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Seasonal reproductive dynamics in common carp (*Cyprinus carpio*): Variations in gonadosomatic index and hormonal profiles

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Abstract

This study investigated the seasonal reproductive cycle and hormonal dynamics in common carp (*Cyprinus carpio* var. *communis*), emphasising variations in the gonadosomatic index (GSI) and gonadotropin hormones (GtH-I and GtH-II). GSI remained low from late summer to December and peaked in May, reaching 9.5±0.46 in males and 16.3±0.12 in females. GtH-I levels showed minimal fluctuations throughout the year and had no significant correlation with GSI. In contrast, GtH-II exhibited marked seasonal variations, peaking in May (1.26±0.03 IU l^{-1} in females and 0.36±0.15 IU l^{-1} in males), with a strong positive correlation with GSI (females: r = 0.91, males: r = 0.84). These findings highlight the pivotal role of GtH-II in regulating gonadal development and reproductive timing in common carp, providing insights into its endocrine control mechanisms.

Introduction

Environmental conditions are crucial for fish reproduction and the survival of offsprings. Seasonal cues initiate the maturation process long before spawning, often taking up to a year. Environmental stimuli signal optimal conditions for fry development, triggering ovulation and spawning once the gametes have matured. Various sensory receptors, including the eye, pineal gland, olfactory organs, taste buds, and thermoreceptors, help fish detect these cues (Sabet *et al*., 2009; Falcon and Munoz-Cueto, 2024). The correlation between gonadal development and plasma levels of gonadotropins and gonadal steroids is a valuable tool for understanding endocrine control in teleost reproduction. In teleost fishes, vitellogenesis and final oocyte maturation are regulated by gonadotropins through steroids secreted by granulosa and theca cells in developing and mature oocytes. Steroid production varies across ovarian cell types during different oocyte development stages. The 17-β estradiol (E2) stimulates hepatic synthesis and secretion of vitellogenin, which accumulates in the oocytes.

The relationship between gonadotropins and sexual maturity has been extensively studied in fishes. Pituitary gonadotropic potency increases during gonadal recrudescence, peaking at reproductive maturity (Schulz *et al*., 2001; Kumar *et al*., 2021). Follicle-stimulating hormone (FSH, homologous to GTH-I) and luteinising hormone (LH, homologous to GTH-II) are two different pituitary gonadotropins that orchestrate gametogenesis, steroidogenesis, and final maturation in teleosts *via* the hypothalamic-pituitary-gonadal (HPG) axis (Aizen *et al.,* 2017; Hollander-Cohen *et al.,* 2018; Santhakumar *et al.,* 2024). While LH is essential for final egg maturation, ovulation, and spermiation, FSH is mostly involved in early gonadal development and gametogenesis. Both hormones typically increase as reproductive maturity approaches. Through neuroendocrine pathways, photoperiodic information and environmental cues control gonadotropin synthesis and secretion, integrating signals that eventually promote sexual development (Santhakumar *et al.,* 2024; Uehara *et al.,* 2024).

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The observed differences in hormone profiles between species and reproductive phases may be explained by complicated regulation of gonadotropin release, including differential neurohormonal control mechanisms beyond standard GnRH stimulation, according to recent research (Uehara *et al.,* 2024). Although patterns can differ by species, environmental context, and assay specificity, plasma gonadotropin levels generally increase with increasing gonadal maturity and frequently peak around spermiation or ovulation in many teleost species.

One of the most biologically dynamic freshwater systems in the north-western Himalaya is Dal Lake (Kashmir Himalaya), a sizable, shallow, temperate lacustrine ecosystem located at an elevation of around 1,580 m above mean sea level. With water temperatures varying from almost freezing in the winter to over 20–25°C in the summer and significant seasonal changes in photoperiod, the lake's environmental circumstances exhibit significant seasonal variation. Reproductive activity is limited to a very brief spawning window due to delayed gonadal recrudescence caused by prolonged winter cold and ice cover during peak winter months. This is followed by rapid physiological activation during spring warming. Tropical and subtropical carp habitats, where heat regimes are rather constant throughout the year, stand in stark contrast to these conditions. The susceptibility of carp reproductive physiology to temperate climatic regimes has been highlighted by earlier research on common carp from Kashmir waterways, which showed clear seasonal variations in gonadal development and reproductive timing (Mohamad *et al*., 2018, 2024). Dal Lake thus offers a natural and ecologically significant environment for studying the gonadal dynamics and seasonal endocrine regulation of the common carp, *Cyprinus carpio* var. *communis*, under intense climate seasonality.

Understanding reproductive endocrinology is essential for successful breeding, providing a scientific basis for influencing maturation and reproduction. However, limited research exists on the reproductive endocrinology of common carp. This study aims to examine seasonal variations in gonadotropin hormone levels in plasma and gonads in relation to gonadal development over a year. Despite extensive research on the reproductive biology of *C. carpio*, the majority of available studies have been conducted under tropical or subtropical conditions or within controlled laboratory and hatchery environments. In contrast, systematic year-round investigations integrating gonadosomatic index with seasonal plasma gonadotropin (GtH-I and GtH-II) dynamics under natural temperate Himalayan conditions remain scarce, particularly for high-altitude lacustrine ecosystems such as Dal Lake, Kashmir. Furthermore, comparative endocrine profiling of both male and female carp populations from a natural lake system across an annual reproductive cycle is limited, restricting region-specific understanding of reproductive timing and hormonal regulation in temperate environments.

Materials and methods

Fish collection and maintenance

Common carp (*C. carpio* var. *communis*), with an average length of 208.23±33.34 mm, were collected monthly from Dal Lake over a 12-month period. A total of 30 adult fish were sampled per month, throughout the annual cycle. Both male and female fish were included in the study, and sex was determined by gonadal examination following dissection. Each month, n=15 males and n=15 females were analysed. Gonadosomatic index (GSI) and plasma gonadotropin (GtH-I and GtH-II) levels were assessed separately for males and females to capture sex-specific seasonal reproductive and endocrine dynamics. The fish were acclimatised at the aquarium station for three days before further processing. To minimise stress, fish were anesthetised by immersion in ice-cold water. After sedation, total length and body weight were measured, and blood and gonadal samples were collected for hormonal analysis.

Blood sampling and plasma separation

Blood samples (0.5–1 ml) were drawn from the caudal artery of each female using heparinised syringes. The collected blood was immediately transferred to chilled tubes to prevent coagulation. Plasma separation was performed by centrifugation at 4°C for 15 min at 1000 rpm. The separated plasma was aliquoted and stored at −35°C until further analysis.

Hormonal analysis

The plasma concentrations of gonadotropin hormones (GtH-I and GtH-II) were determined using the radioimmunoassay (RIA) technique, following the protocol described by Goos *et al.,* 1986. This method utilises radioactively labeled hormones to precisely quantify specific gonadotropin levels in plasma samples. Plasma GtH-I concentrations were quantified using a validated immunoassay. Samples that produced values below the assay detection limit were noted as non-detectable, indicating quantities below the method's sensitivity threshold rather than the hormone's total absence. Standard curves were generated for each assay run to ensure accuracy, and all samples were analysed in duplicate to minimise variability. The assay was conducted under controlled laboratory conditions to maintain reproducibility.

Statistical analysis

All data were analysed using appropriate statistical software. Descriptive statistics were computed, and one-way ANOVA was performed to compare gonadotropin levels across different months. Data were examined for normality (Shapiro–Wilk test) and homogeneity of variance using Chi square test prior to ANOVA. When data satisfied parametric assumptions, differences among months were analysed using one-way ANOVA, followed by appropriate *post-hoc* comparisons**.** When assumptions of normality and/or homoscedasticity were violated, data were analysed using non-parametric tests, and Dunn's *post-hoc* test was applied for multiple comparisons. Statistical significance was accepted at $p<0.05$. Pearson's correlation analysis was conducted to assess the relationship between gonadotropin levels and gonadosomatic index (GSI). Results were expressed as mean±standard error (SE).

Results

Annual cyclical changes

Gonadal development

Seasonal reproductive development in both sexes of common carp (*C. carpio*) commences in winter (December–February), in preparation for the peak spawning period in spring. Specimens were collected throughout the year, providing a comprehensive dataset on gonadal development (Table 1; Fig. 1, 2). The GSI remained low from late summer (August–December) and progressively increased, reaching its peak in May. A sharp post-spawning decline was observed in June and July for both sexes. The highest GSI values were recorded in May, averaging 9.5±0.46 (mean±SD) in males and 16.3±0.12 in females. In females, the mean GSI ranged from 2.5±0.73 (June) to 16.3±0.12 (May), whereas in males, it varied from 3.5±4.01 (June) to 9.5±0.46 (May).

GtH-I (Gonadotropin-I)

The annual hormonal profile revealed no significant fluctuations in GtH-I levels across the year in both sexes (Table 2; Fig. 3, 4). Instead of actual hormonal absence, the occurrence of undetectable GtH-I levels during particular months most likely reflects extremely

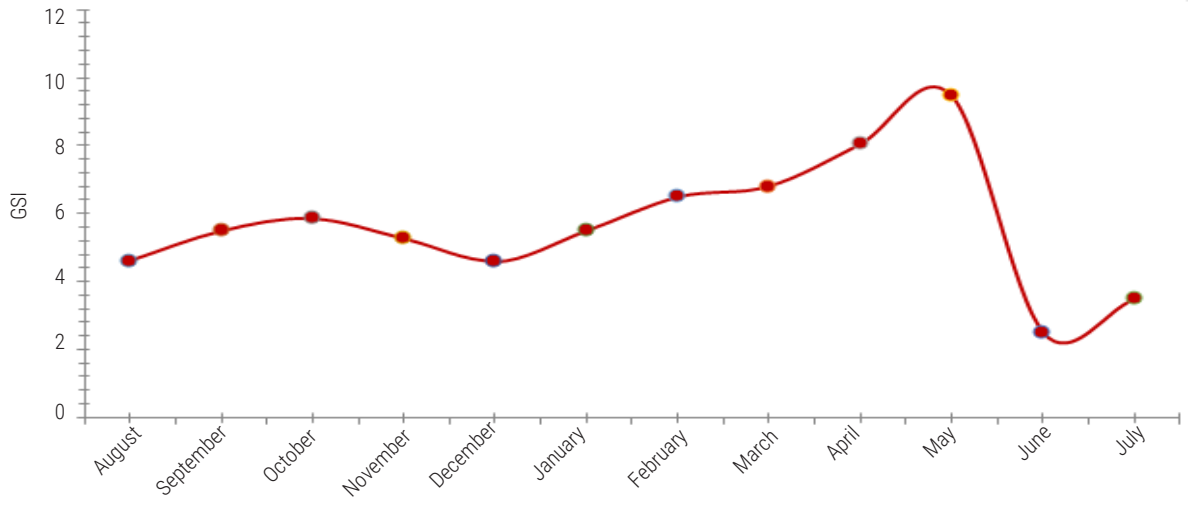

Fig. 1. Annual variation in the GSI in male *C. carpio*

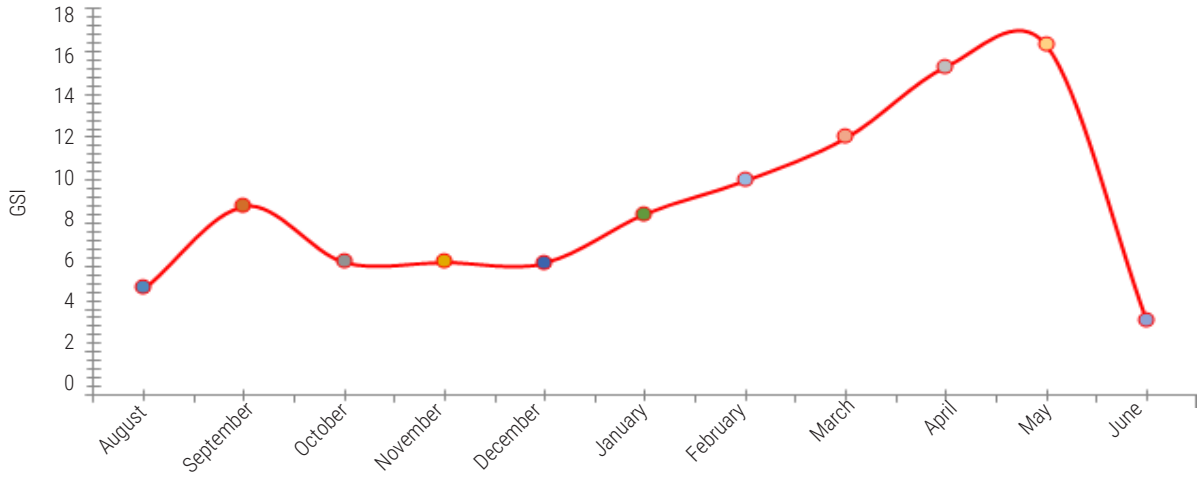

Fig. 2. Annual variation in the GSI in female *C. carpio*

Table 1. Monthly variation in GSI of female and male *C. carpio* var. *communis*

Month	Females (Mean±SD)	Males (Mean±SD)
August	5.00 ± 2.30	4.60 ± 0.22
September	8.80 ± 2.15	5.50 ± 1.90
October	6.20 ± 0.85	5.87 ± 0.31
November	6.20 ± 0.22	5.28 ± 0.98
December	6.15 ± 0.77	4.59 ± 0.10
January	8.44 ± 1.51	5.50 ± 0.06
February	10.00 ± 7.40	6.50 ± 0.14
March	12.00 ± 0.35	6.80 ± 0.52
April	15.30 ± 0.94 **	8.05 ± 0.41 *
May	16.30 ± 0.12 **	9.50 ± 0.46 **
June	3.50 ± 4.01	2.50 ± 0.73
July	3.66 ± 2.77	3.48 ± 2.31

Values are expressed as Mean±SD. Statistical significance is indicated as **p<0.01 (highly significant, marked as**), and *p<0.05 (significant, marked as*).

Table 2. Annual profile of serum gonadotropin GtH-I in female and male *C. carpio*

Month	Gonadotropin GtH-I (Mean±SD) (Female)	Gonadotropin GtH-I (Mean±SD) (Male)
October	Non-Detectable	0.02 ± 0.01^{b}
November	Non-Detectable	0.0003 ± 0.0005^{b}
December	Non-Detectable	0.11 ± 0.01^{b}
January	0.035 ± 0.02^{a}	0.0003 ± 0.0005^{b}
February	0.0005 ± 0.0007^{a}	0.0003 ± 0.0005^{b}
March	0.02 ± 0.02^{a}	0.0005 ± 0.0007^{b}
April	0.50 ± 0.09^{a}	0.19 ± 0.15^{b}
May	0.05 ± 0.02^{a}	0.005 ± 0.005^{b}
June	0.00033 ± 0.00058^{a}	0.146 ± 0.02^{b}
July	0.243 ± 0.23^{a}	0.125 ± 0.09^{b}
August	0.30 ± 0.02^{a}	0.02 ± 0.02^{b}
September	0.44 ± 0.01^{a}	0.013 ± 0.015^{b}

Values are expressed as Mean±SD. "Non-detectable" refers to undetectable levels of GtH-I in the serum. Superscripts (^{a}, ^{b}) indicate statistically significant differences between females and males for each month.

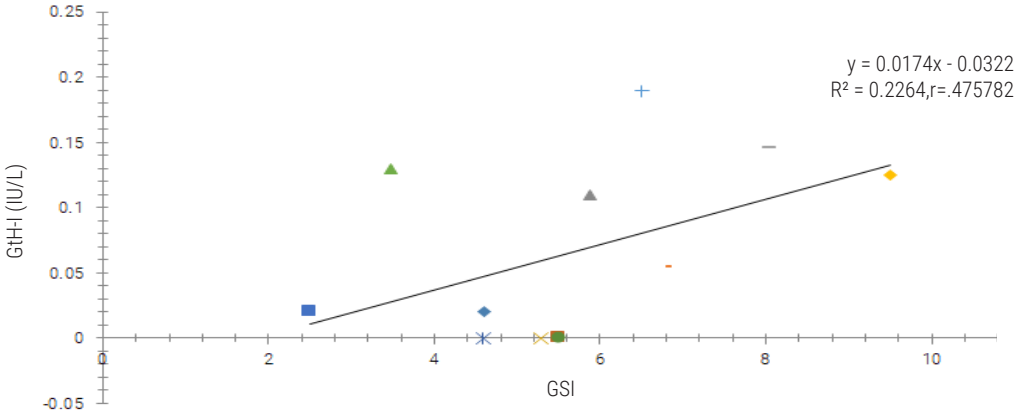

Fig. 3. Relationship between serum GTH-I levels and male GSI's of *C. carpio*

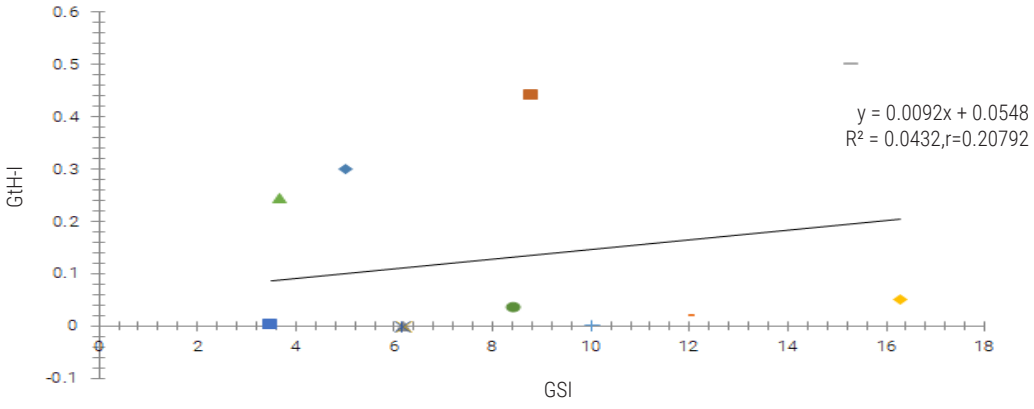

Fig. 4. Relationship between serum GTHI levels and female GSI's of *C. carpio*

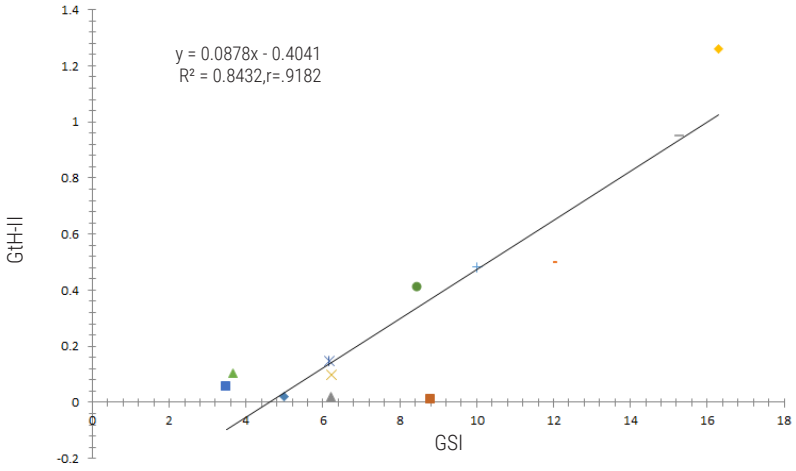

Fig. 5. Relationship between serum GtH-II level and female GSI of *C. carpio*

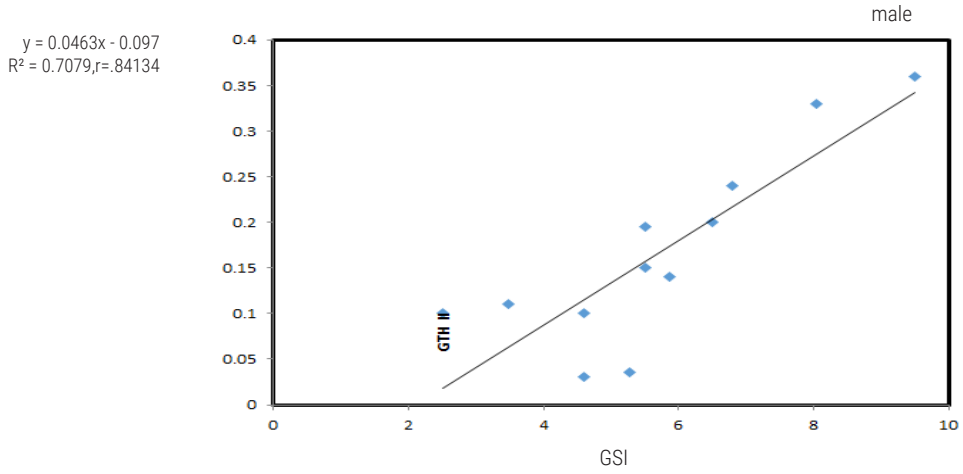

Fig. 6. Relationship between serum GTH-II levels and male GSI of *C. carpio*

low circulating concentrations during reproductive quiescence stages, falling below assay sensitivity. GtH-I levels remained consistently low, with undetectable concentrations in certain months, particularly in females. In males, GtH-I levels ranged from 0.0003±0.0005 IU l^{-1} (September–December) to a maximum of 0.19±0.15 IU l^{-1} (February). In females, values fluctuated between 0.0003±0.0005 IU l^{-1} (June) and 0.5±0.09 IU l^{-1} (April). Statistical analysis showed no significant correlation between GSI and GtH-I in either sex (females: r = 0.20, p > 0.05; males: r = 0.48, p>0.05) (Table 3; Fig. 4, 5).

GtH-II (Gonadotropin-II)

The serum concentrations of GtH-II exhibited pronounced seasonal fluctuations in both sexes (Table 3; Fig. 5, 6). GtH-II levels were lowest from late summer (August–November) but began rising

Table 3. Monthly variation in GtH-II levels of *C. carpio* var. *communis*

Month	GtH-II (Mean±SD) (Male)	GtH-II (Mean±SD) (Female)
August	0.10 ± 0.02^{a}	0.02^{b}
September	0.15 ± 0.0005^{b}	0.01^{ab}
October	0.14 ± 0.01^{ab}	0.022^{bc}
November	0.036 ± 0.005^{c}	0.10^{a}
December	0.03 ± 0.05^{c}	0.15^{d}
January	0.195 ± 0.007^{bc}	0.44^{e}
February	0.20 ± 0.007^{d}	0.485^{f}
March	0.24 ± 0.007^{bd}	0.50^{be}
April	0.33 ± 0.179^{bcd}	0.95^{bcd}
May	0.36 ± 0.153^{e}	1.26^{ef}
June	0.10 ± 0.12^{f}	0.055^{cd}
July	0.11 ± 0.08^{ef}	0.011^{c}

Values are expressed as Mean±SD. Different superscript letters (^{a}, ^{b}, ^{c}, *etc.*) indicate statistically significant differences between months.

from December onwards, peaking in May. The highest recorded levels were 1.26±0.03 IU l^{-1} in females and 0.36±0.15 IU l^{-1} in males, followed by a significant decline post-spawning.

A strong positive correlation was observed between GSI and GtH-II levels in both sexes. Fig. 5 highlight a significant correlation between GSI and GtH-II in females ($r=0.91$, $p<0.01$) and in males ($r=0.84$, $p<0.01$) (Fig. 6) , underscoring the critical role of GtH-II in reproductive maturation and spawning in common carp. These findings suggest that while GtH-I plays a minimal role in gonadal development, GtH-II is the primary regulator of reproductive activity, closely mirroring changes in GSI and spawning behaviour.

Discussion

The reproductive cycle of common carp (*C. carpio*) under temperate conditions in Kashmir follows a distinct seasonal pattern. Although gonadal maturation commences at the onset of winter, severe cold conditions induce gonadal diapause until mid-February. The highest gonadosomatic index (GSI) and peak spawning activity occur in spring (May), corroborating previous studies that report the breeding season extending from April to June (Mohamad *et al*., 2018).

Gonadotropin I (GtH-I) is primarily involved in vitellogenesis, while Gonadotropin II (GtH-II) regulates follicular oocyte maturation (FOM) (Levavi-Sivan *et al*., 2010). The principal function attributed to GtH-I is the uptake of vitellogenin by oocytes (Tyler *et al*., 1997). Annual profiling revealed consistently low and, at times, undetectable GtH-I levels, particularly in females. In males, GtH-I levels ranged from 0.000333±0.0005 IU l^{-1} (September, November, December) to 0.19±0.155 IU l^{-1} (February), whereas in females, values fluctuated between 0.000333±0.0005 IU l^{-1} (June) and 0.5 ± 0.09 IU l^{-1} (April). This pattern aligns with findings in common carp (Hollander-Cohen *et al*., 2017) and sea bream (*Sparus aurata*) (Gen *et al*., 2003). The lack of significant variation in GtH-I levels may be attributed to its shorter synthesis and secretion cycle compared to GtH-II (Hollander-Cohen *et al.,* 2018). In zebrafish (*Danio rerio*), follicle-stimulating hormone (FSH) exhibits a brief rise during the ovulatory cycle (So *et al.,* 2005). In contrast, GtH-II levels demonstrated distinct seasonal fluctuations. From August to November, levels remained low but gradually increased from December to March, peaking in April and May with the presence of fully developed follicles. This trend is consistent with previous studies, which suggest that vitellogenesis begins in late summer, with GtH-II reaching its maximum before spawning in April and May (Yaron and Zermonsky, 1986). A resting ovarian phase is observed during June and July.

A strong correlation between GtH-II levels and gonadal development was evident, with GtH-II rising in tandem with GSI, reaching peak concentrations during the spawning season. Unlike GtH-II, GtH-I exhibited no significant relationship with GSI, reinforcing its limited role in gonadal maturation. The current study is observational in nature and does not involve experimental endocrine manipulation, therefore even though GtH-II levels clearly exhibited a seasonal correlation with advanced gonadal maturation and spawning stages, these relationships should be considered as correlative rather than causative. Other temperate carp populations have shown similar seasonal patterns of higher GtH-II throughout reproductive maturity, which supports the hormone's function as an indication of reproductive preparation rather than direct causation. These results align with previous data that show pituitary gonadotropic potency peaks during reproductive maturity. In temperate teleosts like rainbow trout, seasonal variations in gonadotropin expression and reproductive gene transcription have been demonstrated to coincide with gonadal development and maturation, linking endocrine activity with advancement through vitellogenesis and spawning stages (Chen *et al*., 2021). In several teleost species, gonadotropin II (LH-like hormone) has been demonstrated to drive steroidogenic pathways and final maturation stages, which is compatible with its seasonal augmentation during late gonadal development phases and spawning (Senthilkumaran and Kar, 2021).

In males, GtH-II levels were lowest during early gonadal growth but increased with GSI, peaking at spermiation. This suggests that higher GtH-II concentrations are necessary for spermiation than for earlier spermatogenic stages (Weil *et al*., 2003; Alavi *et al*., 2012). In females, plasma GtH-II levels increased progressively with ovarian development, peaking at ovulation (Yamazaki, 1965). The proliferation of pituitary gonadotrophs parallels gonadal development (Hossain *et al.,* 2024). However, some studies have noted gonadal development at relatively low gonadotropin levels), possibly compensated by increased gonadal blood flow.

Seasonal fluctuations in GtH levels may be associated with ultrastructural changes in gonadotropin-secreting cells. Elevated GtH-II levels during the breeding season likely result from the accumulation of granules and reduced intracellular matrix substance (IMS) activity, which plays a role in GtH degradation *via* lysosomal enzymes (Peute *et al*., 1987). During the resting phase, GtH-II concentrations decline as gonadotrope cells shrink and IMS activity increases (Van Oordt *et al.,* 1986). Unlike GtH-II, GtH-I demonstrated no correlation with GSI, suggesting that GtH-II alone is sufficient for ovarian activation in common carp (So *et al.,* 2005).

When plotted against GSI, GtH-II exhibited a single peak corresponding to the spawning season. In contrast, studies on salmonids have reported two distinct peaks, one during vitellogenesis and another at ovulation. In the present study, however, GtH-II levels in common carp exhibited a gradual increase, peaking at reproductive maturity and spawning, thereby aligning with the species' reproductive strategy in temperate systems. Seasonal endocrine regulation similar to this pattern has been observed in other teleosts, where reproductive axis–related gene expression rises with late gonadal development and spawning readiness (Palomino *et al.*, 2025). Common carp's reproductive endocrinology varies significantly between tropical and temperate climates. According to Migaud *et al.* (2010) and Zohar *et al.* (2010), carp in tropical and subtropical climates frequently show longer or numerous spawning periods, which are supported by generally stable thermal regimes and extended photoperiods. This leads to less noticeable seasonality in gonadotropin production. In contrast, reproductive activity is strictly limited to a brief seasonal window in temperate Himalayan conditions like those found in Dal Lake, where GtH-II dynamics are closely correlated with

rapid spring warming and increasing photoperiod. In temperate teleosts, including cyprinids, temperature and photoperiod are known to be the main environmental factors controlling pituitary activation and gonadotropin release (Doyle *et al.,* 2021; Falcon and Munoz-Cueto, 2024). These contrasts highlight the strong influence of environmental seasonality on endocrine regulation and underscore the ecological specificity of GtH-II dynamics in temperate carp populations from Kashmir.

This study reveals distinct seasonal patterns in the reproductive cycle of common carp, with peak gonadal development occurring in spring. The strong correlation between GtH-II levels and GSI underscores its pivotal role in regulating gonadal maturation and reproductive timing, whereas GtH-I appears to have a minimal influence. These findings enhance our understanding of the endocrine mechanisms governing seasonal spawning in common carp, offering valuable insights for optimising breeding management strategies in aquaculture. Improved knowledge of these hormonal dynamics may aid in developing more effective reproductive control techniques for carps and related species.

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