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The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(7): 2938-2942

www.thepharmajournal.com
Received: 03-04-2023
Accepted: 07-06-2023

Ningthoukhongjam Soranganba
Department of Fisheries Resource Management, College of Fisheries, G.B.P.U.A & T, Pantnagar, Uttarakhand, India

Corresponding Author:
Ningthoukhongjam Soranganba
Department of Fisheries Resource Management, College of Fisheries, G.B.P.U.A & T, Pantnagar, Uttarakhand, India

Seasonal changes in phospholipid-lipoprotein with relation to reproductive cycle of Amur common carp

Ningthoukhongjam Soranganba

Abstract
Phospholipids play a critical role in fish overall physiology and the role played in reproduction and its seasonal changes in different age groups and sexes have not been reported in common carp. The study was carried out to assess the seasonal change in phospholipid (PHO) lipoprotein concentration in amur common carp during four seasons (spring, summer, autumn and winter) among different age groups and sexes with relationship to breeding behaviour. The assumption on the homogeneity of variances was tested for serum, muscle, gonadal and hepatic PHO was tested and was not satisfy based on Levene's *F* test. ANOVA (2-way) for serum, muscle, gonadal and hepatic PHO level were analysed for different age groups and sexes and observed significant effect among different age and seasons. Similar observations can also be seen between age and seasonal interactions. Similar observation could be seen between all seasons and target samples while conducting Tukey's HSD *post-hoc* test for checking the means differences which correlates with the gonadal development and maturity. The above findings revealed that age and seasons directly correlates with the PHO level which forms an integral component for egg development and thus the gonadal cycle. Judicious selection of matured brood and dietary PHO supplement could help achieve better reproductive operation.

Keywords: Amur common carp, phospholipids (PHO) seasonal reproduction

Introduction
Lipids are stored energy providing components for membrane synthesis and precursors of steroid hormones in substantial quantities in various tissues including muscle, liver and mesenteric depots. Lipids requirements encompassed a gross requirement for energy and more specific requirements for functional lipid classes just like intact phospholipid (NRC, 2011) [19]. Phospholipids are a kind of complex polar lipids which widely existed in organisms, as an indispensable component of lipoprotein and a precursor of the neurotransmitter acetylcholine, it is involved into the regulation of lipid transport (NRC, 2011) [19]. Variation in lipid classes in gonads, muscles and liver of adult fish are directly associated with the sexual maturity and spawning of the fish (Mourente *et al.*, 2017; Huynh *et al.*, 2017) [16, 10]. Beamish *et al.*, (1996) [3] proposed about the assessment of reproductive age in females using body size and accumulation of lipids that provided the energy for reproduction. Biochemical profiles changed in relation to sex and season in *Barilius bendelisis* were reported by Sharma *et al*., (2018) [23] and in common carp by Soranganba and Singh (2018) [26]. Petenuci *et al.*, (2016) [21] showed significant variation in different class lipid based on the season with decreased of saturated fatty acid contents during flooded periods whereas significant increase in polyunsaturated fatty acid contents during the same period in from the Amazon Basin. Other than maintenance, significant quantities of lipids reserved in liver and muscles were mobilised and transferred to gonads, especially ovaries. During maturation and spawning, this lipids are transported through blood serum complexes with specific proteins (apolipoproteins) as particles, known as lipoproteins in striped bass, *Morone saxatilis* (MacFarlane *et al.*, 1996) [15], Atlantic bonito *Sarda sarda* (Zohar *et al.*, 2010) [33], carangids, *Scomberoides lysan* (Sutharshiny and Shivashanthini, 2011) [27] and Nile tilapia *Oreochromis niloticus* (Singh *et al.*, 2012) [24].
Phospholipids are also an important source of energy in freshwater fish and main constituents of biological membranes playing an essential role in the regulation of biophysical properties, protein sorting and cell signaling pathways (Adeyeye and Oyarekua, 2011) [1]. Vitellogenin is a glycolic a phosphoprotein containing about 20% lipid, mainly phospholipid, triglycerides and cholesterol.

Studies have shown about energetic lipids (e.g. triglycerides) mobilization from tissues preferentially to structural lipids (e.g. phospholipids) during starvation (Henderson and Tocher, 1987) [9]. Other than maintenance, significant quantities of lipids reserved in liver and muscles were mobilised and transferred to gonads, especially ovaries. During maturation and spawning, this lipids are transported through blood serum complexes with specific proteins (apolipoproteins) as particles, known as lipoproteins in striped bass, *Morone saxatilis* (MacFarlane *et al.*, 1996) [15], Atlantic bonito *Sarda sarda* (Zoaboukas *et al.*, 2006) [32], carangids, *Scomberoides lysan* (Sutharshiny and Shivashanthini, 2011) [27] and Nile tilapia *Oreochromis niloticus* (Singh *et al.*, 2012) [24]. Yang *et al.*, (2022) [30-31] also reported the importance of dietary maintenance of phospholipids and its subsequent effect on growth performance.

Material and Methods

Tissue samples of muscles, liver and gonads needed an extraction procedure before analysis. The lipid extraction of the target tissues was carried out using modified Folch (1957) [7] and Cequier-Sánchez *et al.*, (2008) [4] method. Mixed the tissue with 10 ml (20 times the tissue volume) of 2:1 ratio dichloromethane and methanol solution and agitated the homogenate for 20 mins using modified digital rocker. Centrifuged the homogenate at 2000 rpm for 10 mins and collected the liquid phase in centrifuged tubes. Washed the solvent with 0.2 volume (2 ml for 10 ml) 0.9% NaCl (sodium chloride) solution (9 gm NaCl in 1000 ml water) and vortexes for some few seconds. After vortex, centrifuged the mixture at low speed of 2000 rpm and separated the two phases. Siphoned off the upper phase and collected the lower dichloromethane containing lipid for analysis.

Estimation

Phospholipids were calculated by the formula given by Covaci *et al.*, (2006) [5] as below:

$$\text{Phospholipid} = \text{Cholesterol} \times 0.73 + 90$$

Statistical Analysis

ANOVA two factors and Correlation analysis - Pearson's correlations using SPSS.

Result and Discussion

Seasonal change in the physiological conditions with significant correlation with GSI in Amur common carp in Tarai region of the Himayalan range was reported by Soranganba (2022) [25].

Table 1: Seasonal Phospholipids (mg/dl) Level of 1+ and 2+ Year's old Amur

Age groups	Sample	Summer season		Autumn season		Winter season		Spring season	
		Male	Female	Male	Female	Male	Female	Male	Female
2+	Muscle	91.56±0.04	91.49±0.10	91.29±0.05	91.20±0.03	91.45±0.04	91.33±0.09	92.05±0.15	91.92±0.10
	Gonadal	97.52±0.11	99.01±0.10	93.74±0.07	94.18±0.10	96.04±0.12	97.01±0.10	100.55±0.13	102.21±0.12
	Hepatic	94.58±0.07	95.08±0.04	99.38±0.13	99.92±0.07	95.31±0.11	96.45±0.11	93.74±0.13	94.14±0.18
	Serum	220.42±1.56	232.46±1.15	201.44±0.33	210.16±0.71	193.78±0.72	214.61±0.82	241.97±1.37	252.09±1.79
1+	Muscle	91.42±0.05	90.77±0.06	91.23±0.04	91.16±0.04	91.35±0.10	91.28±0.11	91.52±0.11	91.36±0.09
	Gonadal	95.06±0.06	95.39±0.07	92.56±0.06	92.87±0.09	93.86±0.04	94.25±0.10	96.27±0.14	96.53±0.14
	Hepatic	92.24±0.03	92.30±0.04	96.83±0.29	96.89±0.26	93.70±0.06	93.57±0.04	91.75±0.08	92.09±0.11
	Serum	180.35±0.52	189.67±0.40	166.28±0.41	176.61±0.71	195.73±0.59	207.91±0.61	212.61±0.88	225.21±0.58

[Data are given as mean±SEM (n=5)]

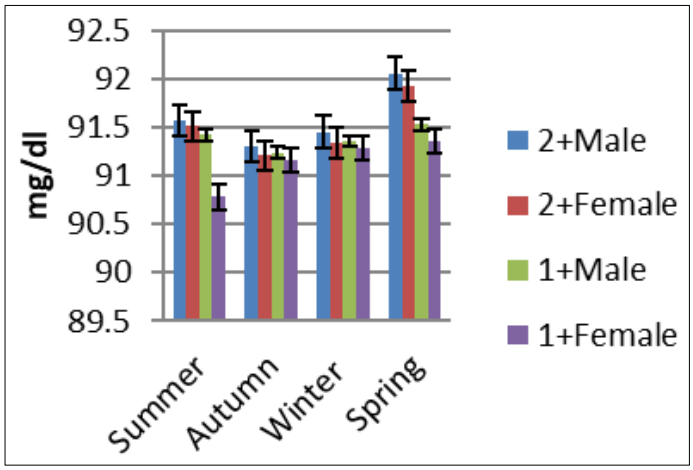

Fig 1: Muscle PHO

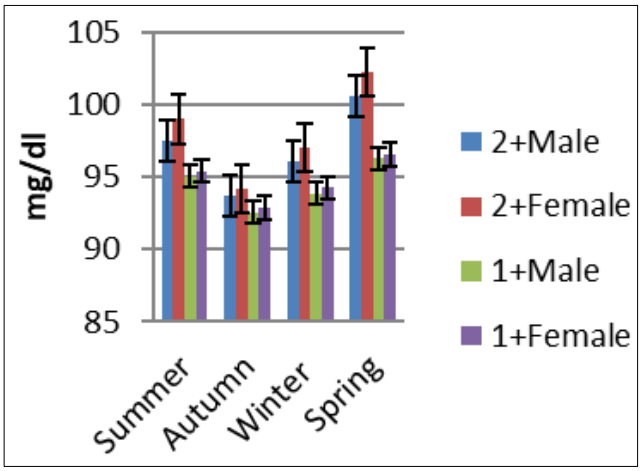

Fig 2: Gonadal PHO

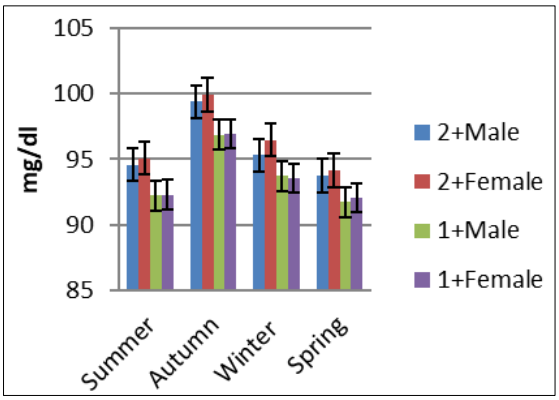

Fig 3: Hepatic PHO

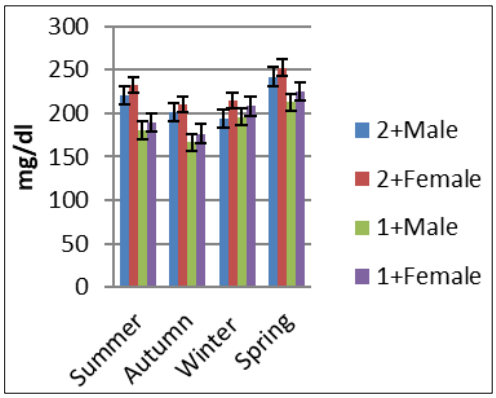

Fig 4: Serum PHO

A descriptive muscle statistics observed across all age groups showed maximum mean PHO level of 91.72±0.08mg/dl during spring followed by winter (91.36±0.04mg/dl), summer (91.32±0.07mg/dl) and autumn (91.23±0.02mg/dl) season respectively. Among the age groups, male 2+ year's group has the highest mean muscle PHO level of 91.59±0.07mg/dl followed by female 2+ (91.49±0.07mg/dl), male 1+ (91.38±0.04mg/dl) and female 1+ (91.15±0.06mg/dl) -year-old groups. Detail in Table and diagrammatically represented in Figure. Seasonal differences might relate to the changes in the muscle PHO level and such changes might differ between age groups and sexes. The assumption on the homogeneity of variances was tested and not satisfy based on Levene's *F* test, F (15, 64) = 2.086, $p<0.05$. ANOVA (two-way) showed age and seasonal changes have a very significant effect with F (3, 80) = 20.161, $p<0.05$, η^2 = 0.486 and F (3, 80) = 25.209, $p<0.05$, η^2 = 0.542 respectively. The interaction between the different age groups and seasonal changes was also highly significant for Amur common carp, F (9, 80) = 4.576, $p<0.05$, η^2 = 0.392. Since age and season has a significant effect on the muscle PHO concentration, a Tukey's HSD *post-hoc* test found that the mean muscle PHO level was not statistically significant between means of spring seasons (91.72±0.08mg/dl) with winter (91.36±0.04mg/dl), summer (91.32±0.07mg/dl) and autumn (91.23±0.02mg/dl) season while the means between the remaining seasons were found to be significant. For age groups, only female 2+ -years-old (91.49±0.07mg/dl) with male 1+ (91.38±0.04mg/dl) and male 2+ (91.59±0.07mg/dl) -years-old were not statistically significant while rest of the means between the age groups were statistically significant ($p<0.05$).

A descriptive gonadal statistics observed across all age groups showed maximum PHO level of 98.89±0.59mg/dl during the spring followed by summer (96.74±0.37mg/dl), winter (95.29±0.29mg/dl) and autumn (93.34±0.15mg/dl) season respectively. Among the age groups, female 2+ year's group has the highest mean gonadal PHO level of 98.1±0.67mg/dl followed by male 2+ (96.96±0.57mg/dl), female 1+ (94.67±0.32mg/dl) and male 1+ (94.44±0.32mg/dl) -year-old groups. Detail showed in Table and diagrammatically represented in Figure. Seasonal differences might relate to the changes in the gonadal PHO level and such changes might differ between age groups and sexes. The assumption on the homogeneity of variances was tested and not satisfy based on Levene's *F* test, F (15, 64) = 1.309, $p<0.05$. ANOVA (two-way) showed age and seasonal changes having a very significant effect with F (3, 80) = 1135.586, $p<0.05$, η^2 = 0.982 and F (3, 80) = 2011.690, $p<0.05$, η^2 = 0.99 respectively. The interaction between the different age groups and seasonal changes was also highly significant with F (9, 80) = 78.523, $p<0.05$, η^2 = 0.917. Since age and season has a significant effect on the gonadal PHO concentration of the species, a Tukey's HSD *post-hoc* test found that the mean gonadal PHO level was statistically significant between all seasons across all age groups ($p<0.05$. There was a highly positive significant correlation between gonadal PHO with gonadal TG.

A descriptive hepatic statistics observed across all age groups showed maximum mean PHO level of 98.26±0.33mg/dl during the autumn followed by winter (94.76±0.28mg/dl), summer (93.56±0.29mg/dl) and spring (92.94±0.24mg/dl) season respectively. Among the age groups, female 2+ year's group has the highest level of 95.76±0.49mg/dl followed by male 2+ (96.4±0.5mg/dl), female 1+ (93.72±0.46mg/dl) and male 1+ (93.63±0.46mg/dl) -year-old groups. Detail showed in Table and diagrammatically represented in Figure. Seasonal differences might relate to the changes in the hepatic PHO level and such changes might differ between age groups and sexes. The assumption on the homogeneity of variances was tested and not satisfy based on Levene's *F* test, F (15, 64) = 5.1, $p<0.05$. ANOVA (two-way) showed age and seasonal changes having a very significant effect with F (3, 80) = 457.135, $p<0.05$, η^2 = 0.995 and F (3, 80) = 1293.836, $p<0.05$, η^2 = 0.984 respectively. The interaction between the different age groups and seasonal changes was also highly significant with F (9, 80) = 3.568, $p<0.05$, η^2 = 0.334. Since age and season has a significant effect on the hepatic PHO concentration, a Tukey's HSD *post-hoc* test found statistically significant means between all the seasons. For age groups, means between female 1+(93.72±0.46mg/dl) and male 1+ year (93.63±0.46mg/dl) old are not significant while the remaining means were statistically significant across all age groups ($p<0.05$).

A descriptive serum statistics observed across all age groups showed maximum mean of 220.11±5.96mg/dl during the spring followed by winter (195.62±3.41mg/dl), summer (193.31±5.74mg/dl) and autumn (182.98±3.93mg/dl) season respectively. Among the age groups, female 2+ year's group has the highest level of 227.06±4.03mg/dl followed by male 2+ (214.09±4.52mg/dl), female 1+ (199.85±4.18mg/dl), male 1+ (188.74±3.97mg/dl) and 0+ (162.99±1.1mg/dl) -year-old groups. Detail showed in Table and diagrammatically represented in Figure. Seasonal differences might relate to the changes in the serum PHO level of the fish and such changes might differ between age groups and sexes. The assumption on the homogeneity of variances was tested and not satisfy

based on Levene's F test, F (19, 78) = 2.897, $p<0.05$. ANOVA (two-way) showed age and seasonal changes having a very significant effect with F (4, 98) = 3334.879, $p<0.05$, η^2 = 0.994 and F (3, 98) = 1687.083, $p<0.05$ η^2 = 0.985 respectively. The interaction between the different age groups and seasonal changes was also highly significant with F (12, 98) = 172.305, $p<0.05$, η^2 = 0.964. Since age and season has a significant effect on the serum PHO concentration of the species, a Tukey's HSD *post-hoc* test found that the mean serum PHO level was statistically significant between seasons across all age groups ($p<0.05$).

Phospholipids are one of the major components of all cell membranes as they can form lipid bilayers. The magnitude of endogenous reserves of specific lipid classes plays a decisive role in the ability to endure energy deficits (Norton *et al.*, 2001) [20]. PHO and CHO were among the principal energy reserves beyond the yolk-sac stage and have been used as a principal indicator of biochemical condition (Doucett *et al.*, 1999) [6]. PHO is the major lipid fraction in the body tissue of fish (Keriko *et al.*, 2010) [12]. Among all the lipid class, PHO was the most dominating and abundant in the present study. Phospholipids are a good source of metabolic energy (Henderson & Tocher, 1987) [9] and essential fatty acids (Fraser *et al.*, 1988; Jhingran, 1991) [8]. The presence of high percentage of PHO was reported in fresh water and marine fish species (Mukhopadhyay *et al.*, 2004; Mukhopadhyay & Ghosh, 2007 [17-18]; including *Cyprinus carpio* (Tapas & Ghosh, 2003) [28]. In the present study, maximum serum PHO level was observed during spring followed by winter, summer and autumn season. Similar observations on seasonal change in PHO levels were reported by Lal & Singh, (1987) [13] in catfish and Lund *et al*., (2000) [14] in striped bass. With the increase in age, the female has higher PHO level in both the groups. The seasonal muscle PHO follows the serum PHO pattern with the male having higher level among the age groups. The gonadal PHO follows the seasonal reproductive cycle of Amur common carp with female dominating the PHO level in both age groups. With significant differences between the seasons across all age groups, the correlations were all positive with GSI. As gonadal tissue requires ample supplies of constituents such as phospholipids for membranes (Aras *et al*., 2008) [2], the observations on gonadal PHO showing positive correlation with GSI was conclusive. Increased in PHO with increased in GSI have been reported by Weigand & Idler (1982) [29]. For hepatic PHO, autumn shows the highest level followed by winter, summer and spring season. Similar low PHO level during spawning, post-spawning and resting phases while sharp increased during the preparatory phase was reported by Lal & Singh, (1987) [13]. Rinchard & Kestemont, (2003) [22] also observed low PHO level during spawning season. Here, females have more PHO concentration in both the age groups. Except for the sex of 1+ year old, significant differences were observed between the age groups among all seasons. Phospholipids required for the vitellogenesis process are known to originate from the liver. The main component of which vitellogenin is composed of content CHO, TG and VLDL. As the liver is the main site for lipid processing, PHO is required for the mobilization of HDL and LDL. Accumulation of PHO during resting phase indicates serious depletion of liver reserves for yolk formation (Rinchard & Kestemont, 2003) [22].

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