



Effect of Seasonal Variations (Breeding and Non-breeding Seasons) on Productive Performance and Reproductive Hormonal Profile in Nile Tilapia (Monosex and Mixed Sex)

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Authors' contributions

This work was carried out in collaboration between all authors. Author SMS designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors SIF and ISA managed the analyses of the study. Authors SMS and ISA managed the practical work and the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Nile tilapia is one of the most widespread fish in Egypt and it is of great economic importance. This study aimed to know the effect of seasonal variations on the body, level of hormones and blood parameters. To conduct this study, Nile tilapia monosex and mixed sex were collected during breeding and nonbreeding seasons. Results revealed that seasonal variations on body parameters are more prominent in body weight, abdominal length, abdominal width, and abdominal circumference especially in female fishes. Reproductive hormones (FSH, LH, prolactin, testosterone, and Estradiol E2) are significantly increased during breeding season. The Erythrogram, total and differential leukocytic count increased significantly during breeding season, while thrombocytes are significantly declined during breeding season. The significant increase of Hepato-somatic_index % and Gonado-somatic index % can be an indicator of breeding season in some fresh-water fish.

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1. INTRODUCTION

The Nile tilapia (*Oreochromis niloticus*) is the most widely cultured tilapia in the world due to its rapid growth, late age of sexual maturity and feeding habits [1].

Methods of reproducing Nile tilapia species are well-reported [2]. In Egyptian Delta, spawning of Tilapia may extend from April to November with a maximum spawning from April to May in early summer [3].

Nile tilapia can spawn three times in the year and therefore three offspring produced in the same season [4]. Rising temperature to 20°C or higher, leads to the spawning operations of Tilapia, which start in April or May [5]. Number of eggs per spawn differs among species, but within same species the number of eggs increased with increasing weight of female [6].

The aquaculture of Nile tilapia (*Oreochromis niloticus*) come back to Antiquated Egypt and it is a standout amongst the most broadly refined freshwater angle overall these days. It was brought into numerous mild, tropical and subtropical locales of the world because of its heavenly meat and high-showcase esteem [7].

The endocrine control of reproduction of both female and male fish works in such a way that the signals from exogenous factors and endogenous physiological factors stimulate the neuroendocrine system, which in turn regulates pituitary and gonadal functions [8].

The hypothalamus-pituitary-gonadal axis is composed of three physiologically connected structures directly involved in the control of reproduction in vertebrates, at all levels of this axis there are a number of key hormones being synthesized and released which act on target tissues within the brain, the liver or the gonad, which has been reviewed in fish [9].

Both sex steroids and growth factors are crucial for the regulation of reproduction, either directly in the gonad (paracrine, autocrine) or through positive and negative feedback mechanisms on the hypothalamus and pituitary [10]. There are some important factors that regulate the reproductive cycles

of bony fishes like salinity [11], seasonal rainfall [12], photoperiod [13], water quality, stocking densities and stress level [14] and temperature [15].

Hematological profile is an important tool which can be utilized for powerful and touchy checking of physiological and obsessive condition of a fish [16]. As well as, blood parameters such as Hematocrit, hemoglobin and red blood corpuscles (RBCs) count are related to environmental factors such as water temperature and salinity [17].

The physiological reaction to natural varieties, for example, saltiness, temperature and broke up oxygen changes in amphibian framework has been researched in fresh and marine water species [18].

The aim of the present study was to assess the effect of seasonal variations (breeding and non-breeding seasons) on some parameters in monosex and mixed sex Nile tilapia (*Oreochromis niloticus*) as Body parameters (body weight, total body length, standard body length, abdominal length, abdominal width, and abdominal circumference); Reproductive hormones (FSH, LH, prolactin, testosterone, and Estradiol E₂); Erythrogram (PCV, Hb, RBCs, WBCs, thrombocytes, MCV, MCH, and MCHC); Leucogram (Total WBCs count and Differential leucocytic count); Thrombocytes (blood platelets) count.

2. MATERIALS AND METHODS

2.1 Materials

All the chemicals used in this experiment were obtained from El Gomhoria Company, Cairo, Egypt, unless otherwise mentioned.

2.2 Source of Fish

The Monosex Nile tilapia (*Oreochromis niloticus*) obtained from the fish farms, Kafr El-sheikh governorate, Egypt. The Mixed sex Nile tilapia (*Oreochromis niloticus*) fish were freshly caught by fish hunter from Ganzour village, Berket El-Sabaa, Menoufia governorate, Egypt.

All fishes have nearly the same mean total and standard body length during the breeding and non-breeding season. All fishes are transported alive in transporting tank which was supplied with natural oxygen to Department of Physiology, Faculty of Veterinary Medicine, University of Sadat city, Menoufia, Egypt.

2.3 Experimental Design

About 240 sexually mature male and female fish (Monosex Nile tilapia and mixed sex Nile tilapia)

depending on their body length as the main determinant of sexual maturity were used in this study according to Hanson et al. [19]; Schulz and Goos [20].

The fishes which collected from the fish farms and by natural catch during the non-breeding season (January – February – March) and the breeding season (April – May – June) were distributed throughout the experimental period as shown in the following Table.

The experimental design during non-breeding and breeding seasons

Season	Non-breeding						Breeding						
	January		February		March		April		May		June		Mean total length (cm)
Month	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	
Sex	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	
Monosex Nile tilapia	20	-	20	-	20	-	20	-	20	-	20	-	
Mixed sex Nile tilapia	10	10	10	10	10	10	10	10	10	10	10	10	
	26.17 ± 0.32						26.21 ± 0.31						26.17 ± 0.32
	24.13 ± 0.33						24.47 ± 0.26						24.13 ± 0.33

2.4 Determination of Body Parameters

The fish weight by grams (g) using digital balance were recorded according to Shalloof and Salama [21] the total fish body lengths (from the tip of the fish mouth to the tip of the caudal fin) by centimeters (cm) using measuring graduated tape and the standard fish body lengths (from the tip of the fish mouth to the base of the caudal fin) were recorded according to Ayandiran and Fawole [22], also the abdominal length is the distance between pelvic fin and anal fin in Nile tilapia (*Oreochromis niloticus*), it is measured using measuring graduated tape by centimeters (cm), the fish abdominal width measured using digital caliber by centimeters (cm) at the midpoint of abdomen, finally the fish abdominal circumference measured using measuring graduated tape by centimeters (cm).

2.5 Blood Sampling

The blood samples were collected as soon as possible for each fish after transportation. Blood was withdrawn from the posterior caudal blood vessels under the lateral line by inserting a needle (25 gauges) of a sterile disposable syringe (3 ml and 5 ml capacity). Blood samples were drawn into a tube containing sodium citrate (0.1 ml of 3.8% sodium citrate / 1 ml blood) for obtaining whole blood for hematological investigations. The plasma was separated from blood cells by centrifugation at 3,000 rpm for 30 minutes. The plasma was stored at -20°C for subsequent analysis [23].

2.6 Hematological Investigations

Hematocrit volume was determined by using microhematocrit method described by Adebayo et al. [24], hemoglobin concentration was measured by the cyan-methemoglobin method [25], red blood cells (RBCs) were counted using an improved Neubauer hemocytometer [26,27], the total number of RBCs was calculated in $10^6/\text{mm}^3$ [28], total White blood cell (WBCs) count were performed by indirect method [29,30] according to the following equation:

$$\text{WBCs count} = (\text{leukocytes number in blood smear} \times \text{RBCs} (10^6/\text{mm}^3) / \text{The number of erythrocytes counted in blood smear})$$

Blood platelets (thrombocytes) count were performed by indirect method [27,28] according to the following equation:

$$\text{Thrombocytes} = (\text{Thrombocytes number in blood smear} \times \text{RBC} (10^6/\text{mm}^3) / \text{The number of erythrocytes counted in blood smear})$$

Differential leukocyte counts (heterophils, eosinophils, basophils, lymphocytes and monocytes) were conducted on May-Grunwald-Giemsa stained blood smears [31], Mean corpuscular volume was determined according to the following equation:

$$\text{MCV (fl)} = \{(\text{PCV \%} / \text{RBCs} (10^6/\text{mm}^3)) \times 10\} [32]$$

Mean corpuscular hemoglobin was determined according to the following equation:

$$\text{MCH (pg)} = \{(\text{Hb g/dl} / \text{RBCs (10}^6/\text{mm}^3)) \times 10\} \quad [32]$$

Mean corpuscular hemoglobin concentration was determined according to the following equation:

$$\text{MCHC (\%)} = \{(\text{Hb g/dl} / \text{PCV (\%)} \times 100) \quad [32]$$

The plasma was used for determination follicle stimulating hormones (FSH), luteinizing hormone (LH), and prolactin hormone (PRL) in both male and female fish and determination of testosterone hormone in male fish and determination of estradiol E2 in female fish.

2.7 Determination of Hepato-somatic Index % and Gonado-somatic Index %

Hepato-somatic index (HSI) was calculated as the ratio between weight of liver and total body weight, expressed as a percentage [33] according to following equation:

$$\text{HSI \%} = \{(\text{weight of liver} / \text{total body weight}) \times 100\}$$

Gonado-somatic index (GSI) was calculated as the weight of the gonads relative to the total body weight, expressed as a percentage [34] according to following equation:

$$\text{GSI \%} = \{(\text{weight of the gonads} / \text{total body weight}) \times 100\}$$

2.8 Determination of Fish Reproductive Hormones

FSH was determined by using ELISA kit specific for fish (Catalogue Number: SL0019Fi, SunLong Biotech Co., LTD) according to Molés et al. [35,36]. LH was determined by using ELISA kit specific for fish (Catalogue Number: SL0024Fi, SunLong Biotech Co., LTD) according to Mateos et al. [37]. Fish prolactin hormone was determined by using ELISA kit specific for fish (Catalogue Number: SL0053Fi, SunLong Biotech Co., LTD) according to Onuma et al. [38]. Testosterone was determined by using testosterone ELISA kit (Catalogue Number: SL2107Hu, SunLong Biotech Co., LTD, China) according to Tietz [39]. Estradiol E2 hormone was determined by using Estradiol E2 ELISA kit (Catalogue Number: SE120049, Sigma-Aldrich,

3050 spruce street, St. louis, USA) according to Tietz [39].

2.9 Statistical Analysis

Data obtained in this study were statistically analyzed for variance using independent sample T - test, significance at $P < 0.05$ probability level). The results were reported as the mean \pm standard error "SE". Statistical analysis was performed using SPSS Version 16.0.1, (SPSS Inc., Chicago, IL, USA).

3. RESULTS

3.1 Seasonal Variations in Body Parameters of Nile Tilapia

The obtained data in Tables 1, 2 and 3 revealed that:

Significant increase ($P < 0.05$) in body weight (g) during breeding season in monosex Nile tilapia, females of mixed sex Nile tilapia, compared to non-breeding season. While, there are non-significant differences ($P < 0.05$) between breeding and non-breeding seasons in males of mixed sex Nile tilapia. Non-significant differences ($P < 0.05$) in total body length (cm) and standard body length (cm) between breeding and non-breeding seasons in Monosex Nile tilapia, mixed sex Nile tilapia. There are non-significant differences ($P < 0.05$) in abdominal length (cm) between breeding and non-breeding -Significant increase ($P < 0.05$) in abdominal width (cm) during breeding season in females of mixed sex Nile tilapia compared to non-breeding season. However, there are non-significant differences ($P < 0.05$) between breeding and non-breeding seasons in monosex Nile tilapia, males of mixed sex Nile tilapia. Significant increase ($P < 0.05$) in abdominal circumference (cm) during breeding season in females of mixed sex Nile tilapia While, there are non-significant differences ($P < 0.05$) between breeding and non-breeding seasons in monosex Nile tilapia, males of mixed sex Nile tilapia.

3.2 Seasonal Variations in Hepato-somatic Index % and Gonado-somatic Index % of Nile Tilapia

The obtained data revealed that:

Significant increase ($P < 0.05$) in Hepato-somatic index % during breeding season in monosex Nile

tilapia, males and females of mixed sex Nile tilapia, compared to non-breeding season (Fig. 1). Significant increase ($P < 0.05$) in Gonadosomatic index % during breeding season in

monosex Nile tilapia, males and females of mixed sex Nile tilapia, compared to non-breeding season (Fig. 2).

Table 1. Seasonal variations in body parameters of Monosex Nile Tilapia

Parameters	Non- breeding season	Breeding season
Body Weight (gm)	325.77 ± 12.45 ^b	340.52 ± 13.12 ^a
Total body length(cm)	26.17 ± 0.3 ^a	26.21 ± 0.3 ^a
Standard body length (cm)	22.02 ± 0.28 ^a	22.28 ± 0.25 ^a
Abdominal length (cm)	7.96 ± 0.19 ^a	8.03 ± 0.17 ^a
Abdominal width (cm)	2.95 ± 0.08 ^a	3.04 ± 0.07 ^a
Abdominal circumference	19.40 ± 0.29 ^a	19.80 ± 0.30 ^a

In the same row, Mean ± SE with different letters superscripts are significantly different at ($P < 0.05$)

Table 2. Seasonal variations in body parameters of male Tilapia

Parameters	Non- breeding season	breeding season
Body Weight (gm)	276.60 ± 7.24 ^a	286.18 ± 6.68 ^a
Total body length(cm)	24.23 ± 0.3 ^a	24.37 ± 0.29 ^a
Standard body length (cm)	20.94 ± 0.34 ^a	21.09 ± 0.32 ^a
Abdominal length (cm)	7.07 ± 0.10 ^a	7.08 ± 0.09 ^a
Abdominal width (cm)	2.57 ± 0.06 ^a	2.64 ± 0.07 ^a
Abdominal circumference	18.69 ± 0.28 ^a	18.79 ± 0.27 ^a

In the same row, Mean ± SE with different letters superscripts are significantly different at ($P < 0.05$)

Table 3. Seasonal variations in body parameters of female Tilapia

Parameters	Non- breeding season	Breeding season
Body Weight (gm)	279.43 ± 6.86 ^b	299.73 ± 6.88 ^a
Total body length(cm)	24.03 ± 0.34 ^a	24.56 ± 0.24 ^a
Standard body length (cm)	20.63 ± 0.33 ^a	21.17 ± 0.30 ^a
Abdominal length (cm)	7.00 ± 0.09 ^a	7.16 ± 0.10 ^a
Abdominal width (cm)	2.51 ± 0.05 ^b	2.83 ± 0.05 ^a
Abdominal circumference	18.45 ± 0.28 ^b	19.89 ± 0.24 ^a

In the same row, Mean ± SE with different letters superscripts are significantly different at ($P < 0.05$)

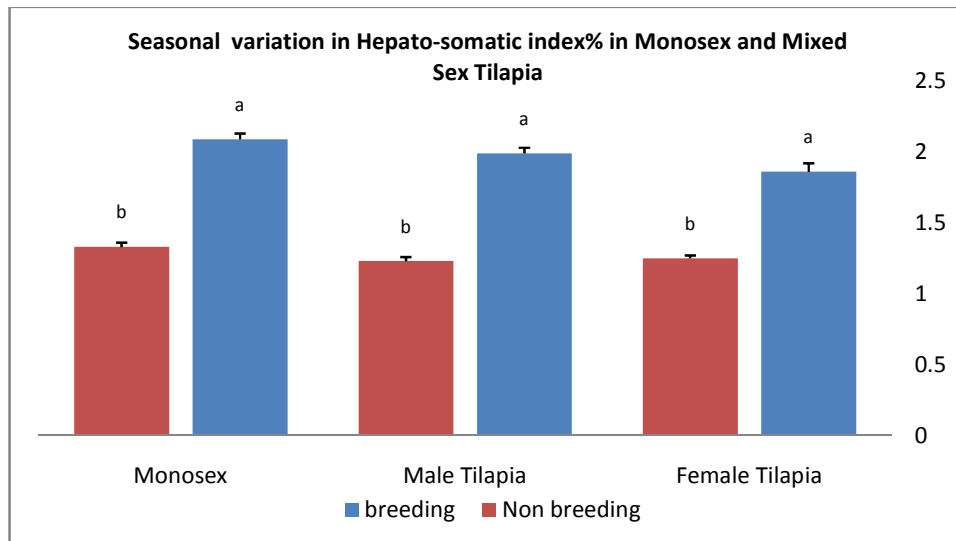


Fig. 1.

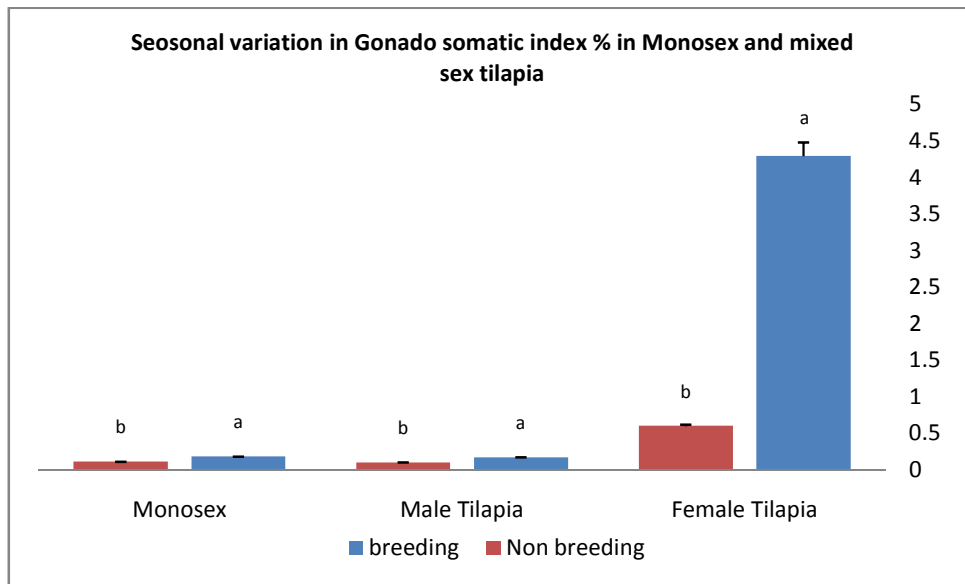


Fig. 2.

3.3 Seasonal Variations in Erythrogram and Leucogram of Nile Tilapia

The obtained data in Table 4, 5, 6 revealed that:

Significant increase ($P < 0.05$) in RBCs count ($10^6/\text{mm}^3$), Hemoglobin (g/dl), and PCV % during breeding season in monosex Nile tilapia, males and females of mixed sex Nile tilapia while a non-significant differences ($P < 0.05$) in MCV (fl), MCH (pg), and MCHC % between breeding and non-breeding seasons in monosex Nile tilapia, males and females of mixed sex Nile tilapia. There is a Significant decrease ($P < 0.05$) in thrombocytes count ($10^3/\text{mm}^3$) during breeding

season in monosex Nile tilapia, males and females of mixed sex Nile tilapia. Significant increase ($P < 0.05$) in total leucocytic count ($10^3/\text{mm}^3$) during breeding season in monosex Nile tilapia, males and females of mixed sex Nile tilapia, Significant increase ($P < 0.05$) in Heterophils % during breeding and season in monosex Nile tilapia, males and females of mixed sex Nile tilapia while non-significant differences ($P < 0.05$) in Eosinophils%, Monocytes%, Lymphocytes%, and Basophils % between breeding and non-breeding seasons in monosex Nile tilapia, males and females of mixed sex Nile tilapia.

Table 4. Seasonal variations in Erythrogram and Leucogram of Mono-sex Tilapia

Parameters	Non- breeding season	Breeding season
RBCs count ($10^6/\text{mm}^3$)	1.89 ± 0.05^b	2.13 ± 0.05^a
Hemoglobin content (g/dl)	8.51 ± 0.22^b	9.60 ± 0.25^a
Mean PCV %	28.33 ± 0.72^b	31.96 ± 0.82^a
MCV (fl)	151.05 ± 4.95^a	150.35 ± 3.64^a
MCH (pg)	45.10 ± 1.49^a	44.80 ± 1.13^a
MCHC%	30.04 ± 0.03^a	30.00 ± 0.03^a
Total leucocytic count ($10^3/\text{mm}^3$)	19.52 ± 1.55^b	26.28 ± 2.47^a
Thrombocytes count ($10^3/\text{mm}^3$)	27.72 ± 2.72^a	21.65 ± 1.61^b
Differential leucocytic count %		
Heterophils %	8.92 ± 0.50^b	11.29 ± 0.61^a
Basophils %	0.38 ± 0.031^a	0.31 ± 0.014^a
Monocytes %	5.47 ± 0.25^a	5.23 ± 0.25^a
Lymphocytes %	80.80 ± 0.56^a	78.51 ± 0.67^a

In the same row, Mean \pm SE with different letters superscripts are significantly different at ($P < 0.05$).

Table 5. Seasonal variations in Erythrogram and Leucogram of Male Tilapia

Parameters	Non- breeding season	Breeding season
RBCs count ($10^6/\text{mm}^3$)	1.80 \pm 0.05 ^b	2.11 \pm 0.05 ^a
Hemoglobin content (g/dl)	9.43 \pm 0.25 ^b	11.11 \pm 0.28 ^a
Mean PCV %	26.99 \pm 0.72 ^b	31.74 \pm 0.79 ^a
MCV (fl)	150.73 \pm 4.63 ^a	150.68 \pm 3.38 ^a
MCH (pg)	52.40 \pm 1.61 ^a	52.75 \pm 1.19 ^a
MCHC%	34.95 \pm 0.03 ^a	35.00 \pm 0.03 ^a
Total leucocytic count ($10^3/\text{mm}^3$)	27.93 \pm 3.53 ^a	21.43 \pm 2.49 ^b
Thrombocytes count ($10^3/\text{mm}^3$)	20.92 \pm 2.46 ^b	30.88 \pm 1.67 ^a
Differential leucocytic count %		
Heterophils %	7.04 \pm 0.21 ^b	10.69 \pm 0.45 ^a
Eosinophils %	4.22 \pm 0.12 ^a	4.44 \pm 0.13 ^a
Basophils %	0.27 \pm 0.015 ^a	0.34 \pm 0.011 ^a
Monocytes %	5.51 \pm 0.27 ^a	6.00 \pm 0.29 ^a
Lymphocytes %	80.85 \pm 0.48 ^a	78.53 \pm 0.65 ^a

In the same row, Mean \pm SE with different letters superscripts are significantly different at ($P < 0.05$).

Table 6. Seasonal variations in Erythrogram and Leucogram of Female Tilapia

Parameters	Non- breeding season	Breeding season
RBCs count ($10^6/\text{mm}^3$)	1.65 \pm 0.04 ^d	1.94 \pm 0.05 ^a
Hemoglobin content (g/dl)	8.67 \pm 0.20 ^b	10.18 \pm 0.28 ^a
Mean PCV %	24.77 \pm 0.59 ^b	29.06 \pm 0.80 ^a
MCV (fl)	150.96 \pm 4.16 ^a	151.21 \pm 4.93 ^a
MCH (pg)	52.83 \pm 1.45 ^a	52.97 \pm 1.72 ^a
MCHC%	34.99 \pm 0.03 ^a	35.03 \pm 0.02 ^a
Total leucocytic count ($10^3/\text{mm}^3$)	21.45 \pm 2.45 ^b	27.89 \pm 2.28 ^a
Thrombocytes count ($10^3/\text{mm}^3$)	26.38 \pm 1.89 ^a	21.13 \pm 1.59 ^b
Differential leucocytic count %		
Heterophils %	8.95 \pm 0.64 ^b	11.02 \pm 0.70 ^a
Eosinophils %	4.65 \pm 0.15 ^a	4.88 \pm 0.12 ^a
Basophils %	0.32 \pm 0.031 ^a	0.28 \pm 0.015 ^a
Monocytes %	5.16 \pm 0.23 ^a	5.33 \pm 0.21 ^a
Lymphocytes %	80.92 \pm 0.63 ^a	78.49 \pm 0.69 ^a

In the same row, Mean \pm SE with different letters superscripts are significantly different at ($P < 0.05$).

3.4 Seasonal Variations in Reproductive Hormones Assay of Some Fresh Water Fishes

The obtained data in Tables 7,8,9 revealed that significant increase ($P < 0.05$) in FSH (ng/ml) during breeding season in monosex Nile tilapia and females of mixed sex Nile tilapia compared to non-breeding season. However, there are non-significant differences ($P < 0.05$) between breeding and non-breeding seasons in males of mixed sex Nile tilapia.

Significant increase ($P < 0.05$) in LH (ng/ml) during breeding season in males and females of mixed sex Nile tilapia compared to non-breeding season. While, there are non-significant differences ($P < 0.05$) between breeding and non-breeding seasons in monosex Nile tilapia.

Significant increase ($P < 0.05$) in prolactin hormone (pg/ml) during breeding season in monosex Nile tilapia and mixed sex Nile tilapia. Significant differences ($P < 0.05$) in Testosterone hormone (ng/ml) during breeding season in monosex Nile tilapia and males of mixed sex Nile tilapia compared to non-breeding season. Significant differences ($P < 0.05$) in Estradiol E2 (pg/ml) during breeding season in females of mixed Nile tilapia compared to non-breeding season.

4. DISCUSSION

The present study has shown changes in the physical and chemical composition of Nile tilapia which appear to be related to the seasons; a result which is in agreement with that of Oliveira et al. [40] and Younis et al. [41] who each

reported that, in different environmental conditions, the body composition of the same fish may vary with respect to differences in water quality, feeding conditions, sex and maturity.

4.1 Body parameters of Nile Tilapia

The findings of the present study revealed significant increase ($P < 0.05$) in body weight (g) in monosex Nile tilapia and females of mixed sex Nile tilapia, also the same occur in abdominal width (cm) and abdominal circumference (cm) in females of mixed sex Nile tilapia during breeding season compared to non-breeding season. However, there are non-significant differences ($P < 0.05$) in total body length (cm) and standard body length (cm) between breeding and non-breeding seasons in all experimental fishes, also the same results occur in abdominal length (cm) and abdominal circumference (cm) in monosex Nile tilapia, male and female of mixed sex Nile tilapia, and also there are non-significant differences ($P < 0.05$) in abdominal width (cm) in males of mixed sex Nile tilapia.

To our knowledge there are no available literatures reported the seasonal variations in body weight, abdominal length, abdominal width, and abdominal circumference and the significant

differences in these values may be attributed to favorable environmental conditions, gonadal growth, increased food consumption, and increased metabolic rate during breeding season.

Seasonal fluctuations in weight and physiological status related to feeding and reproduction were observed in many species of fish living in temperate ecosystems [42,43].

Uka and Edun [44] and Uka and Sikoki [45] reported that the overall length is an important factor in determining the success of spawning in both sexes of *Sarotherodon melanotheron* and *Tilapia guineensis* respectively as a result of significant positive correlation of total length on gonadosomatic index in both sexes.

4.2 Hepato-somatic Index % (HSI) and Gonado-somatic Index % (GSI) in Nile Tilapia

The obtained results revealed significant increase ($P < 0.05$) in Hepato-somatic index % and Gonado-somatic index % during breeding season in monosex Nile tilapia, males and females of mixed sex Nile tilapia.

Table 7. Seasonal variations in reproductive hormonal profile of Monosex Tilapia

Parameters	Non- breeding season	breeding season
FSH (ng/ml)	11.55 ± 1.09 ^b	15.75 ± 0.69 ^a
LH (ng/ml)	1.82 ± 0.13 ^a	2.03 ± 0.09 ^a
prolactin (pg/ml)	146.00 ± 3.48 ^b	231.00 ± 6.05 ^a
Testosterone hormone	0.58 ± 0.014 ^b	0.76 ± 0.030 ^a

In the same row, Mean ± SE with different letters superscripts are significantly different at ($P < 0.05$)

Table 8. Seasonal variations in reproductive hormonal profile of Male Tilapia

Parameters	Non- breeding season	Breeding season
FSH (ng/ml)	16.65 ± 0.75 ^a	17.30 ± 0.51 ^a
LH (ng/ml)	1.57 ± 0.09 ^b	1.93 ± 0.12 ^a
prolactin (pg/ml)	173.00 ± 6.02 ^b	262.00 ± 5.12 ^a
Testosterone hormone	0.67 ± 0.026 ^b	0.81 ± 0.023 ^a

In the same row, Mean ± SE with different letters superscripts are significantly different at ($P < 0.05$)

Table 9. Seasonal variations in in reproductive hormonal profile of Female Tilapia

Parameters	Non- breeding season	Breeding season
FSH (ng/ml)	15.75 ± 0.65 ^b	19.55 ± 0.57 ^a
LH (ng/ml)	1.35 ± 0.07 ^b	2.00 ± 0.10 ^a
prolactin (pg/ml)	160.00 ± 5.96 ^b	255.00 ± 8.88 ^a
estradiol E2 (pg/ml)	94.24 ± 2.92 ^b	127.93 ± 5.94 ^a

In the same row, Mean ± SE with different letters superscripts are significantly different at ($P < 0.05$)

The current results are consistent with Wang et al. [46] who reported that fat and protein storage occur in the liver prior to spawning, in addition to Osman et al. [47] who showed that the increase in the liver wt. was due to physiological changes that occurred during the pre-spawning period. HSI in both genders of *Tilapia zillii* showed higher values during the period from June to September with a peak in July (breeding season), while the lower values occurred during the period from October to February (non-breeding season) [48].

The present study is in accordance with Shallof and Salama [21] who reported that the gonadosomatic values during the breeding period of female Nile Tilapia *Oreochromis niloticus* exhibited several peaks in March, April, June and September.

These results supported by El-Zoghoby et al. [49] who reported that the results of GSI for both sex of some water fishes reached their minimum values during the winter season where the gonads were in the rest season and increase significantly during spring season and reached the maximum values during summer where the gonads were in the spawning season and began to decline once Others during autumn season.

Gaber [50] in Bagrus bayad fish species reported that GSI significantly increased during breeding season and declined during non-breeding season.

4.3 Erythrogram and Leucogram in Nile Tilapia

Information about the hematological profile is an important tool which can be used for effective and sensitive monitoring of physiological and pathological condition of a fish [16].

The findings of the present study showed significant increase ($P < 0.05$) in RBCs count ($10^6/\text{mm}^3$), Hemoglobin (g/dl), and PCV % during breeding season in monosex Nile tilapia, males and females of mixed sex Nile tilapia. However, there are non-significant differences ($P < 0.05$) in MCV (fl), MCH (pg), MCHC % between breeding and non-breeding seasons in monosex Nile tilapia, males and females of mixed sex Nile tilapia.

The results of the present study matched the findings of [16,24,51] which reported that some

levels of the blood parameter may increase to meet high energy demand of fish during summer season. While, the lowest value during winter may be due to low ambient temperature and low metabolic rate.

These results also are in agreement with [52,53] who reported that some blood parameters, like hematocrit, hemoglobin, erythrocytes, total leucocytes and lymphocyte, of some fish species like (*Chalcalburnus mossulensis*, *Cyprinion macrostomus* and *Alburnoides bipunctatus*) increased in summer, suggesting that these changes could be attributed to seasonal water temperature and dissolved oxygen variations.

The results of the present study also revealed significant increase ($P < 0.05$) in total WBCs count ($10^3/\text{mm}^3$), Heterophils % during breeding seasons in Monosex Nile tilapia, males and females of mixed sex Nile tilapia. But there are non-significant differences ($P < 0.05$) in Eosinophils %, Monocytes %, Lymphocytes %, and Basophils % between breeding and non-breeding seasons in Monosex Nile tilapia, males and females of mixed sex Nile tilapia.

Leucocytes act as the first line of defense against any type of pathogen making the organism immune enough to fight any possible stress [54].

The results of this study confirm those of Gupta et al. [55] which reported that the total WBCs count increase in fish during spring and summer with a peak in June seems to be a response to elevated water temperature

Immunodepression appears to be caused by lower water temperature during the winter and is therefore responsible for the low count of leucocytes as lower temperature passively affects the cellular and humoral response [54].

On the other hand, Zaragabadi et al. [56] studied the effect of rearing temperature on the blood parameters of *Huso huso* fish juvenile and reported that some blood hematological parameters such as the number of eosinophils increased while white blood cell count decreased with increasing temperatures, this may be attributed to species difference in fish

The findings of the present study revealed significant decrease ($P < 0.05$) in thrombocytes

count ($10^3/\text{mm}^3$) during breeding season in monosex Nile tilapia, males and females of mixed Nile tilapia.

These findings agreed with Gupta et al. [55], who reported that a significant increase in thrombocytes, during autumn and winter and decrease through spring and summer in *Tor putitora* fish. This may be attributed to winter season which appear to be immunodepressant for fishes.

4.4 Reproductive Hormones in Nile Tilapia

The results of the present study showed:

Significant increase ($P < 0.05$) in FSH (ng/ml) during breeding season in all studied fish species except male of mixed sex Nile tilapia compared to non-breeding season. Significant increase ($P < 0.05$) in LH (ng/ml) during breeding season in males and females of mixed sex Nile tilapia compared to non-breeding season. While, there are non-significant differences ($P < 0.05$) between breeding and non-breeding seasons in monosex Nile tilapia, Significant increase ($P < 0.05$) in prolactin hormone (pg/ml) during breeding season in monosex Nile tilapia and mixed sex Nile tilapia, Significant differences ($P < 0.05$) in Testosterone hormone (ng/ml) during breeding season in monosex Nile tilapia and males of mixed sex Nile tilapia compared to non-breeding season, Significant differences ($P < 0.05$) in Estradiol E2 (pg/ml) during breeding season in females of mixed Nile tilapia compared to non-breeding season.

The results of the current study are consistent with Abbasi et al. [57], who reported that seasonal changes in the concentrations of sex hormones and their importance in reproduction have been reported for several species of fish (teleosts) which may be attributed to annual fluctuations of hormones related to reproduction, nutrition and growth cycles in fish. The annual rhythm of hormones is closely related to factors such as temperature, environment, fish species and length of day [58].

Also, these results are in agreement with Guerriero [59] who investigated the plasma concentrations of estradiol (E2) and testosterone through the maturation of the gonads and spawning of some species of fish and recorded less concentration than

January (Ogonia) to March (previtellogenic oocytes), while plasma E2 estradiol and testosterone concentrations increased in April, when the ovaries mostly contained oocytes.

The results of this study were parallel to studies Kousha et al. [60] and Bahmani et al. [61], which referred to seasonal variations in circulatory plasma levels of sex steroid hormones during the reproductive cycle of a variety of teleosts.

Also, the results are consistent with several studies that show that prolactin levels change during reproductive cycles: For example, Nile tilapia plasma levels are the highest in females after spawning, during vitellogenesis [62]. prolactin is directly stimulating testosterone production in courting male Mozambique tilapia testicular tissue during breeding season [63].

On other hand, the present investigations are contradictory to the results of Sehafii et al. [64] which revealed that sex steroid hormones (17 β -estradiol and testosterone) reach their peaks during fall and winter which coincided with the non-breeding season of Indian Major Carp, *Catla Catla* fish and the reasons for these contradictory results may be due to species differences and different environmental conditions. Reproduction in fish is under hormonal regulation by the hypothalamus–pituitary–gonadal axis. The brain is stimulated by environmental factors like flooding, water temperature, feeding, rainfall and photoperiod to release gonadotropin releasing hormone [65].

5. CONCLUSION

The present study revealed that the body parameters (body weight, abdominal width, and abdominal circumference), the reproductive hormones (FSH, LH, prolactin, testosterone, and Estradiol E2), the Erythrogram, total and differential leukocytic count, and Hepato-somatic index % & Gonado-somatic index % are significantly increased during breeding season especially in female fishes while, the thrombocytes count is significantly decreased during breeding season.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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