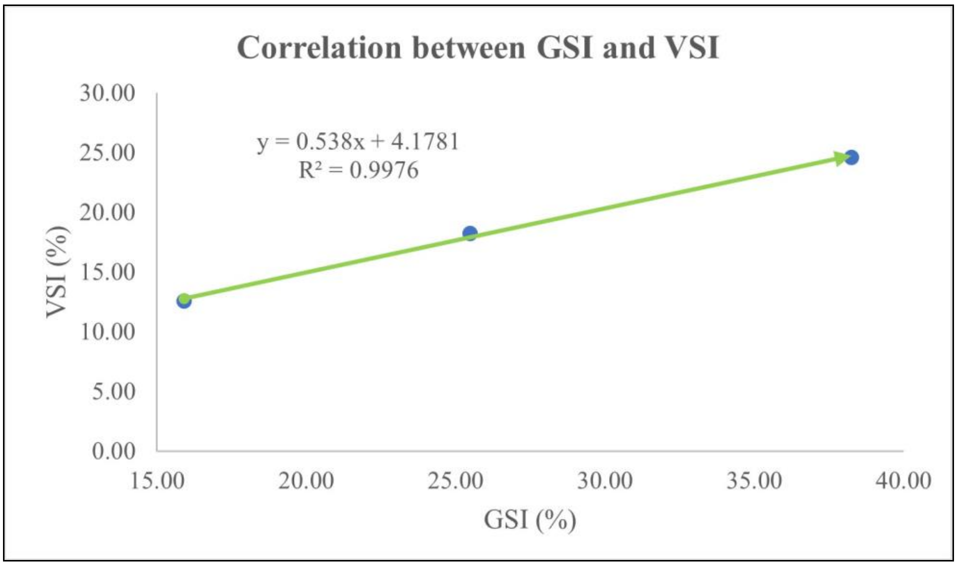

Figure 7. Correlation between gonadosomatic and viscerosomatic indexes.

Correlation between gonadosomatic index (GSI) and hepatosomatic index (HSI). In female Siamese fighting fish, there was a correlation (y = 0.867x + 1.3753) between GSI and HSI. Two possibilities were pronounced, first, the increase in GSI was followed by HSI. Second, the increase in GSI should be supported by more energy derived from HSI, especially during vitellogenesis. Regression coefficient was, moreover, positive (R^2 = 0.9931 or 99.31%), meaning that GSI was 98.8% influenced absolutely by HSI, as shown in Figure 8 below.

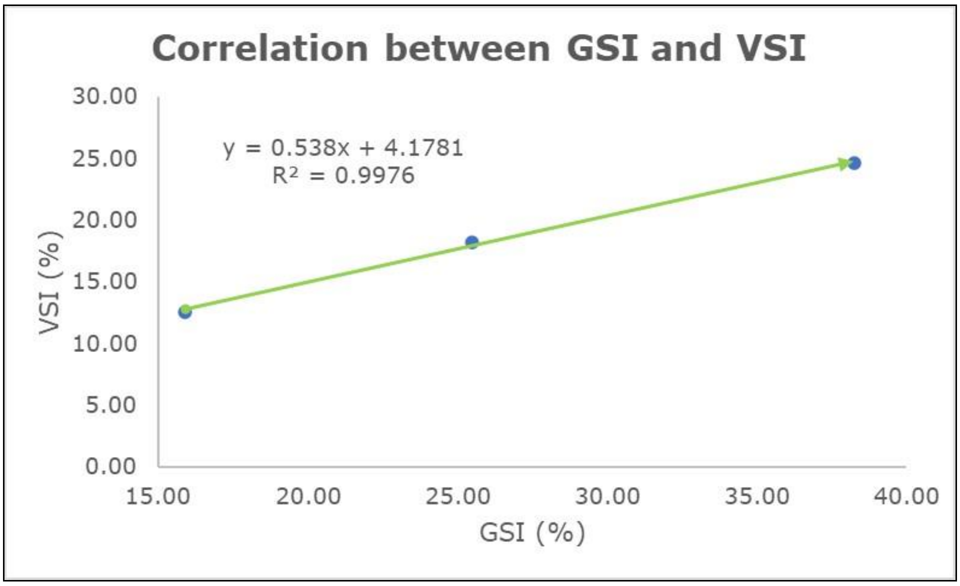

Figure 8. Correlation between gonadosomatic and hepatosomatic indexes.

Discussion. The photoperiod, as reported by Abdollahpour et al (2020), plays an important role in reproduction, growth, and survival of fish, comprehensive intensity and spectrum of light. Meanwhile, Bayarri et al (2004) evoked that photoperiod affected fish physiological activity through melatonin production from the pineal gland. If melatonin, known as an antigonadotropin, was high in the blood is high, the gonadal development should cease. Bairwa et al (2013) stated that high melatonin levels correlate with low levels of gonadotropin-releasing hormone (GnRH), resulting in gonadotropin production decrease. If gonadotropin production decreases, gametogenesis would be encumbered, and vice versa, if melatonin levels in the blood were low, gametogenesis could be enhanced, due to the high levels of GnRH. Biswas et al (2005), and Rodríguez et al

(2004), shared that if photoperiod stimulated fish, development and maturation of the gonads accelerated, whereas if it was inhibitor, gonadal maturity delayed. Photoperiod, therefore significantly, affected reproductive activity. Bairwa et al (2013), and Amano et al (2000), concluded that photoperiod affected hormone melatonin intensity, in term of low hormone melatonin occurred in short light period, and vice versa, high melatonin in long light period.

This study confirmed that photoperiod affected reproductive female Siamese fighting fish, through morphoanatomical indexes analysis. The fish reared under photoperiod regime of 14L:10D, for 45 days, showed high GSI, HSI, and VSI. These indexes were positively increased under long photoperiods. This study obtained high GSI in treatment P2, being 38.25%, which was then followed by treatment P1, being 25.48%. Mahmudah et al (2019) indicated that high GSI signified the female fish matured gonads and were apt to spawn. The GSI value will increase and reach its maximum limit when the fish are about to spawn (Fitriani et al 2022; Nabila et al 2022). Additionally, Effendi (1997), and Nurhidayat et al (2017), detailed that female GSI would be greater than in male fish. This was caused by differences in the structure and growth of gonads. Ovaries grew, structurally and pro forma, more than testis. Ovaries contained matured eggs and could fill up the entire abdominal cavity (Agustin et al 2020; Mukti et al 2020).

The HSI value in this study showed a fairly high value in the P2 and P1 treatments, HSI being 34.93% and 22.53%, successively. Sari et al (2017) stated that such levels of HSI indicated rolling development and maturation of ovary. HSI thus illustrated the reproductive performance of fish. Sulistyo et al (2000) remarked that HSI increased along with spawning process and decreased in post-spawn period. Liver played a very important role in reproduction since it is directly involved in the vitellogenesis and egg yolk formation. Liver contained proteins that were completely delivered to ovary, resulting in a dramatical HSI decrease from onset ovarian development, final maturation, and until ovulation. Lucifora et al (2002) said that female fish contained large amounts of fat, as stored energy, during the previtellogenesis stage and were then spent in vitellogenesis.

VSI, in P2 and P1 treatments, was 24.61% and 18.23%, consecutively. Female fish underwent certain dark periods, resulting in passive movement and are more responsive to food so that provided more energy to be stored in the form of fat around visceral organs (Azizi 2022). Pertiwi et al (2024) stated moreover that stored energy in form of fat around visceral organs was essential in supporting ovarian development. Correlation between GSI and VSI, being proportionally inverse, justified it and the fish were inactively feeding. Damsgård et al (1999) associated also decrease in somatic growth with food intake, in subsequently meat quality of fish being worst. Pazos et al (2003), and Aragón-Flores et al (2017), marked that energy, stored in muscles, digestive tracts, liver, was mainly in form of protein, fat, and rarely carbohydrates. Silverstein et al (1999) noticed that fish being in reproductive stage contained higher fat in storing organs than those in post-reproductive period.

Vitellogenesis correlated with GSI, HSI, and VSI in some traits. Proportion of ova stages during vitellogenesis increased with those indexes. Ova stages in vitellogenesis were intently corresponding ovarian maturation, specifically with GSI augment. Rodrigues et al (2017) reported that, in silver catfish (*Rhamdia quelen*), vitellogenesis caused ovarian mass increased due to ova maturation and vitellogenin inclusion. Costa et al (2010), and Hutagalung et al (2023), confirmed that vitellogenin production and energy reserves caused an increase in liver size, or HSI. Vitellogenin, and oocyte final maturation, required an excessive amount of energy, partly provided by energy reserves stored in visceral organs. In addition, high HSI of female fighting fish also indicated high number of oocytes and vitellogenin production.

Conclusions. Different photoperiod regimes, with 14L:10D and 10L:14D, altered morphoanatomical indexes which differed in levels of female Siamese fighting fish. These indexes correlated with different ovarian development and vitellogenesis. Ova proportion stages were in parallel with GSI, HSI, and VSI. Moreover, GSI altered with HSI and VSI changes.

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Conflict of interest. The authors declare that there is no conflict of interest.

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