

## Comparison of some metabolic and sex hormones in selected vertebrates in different seasons

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Received: Feb. 20, 2020; Accepted: Mar. 29, 2020; Available online : May 15, 2020

### ABSTRACT

Different environmental factors affect animal physiological status. Day length and temperature are the two main factors that have been proved to cause changes in different hormones. The current study aimed to compare seasonal changes of some hormones such as testosterone (T), estradiol-17- $\beta$  (E<sub>2</sub>) and thyroid hormones (T<sub>4</sub>, thyroxine; and T<sub>3</sub>, triiodothyronine) in selected vertebrates (amphibians, fish and mammals). These hormones were assayed by enzyme-linked immunosorbent assay (ELISA).

The present results indicated that; T<sub>3</sub> had the highest concentration in both sexes of amphibians during winter and the lowest one was found in the investigated mammals of both sexes during summer and autumn. Regarding, T<sub>4</sub> also showed the highest concentration in both sexes of amphibians during winter and the lowest one was recorded in male mammals during summer. In relation to E<sub>2</sub>, in females; the highest and detectable concentration was observed in fishes during summer and the lowest one was found in mammals during winter. On the other hand, in males, its highest concentration was found in birds during spring, and the lowest one was recorded in amphibians during winter. Testosterone showed the highest concentration in female birds during summer, while the lowest one was recorded in amphibians during summer. In males, the highest T level was observed in fishes during summer, while the lowest one was found in amphibians in the same season.

According to the results, significant changes in levels of steroid hormones, as well as thyroids in the selected animals from different classes were closely correlated to the length of the day and seasons.

**Keywords:** Seasonal changes, Steroid hormone, estradiol-17 $\beta$ , Testosterone, Thyroid hormones, Thyroxine, Triiodothyronine.

### INTRODUCTION

Different environmental factors affect animal physiological status. Photoperiod (day length) and temperature are two main factors that have been proved to cause changes in different hormones during seasons. Photoperiodism is the ability of plants and animals to measure environmental day length (photoperiod), a process that underlies the so-called biological calendar (Nelson *et al.*, 2010).

Photoperiodism defines the use of the annual cycle of day and night length to coordinate functions such as reproduction, fattening, hibernation, and migration with predictable changes in the environment, for example in food availability or climatic conditions.

Seasonal changes in physiology and behavior typically are innately timed long-term processes, requiring weeks or months to wax and wane. Therefore, additional to photoperiodic readout mechanisms, living

creatures have evolved endogenous long-term timing devices, which allow them to anticipate forthcoming seasonal changes (Dardente *et al.*, 2014).

It is well known that photoperiod affects the physiological behavior of animals by adjusting the daily and seasonal changes, and the animals develop specific adaptive periodic activities through rhythm oscillation (Goldman, 2001; Reppert and Weaver, 2002; Tomioka *et al.*, 2012; Partch *et al.*, 2014).

The purpose of this study is comparing the seasonal hormonal changes among selected vertebrates (amphibians, fishes, birds and mammals) in relation to photoperiod in the field.

## MATERIALS AND METHODS

### Study animal

A license for capturing and sampling was obtained from the Egyptian Environmental Affairs Agency (EEAA).

### Experimental animals

This study was conducted between May 2016 to May 2017. One hundred and four wild animals representing four different vertebrate groups were collected from Damietta governorate. These include 13 males and 13 females of each of the investigated groups of amphibians, fishes, birds, and mammals. Fishes were represented by the African catfish (*Clarias garipinus*), amphibians were represented by the maculated toad (*Bufo regularis*), birds were represented by pigeon (*Columba livia domesticaes*) and mammals were represented by the brown rat (*Rattus norvegicus*).

### Blood sampling

During four seasons (spring, summer, autumn and winter); the selected species were captured and weighed to the

nearest gram. Blood samples were collected from the caudal vein of the African catfish and brown rat, from the heart of the maculated toad, and from the brachial vein of pigeon. Blood samples were collected within 3 minutes maximum to reduce the handling stress, and samples represented no more than 1% of the body weight (lumeij, 1997). Blood samples were allowed for 30 minutes at room temperature before centrifugation at 1207 xg for 20 minutes. The sera samples then stored at -20 until assayed for selected hormones (estradiol 17- $\beta$  ( $E_2$ ), testosterone, free thyroxine ( $FT_4$ ), and triiodothyronine ( $FT_3$ ) later on the same day.

### Hormonal assay

Serum levels of estradiol-17 $\beta$  ( $E_2$ ), testosterone, free thyroxine ( $FT_4$ ), and triiodothyronine ( $FT_3$ ) in both sexes were determined by enzymatic immunoassay ELISA using commercially available kits for a quantitative measurement. Each hormone was read from separate standard curves and each sample was adjusted for percentage recovery of the internal standard. The analysis was run in duplicates.

### Statistical analysis

Values are represented by means  $\pm$  SE. The results were analyzed using the XLSTAT program. One-way ANOVA followed by Turkey test was applied to test the significant hormonal differences between classes during seasons. Probability (p) levels of  $\leq 0.05$  were considered statistically significant.

## RESULTS

### Day length:

During the sampling period, the longest average day length was 13h 52m during spring, while the lowest length was recorded during winter 10h 27m (Table 1).

**Table (1). Data of sampling date, seasonal minimum, maximum and mean temperature and seasonal day and night length.**

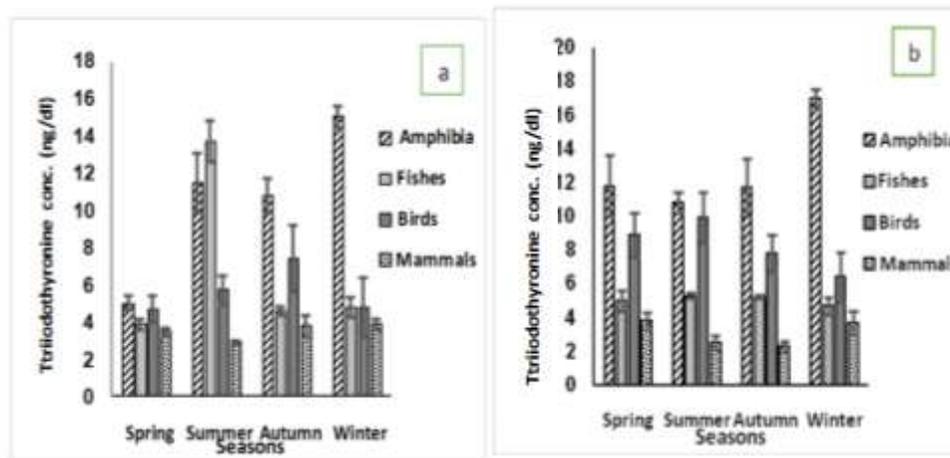
Season	Sampling date	Minimum temp	Maximum temp	Mean Temp	Day length	Night length
Spring	21/5/2016	19	25	24	13h 52m	10h 08m
Summer	21/8/2016	25	30	28	13h 07m	10h 54m
Autumn	1/11/2016	20	24	22	10h 55m	13h 05m
Winter	21/1/2017	14	18	16	10h 27m	13h 33m

**Hormonal change:****Free Triiodothyronine (FT<sub>3</sub>):**

Seasonal levels of FT<sub>3</sub> in the investigated samples of amphibians, fishes, birds, and mammals are shown in Figure (1). In females, FT<sub>3</sub> levels showed significant variations in different classes during summer, autumn and winter ( $P \leq 0.01$ ), where the highest FT<sub>3</sub> concentration was found in amphibians during winter and the lowest one was recorded in mammals during summer

(mean  $\pm$  SE=  $15 \pm 0.6$  and  $2.9 \pm 0.1$  ng/dl; respectively, Fig.1a).

On the other hand, FT<sub>3</sub> levels in males showed significant variations between different classes in all seasons ( $P \leq 0.01$ ). The highest concentration was found in amphibians during winter, while mammals showed the lowest concentration during autumn (mean  $\pm$  SE=  $17 \pm 0.5$  and  $2.30 \pm 0.3$  ng/dl; respectively, Fig.1b).



**Fig. 1.: Serum concentrations of FT<sub>3</sub> (ng/dl) in different classes during different seasons. (a) In females and (b) In males. Values are represented by means  $\pm$  SE**

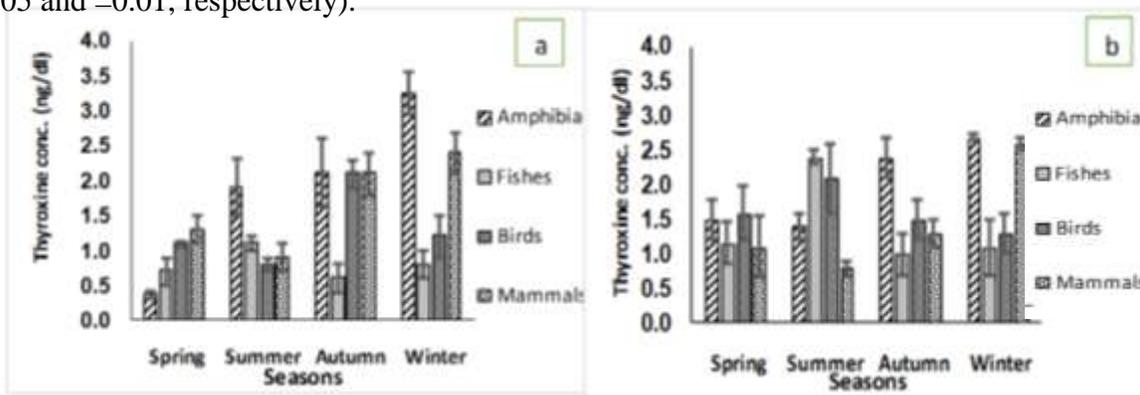
**Thyroxine (FT<sub>4</sub>):**

Seasonal levels of FT<sub>4</sub> in amphibians, fishes, birds, and mammals are shown in Figure (2). In females FT<sub>4</sub> levels showed significant changes between

different classes during summer, autumn and winter ( $P=0.05$ ,  $=0.01$  and  $=0.002$ , respectively). Amphibians showed the mean highest FT<sub>4</sub> concentration was recorded during winter and the lowest one was during

spring ( $3.26 \pm 0.3$  and  $0.39 \pm 0.1$  ng/dl, respectively, Fig. 2a). In relation to males FT<sub>4</sub> mean levels showed significant variations between different classes during summer, autumn and winter ( $P = 0.006$ ,  $=0.05$  and  $=0.01$ , respectively).

The mean highest FT<sub>4</sub> concentration was found in amphibians during winter, while the lowest one was recorded in mammals during summer ( $2.7$  and  $0.8 \pm 0.1$  ng/dl, respectively, Fig. 2b).



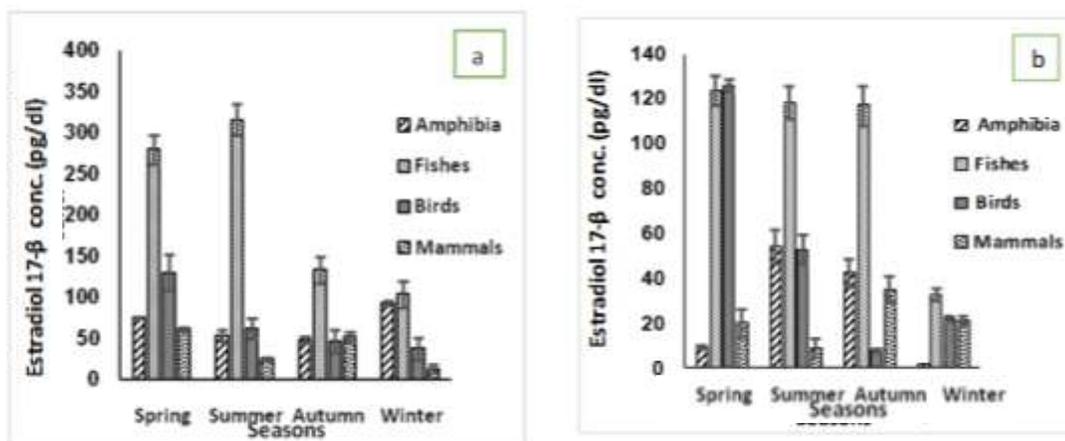
**Fig. 2: Serum concentrations of FT<sub>4</sub> (ng/dl) in different classes during different seasons. (a) In females and (b) In males. Values are represented by means  $\pm$  SE**

### Estradiol 17- $\beta$ (E<sub>2</sub>):

Seasonal mean levels of estradiol 17- $\beta$  (E<sub>2</sub>) in amphibians, fishes, birds and mammals are shown in Figure (3). In females E<sub>2</sub> mean levels showed significant differences between selected animals in all seasons ( $P \leq 0.01$ ). The highest E<sub>2</sub> mean concentration ( $315.8 \pm 18.9$  pg/dl) was observed in fishes during summer, while the lowest one ( $13.1 \pm 4.8$  pg/dl) was in

mammals during winter (Fig. 3a).

Regarding male E<sub>2</sub> mean levels showed significant variations between different classes in all seasons ( $P \leq 0.01$ ). The highest E<sub>2</sub> mean concentration was found in birds during spring, while the lowest one was in amphibians during winter ( $125.8 \pm 2.6$  and  $1.2 \pm 0.1$  pg/dl, respectively, Fig. 3b).

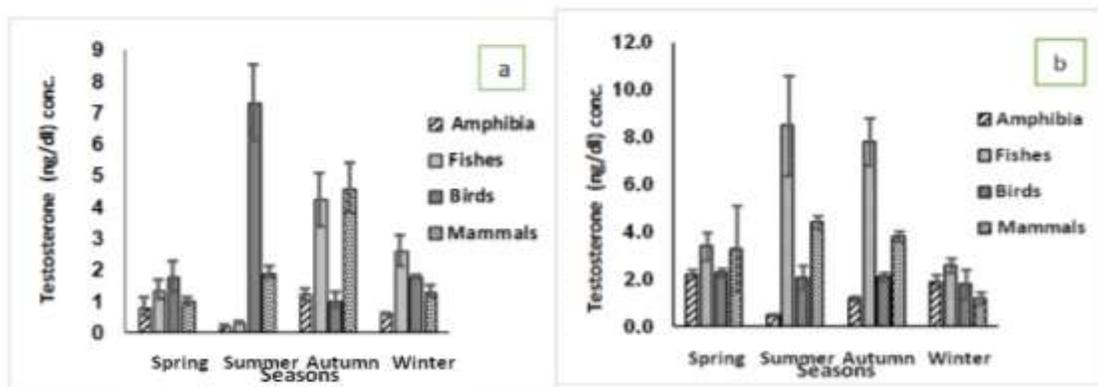


**Fig.3: Serum concentrations of estradiol 17- $\beta$  (E<sub>2</sub>) (pg/dl) in different classes during different seasons. (a) In females and (b) In males. Values are represented by means  $\pm$  SE.**

**Serum testosterone (T):**

Seasonal mean levels of serum testosterone in amphibians, fishes, birds, and mammals are shown in Figure (4). In females testosterone levels showed significant variations between different classes during summer, autumn, and winter ( $P \leq 0.02$ ). The highest T mean concentration was found in birds during summer, while the lowest one was in amphibians during

summer ( $7.30 \pm 1.1$  and  $0.2 \pm 0.1$  ng/dl; respectively, Fig.4a). For males, testosterone mean levels showed significant differences between different classes during summer, autumn, and winter ( $P \leq 0.01$ ). The highest testosterone mean concentration was observed in fishes during summer ( $8.5 \pm 2.1$  ng/dl), while the lowest one was found in amphibians during summer ( $0.5 \pm 0.2$  ng/dl Fig. 4b).



**Fig. 4: Serum concentrations of testosterone (ng/dl) in different classes during different seasons. (a) In females and (b) In males. Values are represented by means  $\pm$  SE.**

**DISCUSSION**

The present study aimed to investigate the seasonal changes in thyroid hormones ( $FT_3$  and  $FT_4$ ) and serum sex steroids (Estradiol 17- $\beta$ , testosterone) in representative animals from both sexes of amphibians, fishes, birds, and mammals during spring, summer, autumn, and winter.

Thyroid hormones (THs), triiodothyronine ( $T_3$ ) and thyroxine ( $T_4$ ), play critical roles in the differentiation, growth, metabolism, and physiological function of nearly all mammalian tissues (Yen, 2001; Cheng *et al.*, 2010) In addition, they are required for amphibian metamorphosis (Furlow and Neff, 2006). Multiple biological effects of THs depend

on intracellular levels of  $T_3$ , which binds to the thyroid hormone receptor and is for the most part generated in peripheral tissues by outer-ring deiodination of  $T_4$  (Galton *et al.*, 2009). Thyroid hormones are key metabolic regulators that coordinate short-term and long-term energy needs (Oetting and Yen, 2007). According to the current results, both sexes of amphibians showed the highest concentration of  $FT_3$  and  $FT_4$  during winter as compared to other classes; this result is in line with Rosenkild (1982) who found that *Bufo bufo* has higher plasma  $T_4$  during hibernation period and spring than summer and autumn. Similar results by Ceusters *et al.*, (1978) showed that at cold temperatures, iodine uptake by the thyroid

increases in *Rana temporaria*. In contrast, another study revealed that FT<sub>3</sub> level changed very little throughout the year but FT<sub>4</sub> level decreased significantly during hibernating phase (winter). The decreased serum thyroxine in the toad suggests that a state of hypometabolism and decreased metabolic rate would allow further conservation of energy during the crucial phase of hibernation (Suman and Jayanta, 2009). Also, Kuhn *et al.* (1985) indicated that thyroid content of T<sub>4</sub> and T<sub>3</sub> was low during winter in *Rana ridibunda*.

The present study showed that both female and male mammals recorded the lowest concentration of FT<sub>3</sub> during summer and autumn, respectively then increased in winter; this finding was in agreement with Thomas and Dora (1996) who found that FT<sub>3</sub> concentration was inversely correlated to photoperiod. Cold exposure also increased serum FT<sub>3</sub> production, utilization, and metabolic rate. Pallavi *et al.* (2011) also suggested that FT<sub>3</sub> is slightly lower in autumn and slightly higher in winter.

Sex steroids are the regulators of reproductive physiology, sexual differentiation and the development of sexual characteristics (Nelson, 2005). Steroid hormones regulate many physiological processes in vertebrates, including reproduction, growth, and homeostatic mechanisms such as water and energy balance. The roles of steroid hormones in reproduction have been well-studied in many vertebrates. Sex steroids, in particular are integral regulators of reproductive behaviors and functions across vertebrates. In many species estrogens (estradiol 17- $\beta$ , E<sub>2</sub>) stimulate sexual behavior and vitellogenesis in females, while androgens (testosterone, T) stimulate sexual behavior and spermatogenesis in males (Norris, 1997). Estradiol 17- $\beta$  is known to be estrogen steroid hormone that secreted by the cells of the ovarian follicles

that promote the development and maintenance of the female sexual characteristics. It plays an important role in the female and male reproductive system. In the current study, the highest concentrations of E<sub>2</sub> were observed in female fishes during summer which may be due to reaching the breeding season. Pavlidis *et al.* (2000) recorded high peak in 17 beta-estradiol plasma concentrations associated with the synthesis of vitellogenic proteins (vitellogenin) in many other teleosts. This result coincided with other studies on *Acipenser Persicus* (Hosseinzade *et al.*, 2012), *Rutilus firrsi kutum* (Heidari *et al.*, 2018), *Labeo rohita* (Suresh *et al.*, 2008). In these studies, levels of E<sub>2</sub> differed during the year and its peak was before spawning.

In the present investigation the highest concentrations of E<sub>2</sub> was found in male birds during spring. Similar studies indicated that domestic birds like pigeon bred throughout the year with a peak in spring and summer (Tomasz, 2004) which may explain the high levels of E<sub>2</sub> in the male birds in the current study. On the other hand, the lowest E<sub>2</sub> levels in the current study were observed in female mammals during winter. This result was in line with Gabry *et al.* (2014) who found that estrogen concentration in adult female rats was significantly higher (P < 0.05) in the long photoperiod group than in the short photoperiod group. The slight differences in hormonal levels between long and short photoperiods may result from a change in the phase of the hormones circadian rhythm rather than a change in their concentration. In contrary, another study revealed that Photoperiod had no effect on estradiol levels in female California mice (Steinman *et al.*, 2011). In relation to males, amphibians recorded the lowest concentrations of E<sub>2</sub> during winter; this is may be due to the cold temperature and hibernation period.

Testosterone is the primary male sex hormone responsible for regulating sex differentiation, producing male sex characteristics, spermatogenesis, and fertility (George and Stephen, 2018). The current results showed that the highest levels of (T) among selected animals were found in female birds during summer. This finding was in line with other studies (Dunmore and Davis, 1963; Murton *et al.*, 1972; Häkkinen *et al.*, 1973; Sengupta, 1974; Dilks, 1975; Johnston, 1984; Dabert, 1987; Johnson and Janiga, 1995) who found that the feral pigeon reproduces in all months of the year, even in winter. Its breeding activity is most intense in the spring and summer and then it decreases markedly in autumn and winter (Janiga and Kocian, 1985) due to deteriorating weather conditions. Skelton *et al.* (1997) reported high concentrations of testosterone male fishes during summer. In the present work the lowest concentrations of testosterone were observed in both sexes of amphibians during summer. Similar investigations on males of other species indicated that levels of testosterone change during seasons and are coordinated with the spermatogenic cycle (Licht *et al.*, 1985).

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### مقارنة بعض الهرمونات الأيضية والجنسية في بعض الفقاريات المختارة خلال المواسم المختلفة

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#### المستخلص

تؤثر العوامل البيئية المختلفة على الحالة الفسيولوجية للحيوانات. فطول النهار ودرجة الحرارة عاملان رئيسيان ثبت أنهما يسببان تغيرات في الهرمونات المختلفة. هدفت الدراسة الحالية إلى مقارنة التغيرات الموسمية لبعض الهرمونات مثل (التستوستيرون ، الإستراديول ، التيرونكسين T4، تراي ايودوثيرونين T3) في بعض الفقاريات المختارة. وقد تم قياس تركيزات هذه الهرمونات باستخدام تحليل الإليزا (الامتصاصية المناعية للإنزيم المرتبط). وقد أظهرت النتائج ارتفاع ملحوظ في تركيز هرمون T3 في كلا جنسي البرمائيات مقارنة بالطوائف الأخرى خلال موسم الشتاء بينما لوحظ أقل تركيز في ذكر وانثى الثدييات في فصلي الصيف والخريف على الترتيب. وقد لوحظ أيضا ارتفاع تركيز هرمون T4 في كلا جنسي البرمائيات مقارنة بالطوائف الأخرى خلال موسم الشتاء بينما لوحظ أقل تركيز في ذكور الثدييات خلال فصل الصيف. أما بالنسبة لهرمون الاستراديول فقد سجلت إناث الأسماك أعلى تركيز للهرمون في فصل الصيف مقارنة بباقي الطوائف الأخرى بينما سجلت إناث الثدييات أقل تركيز خلال فصل الصيف. سجلت ذكور الطيور أعلى تركيز للهرمون خلال فصل الربيع ووجدت أقل نسبة للهرمون في ذكور البرمائيات خلال فصل الشتاء.

وقد أظهرت النتائج وجود أعلى تركيز للهرمون التستوستيرون في إناث الطيور خلال فصل الصيف وأقل تركيز في إناث البرمائيات خلال نفس الفصل أما بالنسبة للذكور فكان أعلى تركيز للهرمون في ذكور الأسماك خلال فصل الصيف مقارنة بباقي الطوائف بينما وجد أقل تركيز للهرمون في ذكور البرمائيات خلال فصل الصيف. وقد أثبتت النتائج أن وجود الاختلافات الجوهرية في تركيزات الهرمونات الجنسية والأيضية مرتبط باختلاف طول النهار والمواسم لبعض الحيوانات المختارة من طوائف مختلفة.