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Reproductive Hormone Performance: Glass Strain Common carp (*Cyprinus carpio*) Reared in The Wet and Dry Seasons

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***Abstract*— Glass strain carp (*Cyprinus carpio*) is highly demanded by farmers due to its delicious, dense meat and fewer spines. To meet the increasing demand for carp, it is necessary to improve the efficiency of aquaculture. The season is known to stimulate one hormone production. The purpose of this study was to determine the effect of season (rainy and dry seasons) on reproductive hormones (follicle stimulating hormone (FSH), luteinizing hormone (LH), testosterone and estrogen, and interstitial cell-stimulating hormone)) in *C. carpio* glass strain. Idi research was conducted using the descriptive method by rearing fish in different seasons (rainy season and dry season). The results showed that the reproductive hormone of C. carpio strain glass increased during the rainy season. During the rainy season, changes in the environment and water flow patterns are detected by the fish's sensory system. The results showed that the highest hormones in a row were: ICSH hormone (12.82 pg/mL), FSH hormone (male: 3.73 ng/mL; female: 12.10 ng/mL), LH hormone (12.48 MIU/ml), testosterone hormone (9.27 ng/mL) and estrogen hormone (21.84 ng/mL). Further research is needed to observe the growth and abnormality of fish larvae produced in different seasons.**

Keywords— *C. carpio*, Hormone, Rain, Reproduction, Season

I. INTRODUCTION[1]

Proteins are essential macromolecules that have a crucial role in maintaining the integrity of structure, function, and metabolic processes in living organisms [1; 2]. Proteins act as the main component of muscle tissue, enzymes, hormones, antibodies, and various other important molecules, thus supporting various biological functions, including enzyme catalysis, substance transportation, intercellular communication, and immune system protection [3]. Protein sources in the diet are generally divided into two categories, namely animal and plant proteins [4; 5]. Animal protein, such as fish, is considered a complete protein source because it contains all essential amino acids in sufficient amounts [6]. In addition, these animal protein sources also contain various other important nutrients, such as iron, vitamin B12, and omega-3 fatty acids, which have an important role in maintaining a healthy body [7].

The human population is predicted to increase from the current figure of around 7.6 billion to nearly 10 billion by 2050 [8; 9]. As the human population increases, the demand for protein also increases [10]. Therefore, an increase in fish production is needed to meet human needs. Between 1961 and 2016, global fish food consumption experienced an average annual growth of 3.2%, which exceeded the population growth rate of 1.6% [11]. This led to per capita fish consumption increasing from 9 kg per person in 1961 to 20.5 kg per person in 2017. This significant increase was mainly influenced by the rapid development of global aquaculture since the 1980s, which now supplies about 50% of the total direct food fish consumption by humans worldwide [12].

Common carp (*Cyprinus carpio*) is a freshwater fish species that can be found in ponds, lakes, rivers, and reservoirs, with a water temperature range between 23°C and 30°C. This species is native to the Ponto-Caspian and Aral Sea regions [13] but has been introduced to various freshwater ecosystems around the world [14; 15]. In 2018, carp contributed 25.2% to global aquaculture production, 35.2% of total aquatic animal production, and 53.2% of cultured finfish production [11]. To meet the increasing global demand for carp, it is necessary to increase efficiency in aquaculture, especially in the aspect of reproduction [16]. Reproduction plays a crucial role in the survival of a species, as it enables the creation of new generations [17; 18]. Cultivation of *C. carpio* in Indonesia began in the mid-19th century in the Galuh region of Ciamis, West Java. After the Ministry of Agriculture established the Inland Fisheries Service in the early 20th century, the spread of *C. carpio* expanded to various regions in Indonesia [19]. In 1927, Jawatan Perikanan Darat began importing *C. carpio* from the Netherlands, including the Galician strain (known as *C. carpio* elephant strain), followed in 1930 by the Franciscan strain (*C. carpio* glass strain). Both strains were in high demand by farmers at that time due to their tasty, dense meat and fewer spines, in addition to faster growth compared to local strains that had been developed earlier. *C. carpio* is known to reproduce seasonally [20; 21]. One of the factors that stimulate fish reproduction is hormones [22]. Hormones serve as chemical signals that enable communication between different types of cells [23; 24]. The cells recognize their tasks and function through receptors, which are protein structures that are specialized to recognize specific molecules [25; 26]. When a hormone binds to its receptor, a series of biochemical reactions will take place, which then trigger a specific biological response [27].

The hypothalamic-pituitary (HP) system in teleost fish serves as the leading center for controlling reproduction through a complex interaction between hormones and neural signals. The hypothalamus produces gonadotropin-releasing hormone (GnRH), which is then released to the pituitary gland

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to trigger the secretion of gonadotropins (GtH), namely follicle-stimulating hormone (FSH) and luteinizing hormone (LH) [28]. FSH plays an important role in the early stages of gonadal development, including the processes of oogenesis and spermatogenesis, while LH functions in the late stages of gonadal maturation, including ovulation and spermiation. These gonadotropins stimulate the gonads to produce sex steroid hormones. This mechanism is controlled by a hormonal feedback system, where levels of steroid hormones such as estradiol and testosterone can provide positive signals to increase GnRH release or negative signals to inhibit it, according to the physiological needs of the fish [29; 30; 31].

Many studies have recorded seasonal concentrations of circulating sex hormones, which play an important role in the reproductive process in diverse teleost fish species [22; 37]. Such studies have shown seasonal fluctuations in sex steroid hormone levels in a number of species, such as in Tilapia (*Oreochromis spp.*) [38], Southern Hake (*Merluccius australis*) [39; 40], midshipman plainfin (*Porichthys notatus*) [41]. Research in fisheries continues to highlight the importance of understanding how environmental factors, such as seasonality, affect reproductive hormones. Similar research has never been conducted on this fish so the information to be conveyed is very important and needed in the future development of *C. carpio*. The purpose of this study is to determine the effect of season (rainy and dry seasons) on reproductive hormones (follicle stimulating hormone (FSH), luteinizing hormone (LH), testosterone and estrogen, and interstitial cell-stimulating hormone)) in *C. carpio* glass strain.

II. Method

The study was conducted for three months (April - June 2024), covering the rainy season and dry season periods. The sample fish (*C. carpio* glass strain) used in this study came from the Punten Aquaculture Fisheries Installation (IPB) and the research was also conducted at that place. A total of 12 brood fish were taken as samples, consisting of 6 males and 6 females, with body lengths ranging from 25 to 30 cm. Rainfall data was obtained from the Meteorology, Climatology and Geophysics Agency (BMKG) of Batu City through the official website (https://dataonline.bmkg.go.id). Reproductive hormone observations included ICSH hormone, follicle-stimulating hormone (FSH), luteinizing hormone (LH), testosterone, and estrogen.

Measurement of hormone levels was carried out on interstitial cell-stimulating hormone (ICSH), follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone and estrogen using the ELISA method as done by Mandala [42]. The study began by preparing all samples and reagents and then conditioning them at room temperature for approximately 30 minutes. A total of 50 µl of standard diluent solution was taken and inserted into the standard well or blank well. Next, 40 µl of the sample was put into the sample well, and then 10 µl of antibody was added to the well. A total of 50 µl of streptavidin-HRP was added to the standard well and sample well, then stirred gently for 1 minute. After that, the wells were covered with adhesive and incubated at 37°C for 60 minutes. After incubation, the adhesive was removed, and each well was aspirated and washed using a wash buffer solution five times. The remaining wash buffer was discarded, and the wells were dried using drying paper. Next, 50 µl of substrate solution A and 50 µl of substrate solution B were added to each well and then incubated for 30 minutes at 37°C with the adhesive covered to protect from light exposure. 50 µl of stop solution was added to each well. Results were measured at 450 nm wavelength using an ELISA reader (Diatek DR-200BC) within 30 minutes to determine the optical density (OD) value. Measurement of hormones ICSH, FSH, and LH were measured from the hypophysis, while testosterone and estrogen were measured from the fish. The data obtained were analyzed simply using Microsoft Excel.

III. RESULTS AND DISCUSSION

1. Rainfall

The results showed that the highest rainfall reached 2546.871429 mm, while during the dry season, the highest rainfall reached 1,357142857 mm (Figure 1). Warmer water temperatures cause an increase in reproductive hormones in the rainy season. Previous studies have shown a number of factors that potentially affect the spawning process of freshwater fish. Environmental factors such as temperature, water flow, water level, and rainfall are known to be closely related to the reproductive cycle of fish [43; 44].

Increasing the temperature too high can also negatively affect reproduction due to the thermal sensitivity of reproductive hormones. Increasing temperature too high can also disrupt the HPG axis at some point by altering hormone synthesis, activity and structural integrity [45]. These disruptions can affect the structure of proteins and hormones, reducing their solubility or ability to bind to receptors. In some cases, hormones can bypass their intended targets and be excreted through the kidneys, leading to the disruption of reproductive processes. Such disruptions in the hormonal cascade caused by high temperatures can reduce reproductive activity, egg size and offspring survival.

In *C. carpio*, flooding events either caused by natural rainfall or artificial attempts to inundate shallow areas have been shown to be a major trigger in initiating the spawning process (Hora, 1945). This stimulates the fish reproductive system by activating the hypothalamus-pituitary-gonadal (HPG) axis. The rainy season also increases the availability of food sources, such as plankton and aquatic insects, which are important energy suppliers for the reproductive process while ensuring adequate resources for offspring survival [46].

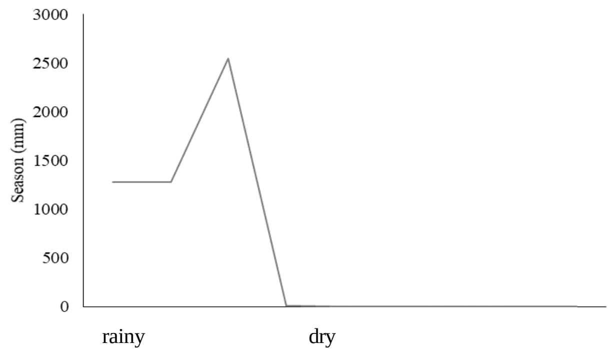

Figure 1. Rainfall during rearing of glass strain *C. carpio*

During the rainy season, environmental changes such as increased water temperature, higher rainfall and changes in water flow patterns are important cues for fish to start their reproductive process. The fish's sensory system then detects these changes, and the information is relayed to the brain. The brain, particularly the hypothalamus, responds to these environmental signals by releasing gonadotropin-releasing hormone (GnRH). GnRH then stimulates the pituitary gland to secrete gonadotropins. This hormone plays a role in regulating gonadal maturation.

2. ICSH hormone in glass-stained *C. carpio*

The action of ICSH in the reproductive process of fish is closely related to the hypothalamus-pituitary-gonadal (HPG) axis. The hypothalamus releases gonadotropin-releasing hormone (GnRH), which then triggers the pituitary gland to secrete ICSH. This hormonal cascade plays a crucial role in gonadal maturation and gamete release. Exogenous GnRH administration is also able to activate the HPG axis, thereby increasing ICSH levels and spurring reproductive activity in various fish species [47; 48]. In addition, the expression of gonadotropin subunits, including ICSH, changes during the reproductive cycle, reflecting the existence of a well-calibrated regulatory mechanism to respond to environmental and physiological cues [49]. The results of the study conducted on male fish showed that ICSH hormones were also different in each season. The highest ICSH hormone of the *C. carpio* glass strain was produced in the rainy season. In the rainy season, the highest ICSH hormone reached 12.82 pg/mL, while during the dry season, the ICSH hormone reached 10.02 pg/mL (Figure 2). ICSH is produced by the pituitary gland in response to the release of Gonadotropin Releasing Hormone (GnRH) produced by the hypothalamus.

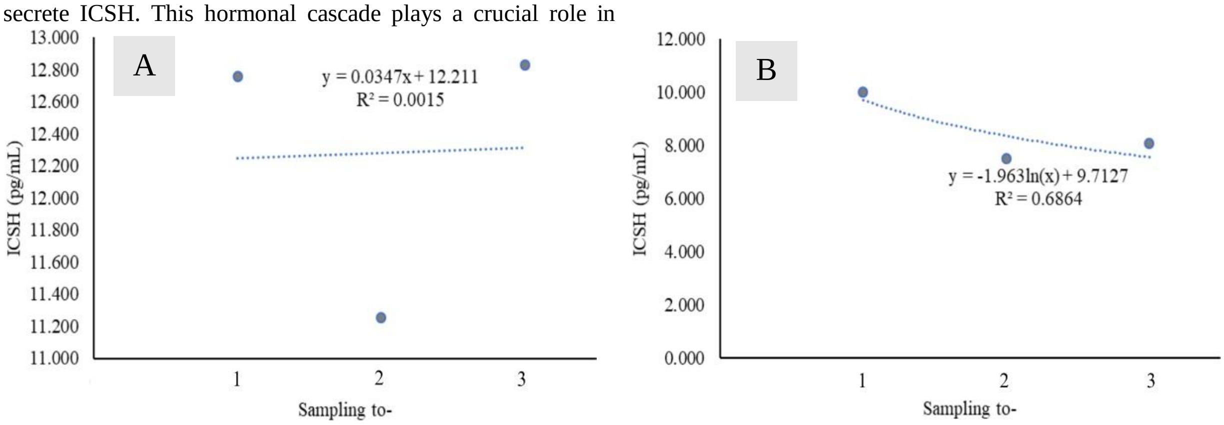

Figure 2. ICSH hormone in *C. carpio* male glass strain. a) Wet season. b) dry season.

Research has also shown that hormonal manipulation, including the use of GnRH analogues, can effectively stimulate gonad development and maturation in farmed fish, thereby increasing reproductive success [50]. This is particularly relevant in aquaculture, where controlled culture is key to maintaining fish stocks and supporting sustainable production. In male fish, ICSH triggers interstitial cells in the testes to produce testosterone, a hormone essential for sperm development [51]. ICSH also works synergistically with other hormones, such as Follicle follicle-stimulating hormone (FSH), to optimize the reproductive process, ensuring the physiological readiness of fish for spawning [50].

ICSH is known to play a role in regulating sex steroid synthesis in the gonads, which is crucial for gamete development. In teleost fish, ICSH stimulates the production of androgens and estrogens, hormones that play a role in spermatogenesis and oogenesis, respectively [52; 53]. This relationship between ICSH and sex steroids is key to successful reproduction, as they regulate processes such as follicular development, ovulation, and sperm maturation [54]. In addition, environmental factors also affect the secretion of ICSH as well as other gonadotropins, which play a role in the reproductive success of fish [55].

Seasonal reproductive patterns in fish are influenced by hormonal changes that are affected by various ecological factors. In addition, environmental factors such as temperature and photoperiod have been shown to play a role in regulating reproductive hormone secretion in fish [56; 57; 58]. Physiological changes due to seasonal changes also affect the metabolic rate and health of the fish, which in turn impacts hormone levels. During the spawning season, the metabolic activity of male fish tends to increase [59; 38]. The nutritional quality of fish food, which varies with the season, is also an important factor in hormone regulation. During the spawning season, the availability of food with high caloric content can support increased levels of reproductive hormones [60].

3. Follicle Stimulating hormone (FSH) of *C. carpio* glass strain

FSH has an important role in the maturation of ovarian follicles in females and spermatogenesis in males, with secretion strictly regulated by many factors, one of which is season [61; 62]. The results of the research conducted showed that the *C. carpio* glass strain reared in different seasons showed different levels of FSH hormone. The results showed the highest male fish FSH hormone during the rainy season (3.73 ng/mL), while during the dry season, the highest hormone (1.06 ng/mL) (Figure 3). Meanwhile, in female fish, the highest FSH hormone during the rainy season is 12.10 ng/mL) while during the dry season, the highest hormone (9.36 ng/mL) (Figure 4). The rainy season is known as the peak spawning season in *C. carpio* [21]. Research on *C. carpio* [63] and *Carassius auratus* [64], FSH mRNA expression also showed a marked increase during the spawning season. FSH functions to support the process of androgen-dependent spermatogenesis through interaction with Sertoli cells to support germ cell development [34]. FSH in Oncorhynchus mykiss ovaries is also known to increase during the rainy season [65].

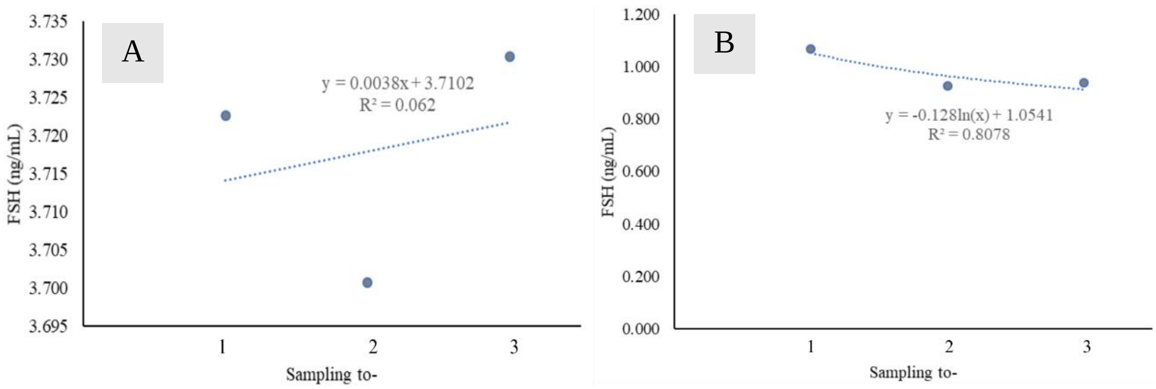

Figure 3. FSH hormone in *C. carpio* male glass strain. a) Rainy season. b) Dry season.

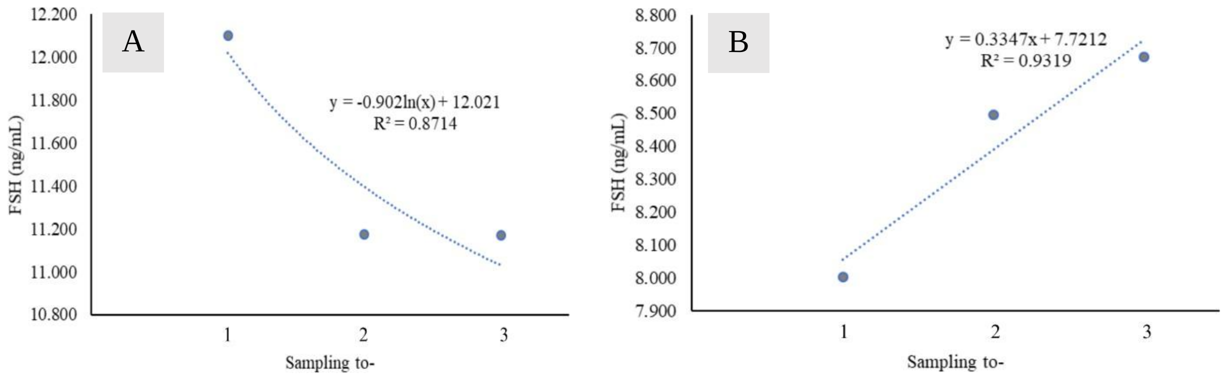

Figure 4. FSH hormone in female *C. carpio* glass strain. a) Wet season. b) dry season.

FSH has a key role in this process by stimulating ovarian follicle maturation in females and egg production. During the rainy season, increased FSH levels will encourage the ovaries to produce estrogen, which in turn supports egg development [66; 67]. The decrease in FSH hormone in the dry season is due to the decrease in gonadotropin levels in circulation. This decrease is often influenced by changes in habitat conditions that are less supportive of the reproductive process. Research conducted by Susatyo [68] on Javanese barb fish showed that hormonally triggered gonad development is strongly influenced by environmental signals that are less supportive during the dry season. In the dry season, gonadotropin inhibitory hormone (GnIH) suppresses the release of FSH and LH. In addition, the expression of the GnRH hormone can also be reduced in response to unfavourable environmental conditions.

In male fish, FSH levels tend to be lower because the reproductive process is more dependent on Luteinizing Hormone (LH) and androgens, which play an important role in spermatogenesis [69; 70]. Studies have also shown that female fish have significantly higher FSH levels during the vitellogenesis phase (Figures 1 and 2), which is the phase in which oocytes undergo yolk accumulation. This phase is controlled by estrogen, which stimulates FSH secretion and promotes ovarian follicle development [71]. The estrogen-sensitive period is crucial for normal ovarian development. Disruption of this period can lead to abnormalities in reproductive function or even sex reversal [72]. The hormonal interactions are complex; FSH plays a major role in the early stages of oocyte maturation, while LH becomes more dominant in the late stages. This indicates a shift in the role of hormones as the fish reproductive cycle progresses [73]. The expression of sex hormone receptors differs between males and females, affecting the response of gonadotropic cells to circulating hormones. In female fish, FSH receptor expression is generally higher, which is in line with the need for FSH levels to support ovarian function [74]. The expression of these receptors not only equates to the action of FSH but also reflects the influence of genetic and environmental factors that determine the sexual differentiation and reproductive strategies of fish [75].

4. Luteinizing hormone (LH) in *C. carpio* glass strain

The results of the research conducted also show that the LH hormone is higher in the rainy season compared to the dry season. The highest LH hormone in *C. carpio* female glass strain reached 12.48 MIU/ml, while during the rainy season, the highest LH hormone reached 9.36 MIU/ml (Figure 5).

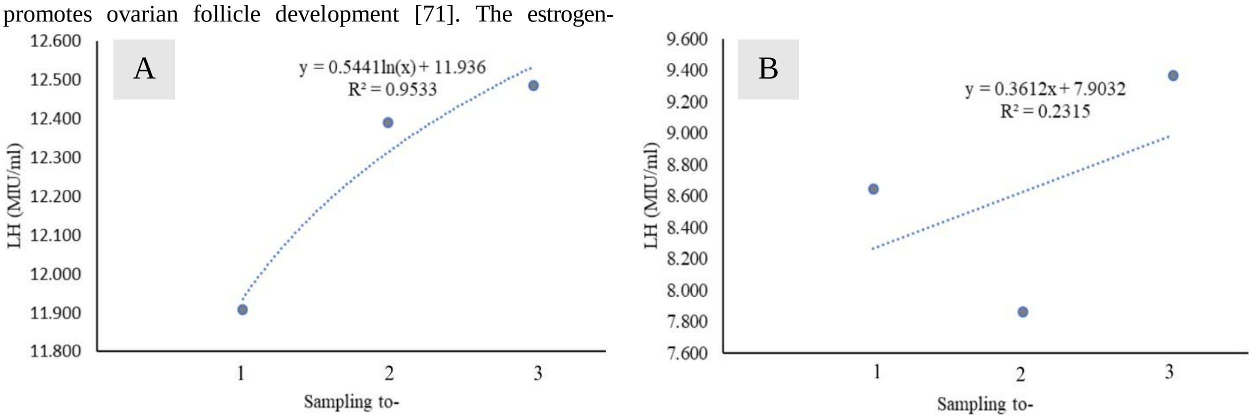

Figure 5. LH hormone in glass strain female *C. carpio*. a) Wet season. b) dry season.

Luteinizing hormone (LH) plays a crucial role in the reproductive physiology of female fish, especially in the process of oocyte maturation and ovulation. LH functions in the final stages of oocyte maturation as well as inducing ovulation. The presence of LH is essential for ovulation because this hormone triggers the synthesis of matrix metalloproteinase 15 (Mmp15), which plays an important role in follicular rupture during ovulation [76]. This confirms that LH is not only responsible for triggering ovulation but also supports the biochemical processes required for the release of mature oocytes. LH deficiency is known to cause infertility [77]. The study conducted also showed that the LH hormone was highest during the rainy season (Figure 3). LH secretion follows a temporal pattern throughout the reproductive cycle, with the peak occurring during the final maturation of oocytes [36]. The process of LH release can be influenced by neurotransmitters such as dopamine and vasoactive intestinal peptide (VIP), which act on the pituitary gland to regulate the secretion of this hormone [78; 79].

Research conducted by Nelson [80] and Ma [81] also showed that the LH hormone in *C. carpio* reaches its peak during the spawning period, which usually coincides with the rainy season. In the rainy season, the inhibitory activity of the GnIH tends to decrease, allowing increased production of LH required for reproductive activity [82; 83]. The balance between hormone stimulatory and inhibitory signals is critical, with the rainy season often providing environmental conditions that favour the dominance of reproductive hormones [84]. Shawky [38] also showed that during the rainy season, fish show increased levels of circulating sex steroids, which are known to stimulate LH secretion. Research conducted on tilapia also showed a significant increase in LH hormone during the breeding season [38]. This shows a close relationship between increased gonadal activity and the maturation process. Hosseinzadeh [22] also showed Indian *C. carpio*, seasonal fluctuations in sex steroid hormones are influenced by environmental factors, including temperature and photoperiod. Nakane [85] also showed that the saccus vasculitis in fish functions as a sensor for seasonal changes in day length, which affects the production of reproductive hormones such as FSH and LH during important phases of gonad development.

5. Testosterone and estrogen hormones in *C. carpio* glass strain

The results showed that season also affects testosterone and estrogen hormones in *C. carpio* strain glass. The highest testosterone hormone during the rainy season reached 9.27 ng/mL, while during the dry season, the highest testosterone hormone reached 8.81 ng/mL (Figure 6). Meanwhile, the highest estrogen hormone was also seen in the wet season (21.84 ng/mL), while the highest dry season reached 20.69 ng/mL (Figure 7).

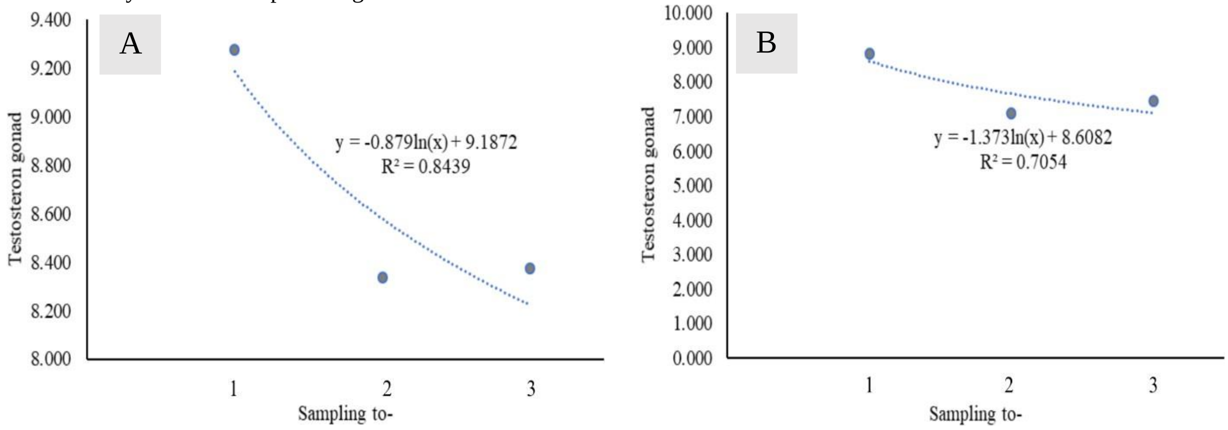

Figure 6. Testosterone hormone in female C. carpio glass strain. a) Rainy season. b) dry season.

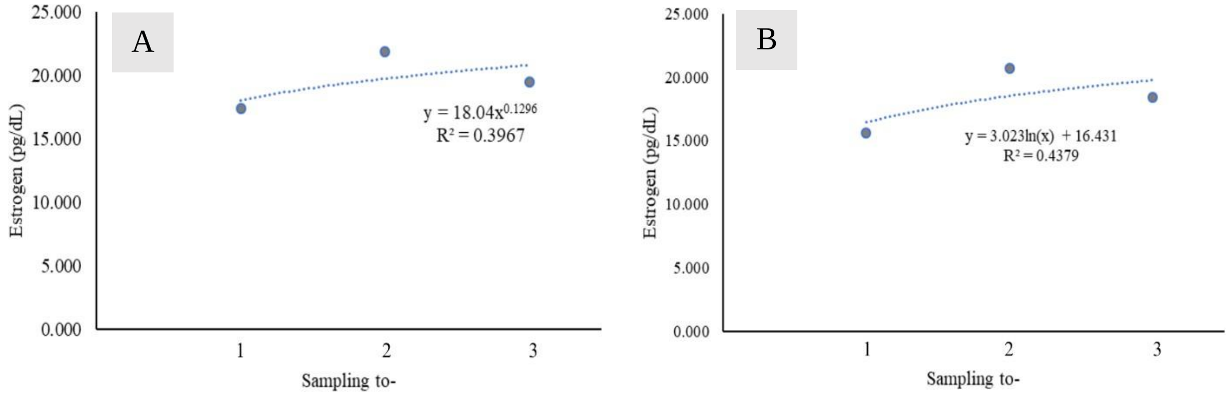

Figure 7. Esterogen hormone in glass strain female *C. carpio*. a) Wet season. b) dry season.

Research on tilapia has also shown that reproductive hormones, including testosterone, increase during the spawning season. This increase is closely related to changes in body parameters and leukocyte counts [38]. Testosterone production physiologically involves the role of various key proteins and enzymes, such as StAR (Steroidogenic Acute Regulatory protein), P450scc (cholesterol side-chain cleavage enzyme), and 3β-HSD (3β-hydroxysteroid dehydrogenase), which play an important role in the process of steroidogenesis in Leydig cells [86]. Regulation of these proteins is key to ensuring testosterone is produced at the right time, with peaks occurring during the spawning season, to support reproductive behaviour and physiological changes required for successful spawning [38]. There are also interactions between testosterone and other hormones, such as cortisol. Elevated levels of testosterone often coincide with high levels of cortisol, likely in response to the stress of mate competition and territory establishment during the breeding season [87]. This suggests that testosterone not only serves to drive reproductive behaviour but also helps prepare fish for energy demands during the spawning process. The expression of androgen receptors also increases as testosterone increases, which in turn affects physiology and behaviour [88]. These receptor-mediated effects are crucial in the emergence of secondary sexual characteristics and aggressive behaviour often seen in male fish during the breeding season [89].

IV. CONCLUSION

The highest reproductive hormones of *C. carpio* strain glass were produced during the rainy season. The highest hormones were successive: ICSH hormone (12.82 pg/mL), FSH hormone (male: 3.73 ng/mL; female: 12.10 ng/mL), LH hormone (12.48 MIU/ml), testosterone hormone (9.27 ng/mL) and estrogen hormone (21.84 ng/mL). During the rainy season, changes in the environment and water flow patterns are detected by the fish's sensory system. This information is then sent to the brain, specifically to the hypothalamus area. The hypothalamus responds to these environmental stimuli by releasing gonadotropin-releasing hormone (GnRH), which in turn triggers the pituitary gland to secrete gonadotropin hormones. Further research is needed to observe the growth and abnormalities of fish larvae produced in different seasons.

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