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## Research Article

# Biological Characteristics in Approaching to Biochemical and Heavy Metals of Edible Fish *Terapon puta*, Cuvier, 1829 from Different Fishing Sites along the Suez Canal, Egypt

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## Abstract

Biological characteristics, biochemical component and heavy metals levels were investigated in *Terapon puta* from three different fishing sites along the Suez Canal. The study aimed to evaluate the seasonal biological activities effect on protein, lipids, glycogen contents and heavy metals level. Length range, age and growth of *T. puta* were differed according to the fishing sites. Suez Bay, Great Bitter Lake and Lake Timsah constituting fish with mean total length of  $10.15 \pm 0.96$ ,  $12.38 \pm 1.74$  and  $11.21 \pm 0.95$  cm, with age groups of I, III, II years, growth rates (K) was found to be 0.88, 0.6, 0.7 per year and ( $L_{\infty}$ ) 13.1, 17.5, 15.5, respectively. The spawning period established to be in summer. Heavy metals Cu, Zn and Cr in water had the highest levels in spring, Cd and Ni in summer while Fe in autumn. Levels of metals in fish muscles had the highest values of Cu and Zn in winter, Pb, Ni, Cd, Co, Cr in spring and summer which coincided the spawning period. Biochemical results in muscles revealed that the highest total protein was recorded in autumn, total lipid and glycogen were in spring. The spawning activity was correlated with increasing of protein and lipids levels in both pre-spawning (spring) and spawning seasons (summer), contrariwise the glycogen attained its lowest levels as it consumed to evaluate the spawning activity.

**Key words:** *Terapon puta*, growth rate, spawning period, protein, lipid, glycogen, heavy metal

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**Competing Interest:** The authors have declared that no competing interest exists.

**Data Availability:** All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Fish occupies a particular position in the aquatic contamination studies, because it plays significant role in establishing water quality guidelines and is considered as a main part of human diet (Safahieh *et al.*, 2011). *Terapon puta* is a commercial fish, caught with different types of fishing gears including gill nets, trammel net, beach seine and bottom trawl. It is widespread in the Indo-Pacific, Red Sea and Persian Gulf, it is a lessepsian migratory species (Golani *et al.*, 2002; Riede, 2004). It is a small-sized species with maximum total length about 16 cm, commonly between 11 and 13 cm, adults inhabit coastal waters, entering brackish estuaries (Allen and Swainston, 1988). Despite the extensive distribution of this species along the indo-pacific waters, a little information on its biological traits has been reported. Ben-Tuvia (1986) in Bardawil lagoon, Egypt, Paxton *et al.* (1989) in Australia studied the general biology of *T. puta*, Sommer *et al.* (1996) investigated the food and feeding behavior in Somalia, Broad (2003) reported the population characteristics in Philippines, Alwany and Hassan (2008) studied the relationship between otoliths size and the fish body size of *T. puta* from the Gulf of Suez and Nandikeswari *et al.* (2013) studied sex ratio and the population characteristics of *T. puta* in India.

The biochemical constituents of fish muscle give aspects on seasonal effects and other external and internal stressors. The live weight of the majority of fish usually consists roughly of water (70-80%), protein (20-30%) and lipids (2-12%) (Weatherley and Gill, 1987). The values vary considerably within and between species, size, sexual condition, maturity, feeding, time of year and activity. There are many studies revealed to composition of fish muscle, *Pesttode serumi* (Das and Mishra, 1990), *Liza aurata* (Abo Hegab *et al.*, 1994), *Saurida undosquamus* and *Nemipterus japonicus* (Abou El-Naga, 2003) and *Rhabdosaragus haffara* (Abou El-Naga and El-Boray, 2004).

Heavy metals constitute a highlighted group of pollutants in aquatic ecosystems because of their accumulative behavior (Turkmen *et al.*, 2009). Since they are not biodegradable, they could enter aquatic food chain (Tuzen, 2003; Altindag and Yig, 2005) and consequently accumulate in organisms positioned in various trophic levels. This condition may also produce health problem in people who consume contaminated seafood (Dural *et al.*, 2007). Recent urbanization of the region has led to the situation that the Suez Canal system is suffering considerably from pollution, since it acts as a big navigation route. Recently, several authors studied the effect of pollution and biochemical components on the biological features (length, weight, growth, maturation and spawning) of fish population (Farkas *et al.*, 2003). The toxic effects of pollutants

may affect behavior, cellular metabolism, endocrine regulations and reproduction of aquatic organisms (Popek *et al.*, 2004).

The Suez Canal is considered to be the shortest link between the east and the west due to its unique geographic location; it is an important international navigation canal linking between the Mediterranean Sea at Port said and the Red Sea at Suez. The unique geographical position of the Suez Canal makes it of special importance to the world and to Egypt as well (El Attar, 2013; Kenawy, 2015).

Three fishing sites along Suez Canal (Suez Bay, Great Bitter Lake and Lake Timsah) had been chosen to study the biological characteristics of *Terapon puta* which may be affected by the changes in the three environments. This study is considered to be the first documentation on the population structure, growth, length-weight, condition factor and reproduction aspects of the *Terapon puta* along the Suez Canal. The aim of the present study was to provide baseline information about biochemical constituents and heavy metals concentration within their effects on the biological characteristics on *T. puta* muscles as a commercial edible fish.

## MATERIALS AND METHODS

**Investigated fishing sites:** Lake Timsah covers about 15 km<sup>2</sup> between 30°32' and 30°36' North latitude and 32°16' and 32°21' East longitude and is located near the middle of the Suez Canal at a point 80 km South of Port Said. The Great Bitter Lake (30°20' N, 32°23' E) are the largest water bodies along the Suez Canal and have a surface area of about 250 km<sup>2</sup>. The Suez Bay includes the northern part of the Gulf of Suez. It extends from the tip of the Gulf to Ras Abo El-Darag on the Western side and to Ras Matamer on the Eastern side with depth range of 1.5-10 m. The three investigated areas (Fig. 1), Lake Timsah, Great Bitter Lake and Suez Bay are represented the artisanal fishing sites along the Suez Canal with small boats of about 3-7 m long that used gill and trammel nets.

**Data collection:** Seasonal random samples of *Terapon puta* were obtained from the commercial fishing boats operating and landing in Lake Timsah, Great Bitter Lake and Suez Bay during 2011/2012.

### Data analysis

**Biological analysis:** In total, 474 individuals were analyzed, 151, 158 and 165 samples from the Suez Bay, Great Bitter Lake and Lake Timsah, respectively. Samples total length (cm), total weight (g) was determined. The gonads were examined macroscopically to determine the sex and the maturity stages, which classified into three main categories according to the

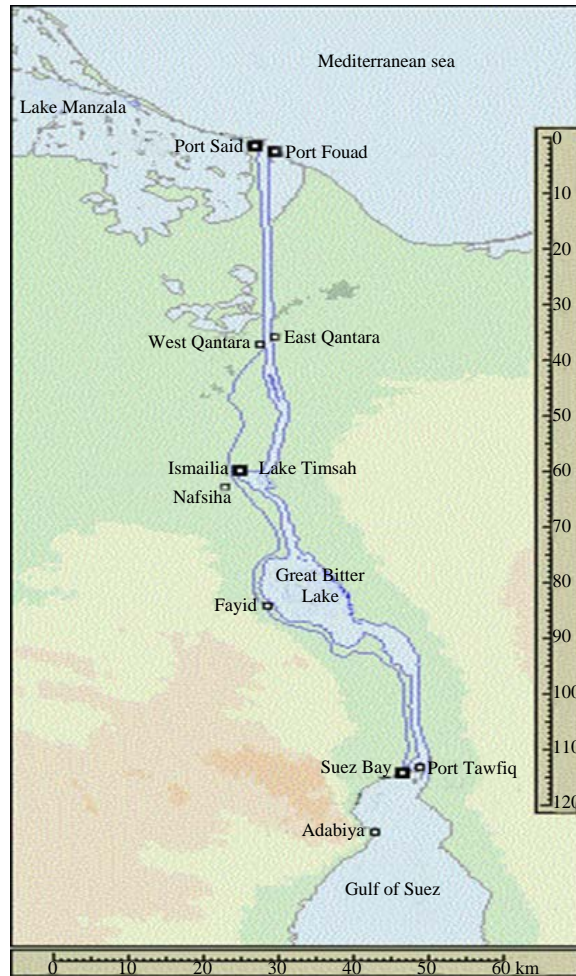


Fig. 1: Suez Bay, Great Bitter Lake and Lake Timsah fishing sites along the Suez Canal

scale of Andreu (1957), pre-spawning, spawning and post spawning stages. Gonado-Somatic Index (GSI) was estimated seasonally and calculated as:

$$GSI = \frac{\text{Gonad weight}}{\text{Total weight}} \times 100$$

Otoliths were taken from each fish and put in a small envelope for age determination. Length weight relationship was computed according to Le Cren (1951), by the equation:

$$W = a L^b$$

where, W is the total body weight in gram, L is total length in cm, a is a coefficient related to body form and b is a factor indicating the growth type. Condition factor (K) was calculated using the equation of Schreck and Moyle (1990):

$$K = W \times 100 / L^3$$

where, K is the condition coefficient, W is weight in grams, L is length in cm and 100 is a factor to bring the value of K near unity. The Von Bertalanffy (1938) growth parameters were estimated by means of ELEFAN-I incorporated in FISAT program (Munro, 1984; Gayanilo *et al.*, 1995). The von Bertalanffy growth equation was used to determine the lengths of *T. puta* at various ages as follows:

$$L_t = L_\infty (1 - e^{-k(t-t_0)})$$

where,  $L_t$  is length at time t,  $L_\infty$  is asymptotic length, k is the growth rate and  $t_0$  is the age at length 0.

**Biochemical analysis:** Total soluble protein was extracted seasonally from the fish samples in each fishing site by saline

solution (0.9 g NaCl%) and measured by biuret reagent according to Gronall *et al.* (1949). Total lipids were determined according to Knight *et al.* (1972). Glycogen was measured by the method of Seifter *et al.* (1950). Water percentage was calculated as the difference between wet weight and dry weight after drying the specimens in an oven at 80°C for 24 h.

**Heavy metals analysis:** Water samples were collected into 1 L capacity plastic bottles from the coastal area (>30 cm subsurface) for measurements of heavy metals with three replicas each season. The metals in seawater samples were pre-concentrated using APDC and MIBK extraction procedure according to standard methods (Brewer *et al.*, 1969; APHA, AWWA and WPCF., 1989). The obtained results were expressed in ppb ( $\mu\text{g L}^{-1}$ ). Fish sample from each site were dissected and the muscles were separated for metal analysis. Five grams of muscles (wet weight) were digested in Teflon cups using 4 mL  $\text{HNO}_3$  and 2 mL  $\text{HClO}_4$  then left at room temperature. After 24 h, samples were heated subsequently at 100°C for 2 h. After cooling, the solutions were then made up to 25 mL with double distilled water (Schuhmacher and Domingo, 1996). The obtained results were expressed in ppm ( $\mu\text{g g}^{-1}$  wet weight). The analyses of metals in all samples were performed by PerkinElmer (Analyst 100) atomic absorption spectrophotometer. The metals were determined by flame technique. The detection limits of the studied metals were the following: Cu (1.5 ppb), Zn (0.85 ppb), Cd (0.18 ppb), Pb (9.7 ppb), Ni (2.06 ppb), Co (8.8 ppb), Fe (4.6 ppb), Cr (3 ppb). Accuracy and precision were verified by using reference materials for biota (MA-A-2/TM) which was within the range of certified values with 95-111% recovery for the metals studied.

**Statistical analysis:** The statistical analysis of variance (ANOVA) and the values for each fishing site were tested by t-test to verify its significance level ( $p < 0.05$ ) in different seasons using the computer program Statistica version 10.

**RESULTS**

**Biological characteristics**

**Fishing site specific length distribution:** Samples of *T. puta* from Suez Bay fishing site ranged in total length between 7.2-11.8 cm with mean length of  $10.15 \pm 0.96$  cm, while in Great Bitter Lake it was ranged from 8.2-16.7 cm with mean of  $12.38 \pm 1.74$  cm and the fish from Lake Timsah varied in length from 8.8-14.2 cm with mean  $11.21 \pm 0.95$  cm (Table 1). The length distributions of *T. puta* clearly showed differences in sizes by fishing site. Figure 2 showed the distributions of length groups from the three fishing sites, it is clear that the length groups 10 and 11 cm constituted the highest value in the three fishing sites, followed by length groups of 12 and 13 cm in Great Bitter Lake and Lake Timsah. The statistical analysis t-test indicated no significant evidence in length distribution between sexes ( $p = 0.112 > 0.05$ ), while it showed significant difference between the three fishing sites ( $p = 0.001 < 0.05$ ).

**Age determination and site specific age composition:** A total of 151, 158 and 165 otoliths were used for age determination of *T. puta* from Suez Bay, Great Bitter Lake and Lake Timsah, respectively. Based on the number of rings on the otoliths, the oldest fishes were 3 years old recorded in Great Bitter Lake, while the youngest fish was 0 groups (7.2-8.4 cm)

Table 1: Total length, total weight, growth coefficient, asymptotic length and maximum age of *Terapon puta* from three different fishing sites along the Suez Canal

Fishing sites	No. of fish	TL range (cm)	Mean L $\pm$ SD	TWt range (g)	Mean Wt $\pm$ SD	K/year	L $\infty$ (cm)	Max. age (year)
Suez Bay	151	7.2-11.8	$10.15 \pm 0.96$	6.0-24.0	$13.50 \pm 4.38$	0.88	13.1	1
Bitter Lakes	158	8.2-16.7	$12.38 \pm 1.74$	7.0-58.2	$23.91 \pm 9.62$	0.60	17.5	3
Lake Timsah	165	8.8-14.2	$11.21 \pm 0.95$	8.0-31.0	$16.45 \pm 4.37$	0.70	15.5	2

SD: Standard deviation, TL: Total length, TWt: Total weight, L $\infty$ : Asymptotic length, K: Growth coefficient

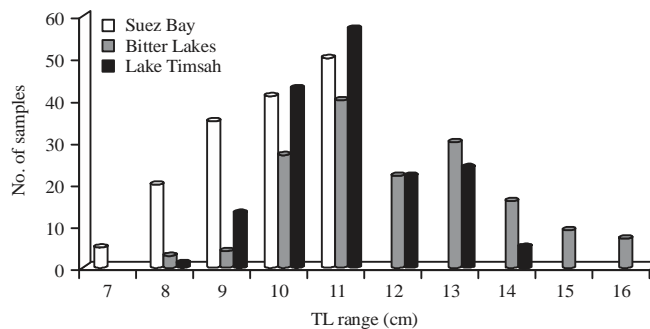


Fig. 2: Fishing sites specific length frequency distribution of *T. puta* along the Suez Canal

and 1 year were recorded in the Suez Bay (8.5-11.0 cm), however, fish from Lake Timsah reached 2 years old. The age composition of *T. puta* indicated that age group one was the most frequent age group constituting over 50% of the total collected samples in all sites, then the percentage decreased as the fish grow in age. Age group 0 was appeared by high percentage 37.1% at the Suez Bay, while it constituted low value in the other fishing site. Results revealed that fish from Suez Bay fishing site constituted the smallest length and age, while that from Great Bitter Lake were the biggest ones (Table 1).

**Fishing site specific length-weight relationship:** The relationship between total length and total body weight of *T. puta* was estimated for the three fishing sites. Results revealed highly correlation ( $r^2$  ranged 0.85-0.90) and the relationship was computed as  $W = 0.008 L^{3.14}$ ,  $W = 0.024 L^{2.72}$  and  $W = 0.018 L^{2.82}$  for fishes from the Suez Bay, Great Bitter Lake and Lake Timsah, respectively. It is clear that the growth in weight from the Suez Bay was characterized by positive allometric growth ( $b > 3$ ), despite in the Great Bitter Lake and Lake Timsah exhibit negative allometric growth ( $b < 3$ ). No significant difference was found between sexes within each fishing site ( $p > 0.05 = 0.114$ ), in the opposite the LWR showed a significant difference between the fishing areas ( $p < 0.05 = 0.007$ ).

**Fishing site specific condition factor:** The seasonal variation in Condition Factor (CF) of *T. puta* was showed almost similar trend along the three fishing sites during the study period. Condition factor scoring a peak value during autumn 1.223, 1.284, 1.575 and winter 1.351, 1.400, 1.452 seasons for

Suez Bay, Great Bitter Lake and Lake Timsah, respectively. It also tended to its lowest value during spring 0.988, 1.041, 1.112 and summer 0.955, 0.992, 1.078 for the three fishing sites, respectively (Fig. 3). Condition factor significantly difference between seasons in all sites ( $p < 0.05 = 0.003$ , means are different), while it exhibit no significant difference between sexes ( $p > 0.05 = 0.302$ , means are the same).

**Gonad maturation and spawning season:** Seasonal variation in gonad maturation of *T. puta* from the three investigated sites was observed; hence, it took place by the same rhythm. The statistical analysis revealed that there were significant differences in gonad maturation between males and females ( $p = 0.0006$ ), between seasons ( $p = 0.0002$ ) and between fishing sites ( $p = 0.0001$ ). Full ripe fishes (spawning stage) were observed in spring and summer with highest abundance during spring; while the post spawning stages were appeared in autumn and winter seasons by over 60% of the total samples. The pre spawning fish were detected in winter season by a considerable value. Seasonal variations in Gonado-Somatic Index (GSI) in males and females as shown in Fig. 4a and b, respectively, confirmed the changes in the gonad maturation in the three fishing sites. It was clear that GSI in males and females increased sharply in spring and continue in summer with a relatively decreasing (spawning stage), then it was decreased in autumn and maintained its lowest value at winter (post spawning stage). Therefore, the fish maturation was considered to be started at spring by a high ratio and extended to summer. In summer a considerable numbers of gonads were found to be released their eggs and seemed to be in the running condition (spawning stag); therefore it can be concluded that *T. puta* is a summer spawner fish.

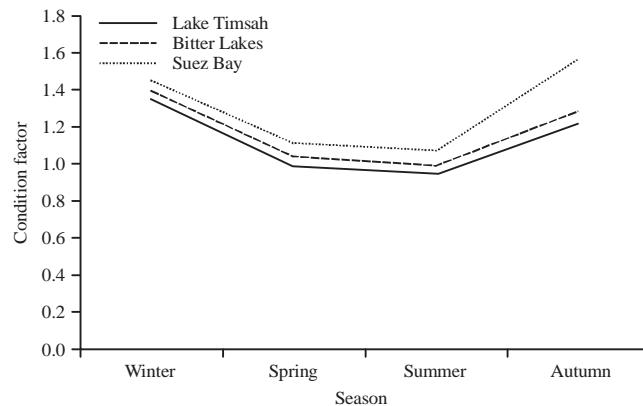


Fig. 3: Seasonal variation in condition factor of *T. puta* from different fishing sites along the Suez Canal

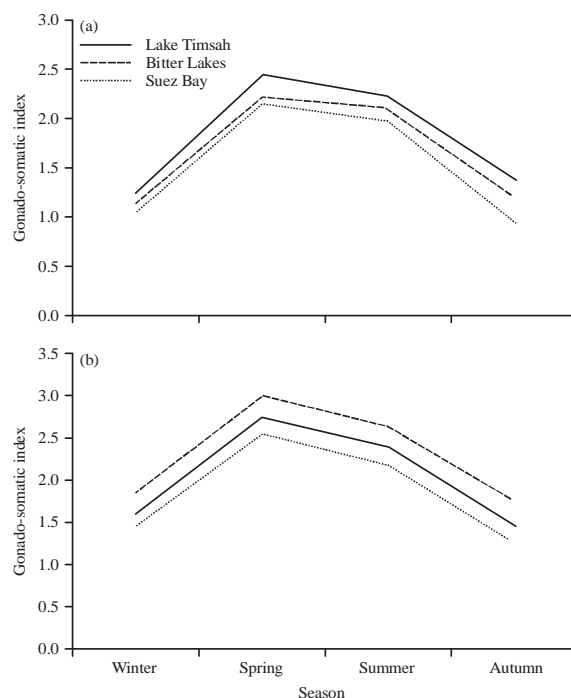


Fig. 4(a-b): Seasonal variations in Gonado-somatic index of *T. puta* (a) Males and (b) Females along the Suez Canal

Table 2: Seasonal variation of water content, total protein, total lipids and glycogen (Mean  $\pm$  SE) in muscle of *Terapon puta* along the Suez Canal

Seasons	Water content	Total protein	Total lipids	Glycogen
<b>Suez Bay</b>				
Winter	76.56 $\pm$ 0.38	9.85 $\pm$ 0.94	1.03 $\pm$ 0.13	0.32 $\pm$ 0.13
Spring	76.17 $\pm$ 0.77	8.90 $\pm$ 0.14	1.45 $\pm$ 0.08	0.37 $\pm$ 0.01
Summer	75.58 $\pm$ 0.72	9.45 $\pm$ 0.75	1.29 $\pm$ 0.08	0.28 $\pm$ 0.02
Autumn	74.69 $\pm$ 0.58	13.73 $\pm$ 0.96	0.94 $\pm$ 0.14	0.22 $\pm$ 0.02
<b>Bitter Lake</b>				
Winter	76.06 $\pm$ 1.37	90.05 $\pm$ 0.16	0.84 $\pm$ 0.08	0.26 $\pm$ 0.04
Spring	71.66 $\pm$ 0.76	8.50 $\pm$ 0.14	2.07 $\pm$ 0.17	0.40 $\pm$ 0.02
Summer	73.84 $\pm$ 1.12	11.50 $\pm$ 0.52	0.83 $\pm$ 0.06	0.33 $\pm$ 0.03
Autumn	73.60 $\pm$ 0.96	12.95 $\pm$ 0.80	1.11 $\pm$ 0.18	0.18 $\pm$ 0.01
<b>Lake Timsah</b>				
Winter	76.16 $\pm$ 0.22	10.55 $\pm$ 0.28	1.02 $\pm$ 0.20	0.27 $\pm$ 0.04
Spring	74.18 $\pm$ 0.60	8.50 $\pm$ 0.34	1.88 $\pm$ 0.14	0.40 $\pm$ 0.02
Summer	76.48 $\pm$ 0.88	11.50 $\pm$ 0.89	1.35 $\pm$ 0.10	0.30 $\pm$ 0.02
Autumn	73.30 $\pm$ 0.44	13.00 $\pm$ 0.78	1.05 $\pm$ 0.13	0.32 $\pm$ 0.08

### Biochemical investigation

**Water content:** The highest value of water percentage in muscle of *T. puta* (76.56, 76.06 and 76.48%) in different sites was recorded in winter while the lowest water content (74.69, 73.60 and 73.30%) was recorded in autumn (Table 2). There is a significant difference in water content between seasons in all sites, winter and autumn (Suez Bay,  $p < 0.032$ ), winter and spring (Great Bitter Lake,  $p < 0.022$ ), while autumn, summer and winter ( $p < 0.011$ ,  $p < 0.000$ ) (Table 3). The annual value of water content in fish muscle was 75.74, 73.79 and 75.03% in Suez Bay, Great Bitter Lake and Lake Timsah, respectively (Table 4). There is a significant difference between fish muscle water content in Suez Bay and Great Bitter Lake ( $p < 0.0023$ ).

**Total protein:** Table 2 showed that, total protein in the muscle of *T. puta* in Suez Bay, Great Bitter Lake and Lake Timsah attained the highest value in autumn  $13.73 \pm 0.96$ ,  $12.95 \pm 0.80$  and  $13.00 \pm 0.78$  g/100 g, respectively. Autumn attain the lowest protein values ( $8.90 \pm 0.14$ ,  $8.50 \pm 0.14$  and  $8.50 \pm 0.34$  g/100 g).

Total protein in autumn was significant difference with winter ( $p < 0.009$ ), spring ( $p < 0.000$ ) and summer ( $p < 0.002$ ) in Suez Bay. In Great Bitter Lake protein value of fish muscle in spring was significant difference with winter ( $p < 0.000$ ), summer ( $p < 0.008$ ) and autumn ( $p < 0.001$ ). In Lake Timsah it was significant difference between summer and winter, spring and between autumn and winter, spring (Table 3). Annual

Table 3: Significant difference between seasons in the muscle water content, total protein, total lipids and glycogen of *T. puta* along the Suez Canal

Seasons	Suez Bay			Bitter Lakes			Lake Timsah		
	Spring	Summer	Autumn	Spring	Summer	Autumn	Spring	Summer	Autumn
<b>Water contents</b>									
Winter			p<0.032	p<0.022			p<0.014		p<0.000
Spring									
Summer									p<0.011
<b>Total protein</b>									
Winter			p<0.009	p<0.000				p<0.001	p<0.000
Spring			p<0.000		p<0.008	p<0.001		p<0.000	p<0.000
Summer			p<0.002						
<b>Total lipids</b>									
Winter	p<0.008			p<0.000			p<0.008		
Spring			p<0.002		p<0.001	p<0.004		p<0.014	p<0.002
Summer			p<0.035						
<b>Glycogen</b>									
Suez Bay									
Winter	p<0.014	p<0.045	p<0.000	p<0.013			p<0.020		
Spring		p<0.000	p<0.000			p<0.000		p<0.002	
Summer			p<0.007			p<0.001			

Table 4: Annual Mean±SE of total protein, total lipids, glycogen and water content in the muscle of *Terapon puta* along the Suez Canal

Parameters	Suez Bay	Bitter Lakes	Lake Timsah
Water content	75.74±0.31*	73.79±0.61*	75.03±0.41
Total protein	10.35±0.49	10.58±0.45	10.44±0.48
Total lipids	1.18±0.06	1.21±0.13	1.32±0.11
Glycogen	0.30±0.01	0.29±0.02	0.32±0.02

\*p<0.032, SE: Standard error

protein means were 10.35±0.49, 10.58±0.45 and 10.44±0.48 g/100 g in Suez Bay, Great Bitter Lake and Lake Timsah (Table 4). It was clear that there was no significant difference between protein contents.

**Total lipids:** Total lipids was recorded the highest values in fish muscle during spring (1.45±0.8, 2.07±0.17 and 1.88±0.14 g/100 g) in Suez Bay, Great Bitter Lake and Lake Timsah, respectively. While the lowest values in fish muscle was recorded in autumn for the Suez Bay and Lake Timsah (0.94±0.14 and 1.05±0.13 g/100 g) and during summer for Great Bitter Lake fish muscle was 0.83±0.06 g/100 g (Table 2). Table 3 illustrates significant difference in Suez Bay between autumn, summer and spring (p<0.002, p<0.035) and in Great Bitter Lake between spring and winter, summer, autumn (p<0.000, p<0.001, p<0.004), also in Lake Timsah, fish muscle lipid was significant difference between spring and winter, summer, autumn (p<0.008, p<0.014, p<0.002). The annual mean of total lipids in Suez Bay, Great Bitter Lake and Lake Timsah were 1.18±0.06, 1.21±0.18 and 1.32±0.11 g/100 g and there is no significant difference between lipid content in different sites (Table 4).

**Glycogen:** Table 2 showed that the highest glycogen content was recorded in spring for all sites (0.37±0.01, 0.40±0.02 and 0.40±0.02 g/100 g) and the lowest glycogen values were

attained in autumn 0.22±0.02 and 0.18±0.01 g/100 g for Suez Bay and Great Bitter Lake, while in Lake Timsah, the lowest glycogen value was 0.27±0.04 g/100 g in winter. Significant difference were exists between autumn and winter, spring, summer (p<0.000, p<0.000, p<0.007), also between summer and winter, spring in fish muscle in Suez Bay. In Great Bitter Lake, there was significant difference between autumn and spring, summer (p<0.000, p<0.001). Glycogen in fish muscle from Lake Timsah exhibit significant difference between spring and winter, summer (p<0.020, p<0.002). The annual mean of fish muscle glycogen in Suez Bay (0.30±0.01 g/100 g), Great Bitter Lake (0.29±0.02) and Lake Timsah (0.32±0.02 g/100 g) were shown in Table 4. Also, there is no significant difference between glycogen in fish muscle collected from the three sites.

**Heavy metals investigation:** The concentrations of Cu, Zn, Cr, Cd, Ni and Fe in water collected from Suez Bay, Great Bitter Lake and Lake Timsah are illustrated in Fig. 5 while Pb and Co were not detected in water samples. Generally, the highest values of Cu, Zn and Cr (2.796, 13.45 and 3.713 µg L<sup>-1</sup>), respectively recorded at spring, Cd and Ni recorded at summer (0.733 and 2.841 µg L<sup>-1</sup>), respectively and Fe noticed at autumn (26.02 µg L<sup>-1</sup>). According to the spatial variation, the highest values of Zn, Cr and Cd were in the Suez Bay, Fe and Ni were recorded in the Great Bitter Lake and Cu was in Lake



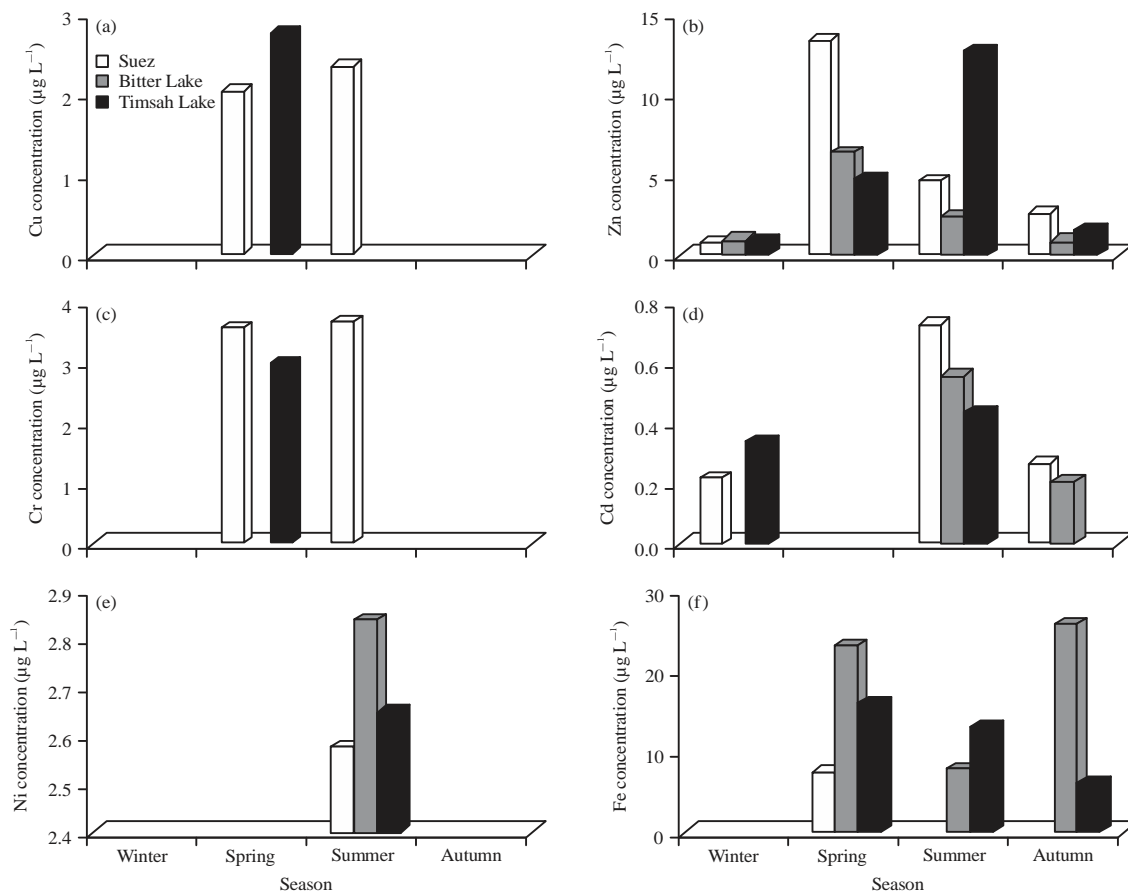


Fig.5(a-f): Seasonal variation of heavy metals (a) Cu, (b) Zn (c) Cr, (d) Cd, (e) Ni and (f) Fe in water ( $\mu\text{g L}^{-1}$ ) collected from Suez Bay, Great Bitter Lake and Timsah Lake during 2011-2012

Table 5: Average  $\pm$ SD of heavy metals in the muscles of *Terapon puta* collected from Suez Bay, Bitter Lakes and Lake Timsah during 2011-2012

Season and stations	Heavy metals							
	Cu	Zn	Pb	Fe	Cd	Ni	Co	Cr
<b>Winter</b>								
Suez Bay	1.53 $\pm$ 0.32	5.32 $\pm$ 2.62	0.38 $\pm$ 0.06	4.36 $\pm$ 1.09	0.08 $\pm$ 0.02	0.07 $\pm$ 0.07	0.07 $\pm$ 0.04	0.42 $\pm$ 0.22
Bitter Lakes	0.93 $\pm$ 0.86	3.97 $\pm$ 1.91	0.22 $\pm$ 0.23	2.72 $\pm$ 2.31	0.05 $\pm$ 0.04	0.07 $\pm$ 0.00	0.06 $\pm$ 0.02	0.32 $\pm$ 0.14
Lake Timsah	0.89 $\pm$ 0.05	2.94 $\pm$ 1.46	ND	2.52 $\pm$ 0.29	0.04 $\pm$ 0.00	0.04 $\pm$ 0.05	ND	0.23 $\pm$ 0.13
<b>Spring</b>								
Suez Bay	0.68 $\pm$ 0.42	2.57 $\pm$ 1.12	0.23 $\pm$ 0.00	1.96 $\pm$ 1.13	0.03 $\pm$ 0.02	0.04 $\pm$ 0.03	0.04 $\pm$ 0.03	0.21 $\pm$ 0.09
Bitter Lakes	0.55 $\pm$ 0.18	1.85 $\pm$ 1.02	0.11 $\pm$ 0.16	1.54 $\pm$ 0.59	0.02 $\pm$ 0.01	0.03 $\pm$ 0.01	0.03 $\pm$ 0.01	0.15 $\pm$ 0.08
Lake Timsah	0.37 $\pm$ 0.26	1.44 $\pm$ 0.58	0.14 $\pm$ 0.03	1.06 $\pm$ 0.67	0.01 $\pm$ 0.01	0.02 $\pm$ 0.02	0.02 $\pm$ 0.02	0.12 $\pm$ 0.05
<b>Summer</b>								
Suez Bay	0.52 $\pm$ 0.11	2.89 $\pm$ 0.76	0.90 $\pm$ 0.19	4.38 $\pm$ 1.52	0.39 $\pm$ 0.13	0.79 $\pm$ 0.25	1.55 $\pm$ 0.49	5.07 $\pm$ 2.43
Bitter Lakes	0.51 $\pm$ 0.06	2.51 $\pm$ 0.61	0.83 $\pm$ 0.19	3.24 $\pm$ 0.42	0.33 $\pm$ 0.03	0.44 $\pm$ 0.09	1.03 $\pm$ 0.10	3.74 $\pm$ 0.99
Lake Timsah	0.63 $\pm$ 0.10	3.72 $\pm$ 1.04	1.28 $\pm$ 0.52	4.83 $\pm$ 1.53	0.54 $\pm$ 0.18	0.63 $\pm$ 0.05	1.82 $\pm$ 0.58	4.84 $\pm$ 1.13
<b>Autumn</b>								
Suez Bay	0.40 $\pm$ 0.22	2.75 $\pm$ 0.57	0.29 $\pm$ 0.10	5.49 $\pm$ 0.73	0.15 $\pm$ 0.05	0.28 $\pm$ 0.14	0.17 $\pm$ 0.09	0.57 $\pm$ 0.23
Bitter Lakes	0.38 $\pm$ 0.07	3.42 $\pm$ 0.27	0.23 $\pm$ 0.04	5.56 $\pm$ 1.36	0.16 $\pm$ 0.03	0.29 $\pm$ 0.04	0.10 $\pm$ 0.02	0.62 $\pm$ 0.15
Lake Timsah	0.37 $\pm$ 0.06	3.14 $\pm$ 0.54	0.23 $\pm$ 0.11	5.79 $\pm$ 0.71	0.13 $\pm$ 0.05	0.28 $\pm$ 0.09	0.09 $\pm$ 0.07	0.72 $\pm$ 0.17

Timsah (Fig. 5). The concentrations of Cu, Zn, Pb, Cr, Cd, Ni, Co and Fe in muscles of *Terapon puta* collected from Suez Bay, Great Bitter Lake and Lake Timsah are shown in Fig. 6 and

Table 5. The maximum concentration of Cu and Zn were observed in Great Bitter Lake, while in Lake Timsah the highest values were recorded for Fe, Cd and Ni. In the Suez Bay the

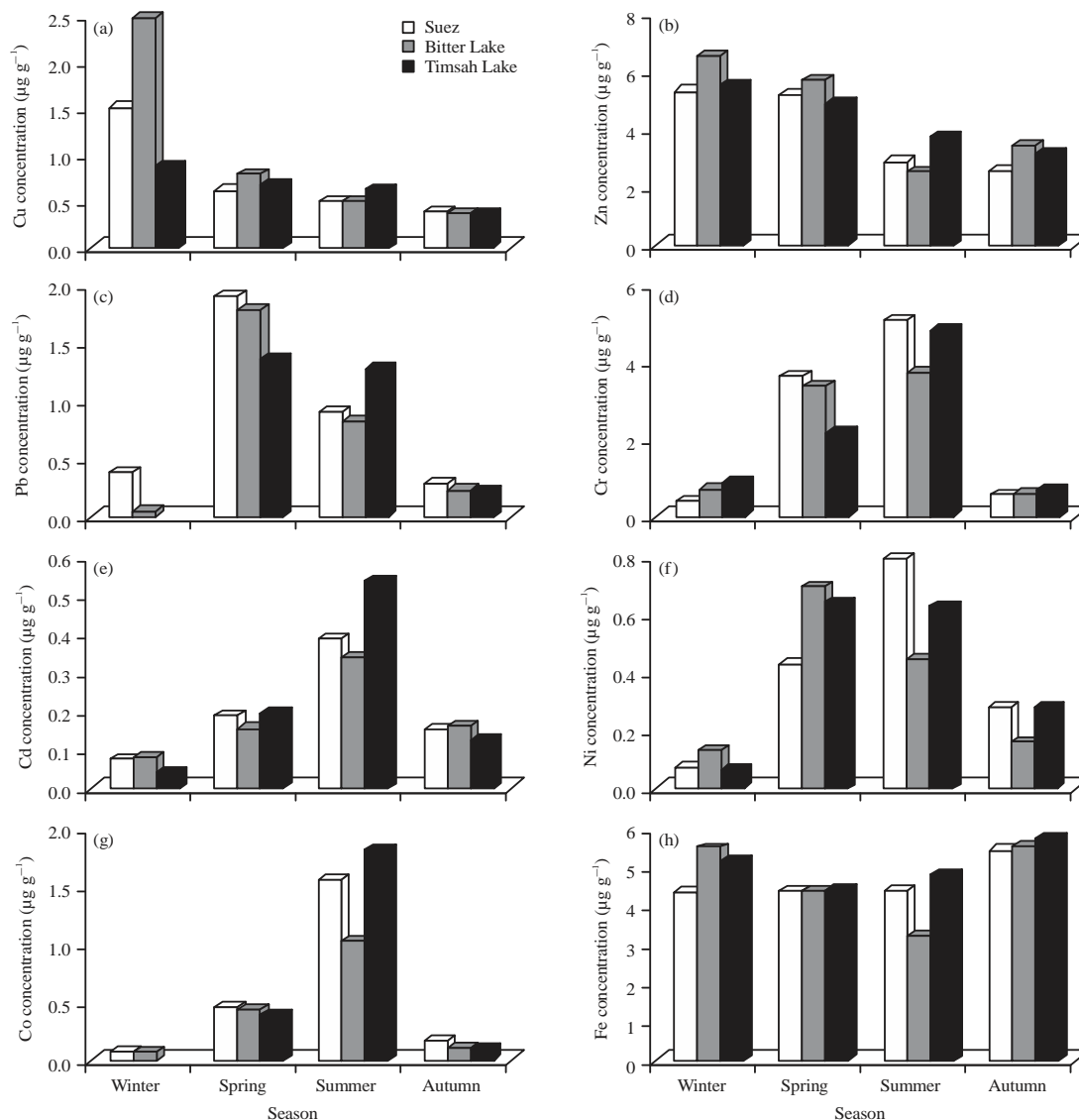


Fig.6(a-h): Seasonal variation of heavy metals (a) Cu, (b) Zn, (c) Pb, (d) Cr, (e) Cd, (f) Ni, (g) Co and (h) Fe in the muscles of *Terapon puta* collected from Suez Bay, Great Bitter Lake and Timsah Lake during 2011-2012

Table 6: Annual Mean±SD of heavy metals in the muscles of *Terapon puta* collected from Suez Bay, Great Bitter Lakes and Lake Timsah

Stations	Heavy metals concentration (µg g <sup>-1</sup> )							
	Cu	Zn	Fe	Pb	Cd	Ni	Co	Cr
Suez Bay	0.769±0.517	3.999±1.468	4.654±0.543	0.874±0.740	0.203±0.133	0.398±0.304	0.563±0.680	2.435±2.307
Bitter Lakes	1.045±0.972	4.532±1.877	4.688±1.108	0.729±0.782	0.185±0.109	0.360±0.265	0.409±0.446	2.119±1.676
Lake Timsah	0.653±0.218	4.326±1.089	5.059±0.580	0.959±0.637	0.225±0.219	0.407±0.282	0.769±0.918	2.161±1.904

maximum concentrations of metals were mentioned for Pb, Co and Cr. From Fig. 6, it could be recognized that Cu and Zn showed their maximum concentration in the fish muscles at winter then their concentrations decreased gradually during the year till they reached their minimum value at autumn in all sites. The Cd, Cr, Co, Ni and Pb showed their high concentrations during spring and summer in the investigated

sites while Fe showed an opposite pattern where the lower values appear to be at spring and summer. According to the spatial variation it could be noticed that Pb, Co, Cd, Ni and Fe exhibited their maximum annual mean at the Lake Timsah while Zn and Cu found to be at Great Bitter Lake. Suez Bay showed the highest annual mean of Cr in the muscles of the collected fish (Table 6).

## DISCUSSION

*Terapon puta* is a commercial edible and desirable fish distributed along the Suez Canal. It is evidence that the size structures and age groups distribution of *T. puta* community are differed by each fishing site; the smallest fish size (7.2-11.8 cm) and age of one year were appeared in the Suez Bay, as it is considered to be the main spawning and nursery area for the most of fish population from the Gulf of Suez (Sabrah *et al.*, 2011). Lake Timsah (max. length 14.2 cm and two age groups) followed the Suez Bay, since it exposed to high fishing pressure as it is intermediated Ismailia city clubs and resorts, with high consumer of fish. Fish of Great Bitter Lake constituted the largest length and age (max. length 16.7 cm and three age groups), as it is a spawning area that contained the mother's fish come from the Mediterranean Sea. The overall length trend is a reduction in large fish and a relative increase in small fish along the Suez Canal. In Australia Allen and Swainston (1988) reported 16.0 cm as a maximum total length of *T. puta*. The biological results recorded in this study could not be compared with previous studies due to it is the first-time to assess the biological characteristics, growth, length-weight and reproduction of *T. puta* along the Suez Canal Coasts. *Terapon puta* is a short life span fish (max. 3 years), so it is exhibited a relatively high growth rate K, which ranged between 0.60 and 0.88 per year.

Gonado-somatic index has been considered as reliable estimate for gonad maturity and spawning of any species. The gonado-somatic index increased with the maturation of fish and reaches to its maximum at the peak period of maturity. Its abrupt decrease indicates the beginning of spawning (Mishra and Saksena, 2012). *Terapon puta* GSI showed peak in spring indicating maturation, then decline in summer indicates the spawning activity, this pattern of changes in GSI of males is almost similar to that of females. The gonads were in the post-spawning stage of maturity during autumn and they were in the pre-spawning stage or resting in winter. The present results revealed that the *T. puta* is a summer spawner. Ben-Tuvia (1986) concluded that *T. puta* have one clear seasonal peak per year and the spawning season occurred in June, July and August in Bardawil lagoon, Egypt. Similar observation was made by Nandikeswari and Anandan (2013) in India, they reported that *T. puta* has spawning period beginning from March to October.

The condition factor (K) is an index reflecting the fitness or general well being of the fish populations (Le Cren, 1951). In the current study (K) attained minimum value in spring and summer (spawning phase), while exhibited maximum value in autumn and winter. The increase in the 'K' value during the

pre-spawning and post-spawning phases (autumn and winter) indicates active feeding behavior, consequently K-values decreased in the spawning period and the recovery takes place in the post-spawning (Rimmer, 1985).

The mean value of water content in the fish muscles were  $75.74 \pm 0.31\%$  from Suez Bay,  $73.79 \pm 0.61$  from Great Bitter Lake and  $75.03 \pm 0.41$  from Lake Timsah. Significant differences were found between seasons and locations (Suez Bay and Great Bitter lake). Cullen *et al.* (2003) reported that water content of fish muscle (*Euphausia superba*) ranged from  $83.61 \pm 0.46$  to  $80.37 \pm 1.68\%$ . The lower percentage of water content the greater of lipid and protein contents (Dempson *et al.*, 2004). Also Abou El-Naga (2003), mention that water content in muscle of fish *Saurida undosquamis*, *N. japonicus* and *S. japonicus* collected from Suez Gulf were  $78.19 \pm 0.38$ ,  $79.07 \pm 0.18$  and  $73.73 \pm 0.29\%$ , respectively. Highest moisture content was 83.50% in May and the lowest moisture content 70.50% in December and there is a significant difference was found (Zafar *et al.*, 2004). Protein content in muscle of *Terapon puta* was ranged from  $9.85 \pm 0.94$  to  $13.73 \pm 0.96$  (Suez Bay),  $8.50 \pm 0.14$  to  $12.95 \pm 0.80$  (Great Bitter Lake) and  $8.50 \pm 0.34$  to  $13.00 \pm 0.78$  (Lake Timsah). There are significant differences between protein content in seasons of locations. The mean protein values Suez Bay ( $10.35 \pm 0.49\%$ ), Great Bitter Lake ( $10.58 \pm 0.45\%$ ) and Lake Timsah ( $10.44 \pm 0.48\%$ ), there is no significant difference between them. Abou El-Naga and El-Boray (2004) reported that protein content of female and male *Rhabdosaragus haffara* attain  $11.96 \pm 0.48$  and  $12.68 \pm 0.60\%$  also the highest protein value was recorded in autumn while the lowest value was recorded in winter. Significant differences were found for protein content among seasons and the highest values  $12.12 \pm 0.31$  (male) and  $13.15 \pm 0.37$  (female) during May. Abou El-Naga (2003) reported that, protein mean value of *Saurida undosquamis* was  $14.66 \pm 0.47\%$ , *N. japonicas* was  $11.85 \pm 0.65\%$  and *S. japonicas* was  $12.74 \pm 0.51\%$  and there are significant differences between seasons. The highest protein value of *S. undosquamis* was recorded in autumn. Zafar *et al.* (2004) mention that the highest protein content of *Scylla serrata* was 20.1% in December and the lowest protein value 14.0% in May with annual mean value  $17.69 \pm 2.14\%$ . Lipid values of present study in Suez Bay, Great Bitter Lake and Lake Timsah were  $1.18 \pm 0.06$ ,  $1.21 \pm 0.13$  and  $1.32 \pm 0.11$ , there is no significant difference was found. While maximum lipid values ( $1.45 \pm 0.08$ ,  $2.07 \pm 0.17$  and  $1.88 \pm 0.14\%$ ) were found in spring to be used as energy supplement in winter and there are significant differences between seasons in different locations. Zafar *et al.* (2004) reported that the annual fat content in *Scylla serrata* was  $0.51 \pm 0.12$ ; there is a significant

difference between seasons. Abou El-Naga and El-Boray (2004) mention that the annual lipid values of female fish were  $1.565 \pm 0.11\%$  and male  $1.84 \pm 0.11\%$ . The highest lipid values were recorded in autumn for both female and males and the lowest lipid values were recorded in winter. Abou El-Naga (2003) investigated total lipids in three fish species collected from Suez Gulf and found that mean lipid content were  $0.60 \pm 0.09$ ,  $0.35 \pm 0.04$  and  $2.54 \pm 0.30\%$ . Results indicated that GSI values were correlated with increased of protein and lipids levels in both pre-spawning and spawning seasons of the fish under study, which may be due to enlarged vitellogenesis in ovary and spermatogenesis in testes that require large amount of lipoproteins under impact of hormones (Shengde and Mane, 2006). Glycogen value in fish muscle of the present study has annual mean  $0.30 \pm 0.01$ ,  $0.29 \pm 0.02$  and  $0.32 \pm 0.02\%$  and there is no significant difference between locations. Spring has the maximum glycogen values in different locations and autumn has the lowest glycogen values summer which may be contributed to its consuming to enhance the spawning process. There are significant differences between seasons for three locations. It recorded its lowest level in spring and summer which may be contributed to its consuming to enhance the spawning process. Abou El-Naga and El-Boray (2004) investigated glycogen content in females ( $0.15 \pm 0.02\%$ ) and males ( $0.18 \pm 0.01\%$ ). The highest glycogen content was recorded in winter for both females and males and the lowest values in summer (males) and spring (females).

From the water results of heavy metals, Suez Bay considered to be the most influenced site with heavy metals especially at summer season comparing to the other sites. Suez Bay is directly affected by many sources of pollution including shipping activity, fishing activity, petroleum refineries, steel factories, cement industry, power plants and the tourism activities. The concentrations of Cu, Zn, Cd and Cr in water at the Suez Bay site considered to be as a result of these activities. Cu and Zn found to leach from the ships and fishing boats where, Ghanem (1986) stated that, Cu antifouling coatings used in ships and fishing boats are the major source of Cu pollution. According to FAO (1992), Cu and Zn found to be in the paints used in the antifouling painting of ships while Cr used as additives in stainless steel production (Moore, 1991). By comparing the concentration of the studied metals with previous studies, it can be notice that present study results of water were lower than that recorded in the previous studies (Table 7). Fish respond sensitively to an increase of heavy metal concentration in water. Being at the top of the aquatic food chain, fish mirror the combination of the biotic and abiotic conditions, in the particular aquatic environment (Dragun *et al.*, 2009; Zubcov *et al.*, 2012). By referring to the water results, high concentrations of metals found to be at summer in most studied metals which can be the reason of increasing the heavy metal content in the fish muscles during this season. According to Ersoy and Celik (2009), seasonal changes of metal concentrations in fish may

Table 7: Comparison between concentrations of metals water samples of present study and other studies

Locations	Concentration ( $\mu\text{g L}^{-1}$ )								References
	Cu	Zn	Fe	Pb	Cd	Ni	Co	Cr	
Suez Bay	ND-2.37	0.85-13.45	ND-7.59	ND	ND-0.73	ND-2.58	ND	ND-3.71	Present study
Bitter lake	ND	1.06-6.42	ND-26.02	ND	ND-0.56	ND-2.84	ND	ND	
Lake Timsah	ND-2.79	0.98-12.79	ND-16.17	ND	ND-0.46	ND-2.65	ND	ND-3.04	
Lake Timsah	0.9	0.25	2.5	0.41	-	-	-	0.5	Ibrahim and Abu El-Regal (2014)
Timsah Lake	0.78-2.21	0.01-3.93	-	0.01-4.99	ND-0.20	-	-	-	El-Wazzan <i>et al.</i> (2013)
Suez Bay	3.06	5.79	8.15	0.80	0.36	1.02	-	-	El-Sawy (2009)
Al-Khadoud									
spring channel	2-5	31-30	980-2210	ND-21	4-4	-	-	-	Al-Kahtani (2009)
Lake Timsah	11-95	74-985	1399-1609	0.00-52	9-82	45-1490	-	-	Eissa <i>et al.</i> (2008)
Lake Timsah (conta. site)	100	170	1270	310	150	540	450	-	Gabr and Gab-Alla (2008)
Lake Timsah (trans. site)	60	90	960	170	30	280	27	-	
*Egyptian Standard	2000	-	-	15	5	20	-	-	Eissa <i>et al.</i> (2008)
Suez Canal	1.71	4.32	35.0	3.52	0.51	1.46	0.33	0.23	El-Moselhy <i>et al.</i> (2005)
Suez Gulf	3.03	17.31	22.55	1.02	0.20	1.58	-	-	Hamed <i>et al.</i> (2003)
Suez Bay	0.321-8.934	4.198-25.541	2.345-160.1	0.069-9.341	0.017-1.387	0.346-3.200	0.067-1.02	-	Mohamedein (2002)
Suez Bay	8.20	19.8	18.7	2.07	0.89	6.73	0.78	0.56	Hamed and El-Moselhy (2000)
Suez Canal	5.12	12.8	16.2	0.45	0.29	2.90	0.69	0.14	Hamed (1996)
USEPA	1500	500	-	50	1000	100	-	-	EPA (1986, 1999)
WHO	2000	5000	-	50	-	20	-	-	WHO (1993)
IOC/UNEP Limits	1450	4750	-	110000	21500	-	-	-	IOC/UNEP (1991)

\*Egyptian standard in Al-Waqae'a Al-Masreya Magazine (2001)

result from factors such as growth cycle, reproductive cycle and changes in water temperature. In the present study Cu and Zn concentrations in muscles decreased gradually from winter to autumn where the spawning season recorded to be in spring and summer. Fish may consume Cu and Zn during spring (pre-spawning phase) and summer (spawning period). All levels of heavy metals were in the lowest levels at winter season due to the lower reproductive activity. Zubcov *et al.* (2012) found that Zn and Cu concentration increased in gonad during spawning while decreased in muscles in that period. Also, Danilov and Shevchenko (1973) found that during fasting, fish lose Zn from muscles. The energy used for gonad formation is originated not only directly from food but also depleted from energy reserved in muscles (McKeown, 1984; Kamler, 1992). Which means it collapse after the spawning period and that can be attributed to their role in the fish gonad maturation and their important role in this physiological process. Zubcov *et al.* (2012) stated that Cu and Zn are essential constituents of many enzymes and are

carefully regulated by physiological mechanisms in most organisms, including fish. It is especially during the last 15-30 days prior to egg laying, trace element concentration in gonads increased 1.5-6 times for Zn and 3-11 times for Cu. The Cd, Cr, Co, Ni and Pb found to have their highest concentrations at spring and summer, these results confirm with increasing these elements in hot seasons.

Figure 7 and 8 showed the seasonal variations in heavy metals levels and the biochemical contents with the seasonal variations in Gonado-Somatic Index (GSI). It is clear that the levels of Cd, Ni, Co and Cr were increased in spring (pre-spawning), recording their highest levels in summer (spawning period), that probably due to the fish tend to accumulate metals during this metabolically active period, while Pb level maintained its highest level in spring than in summer. Most probable, the dynamics of trace element accumulation is determined by the growth rate of fish, which is the highest during summer (Zubcov *et al.*, 2009) and by changes in the feeding rate and lipid dynamics of the tissues

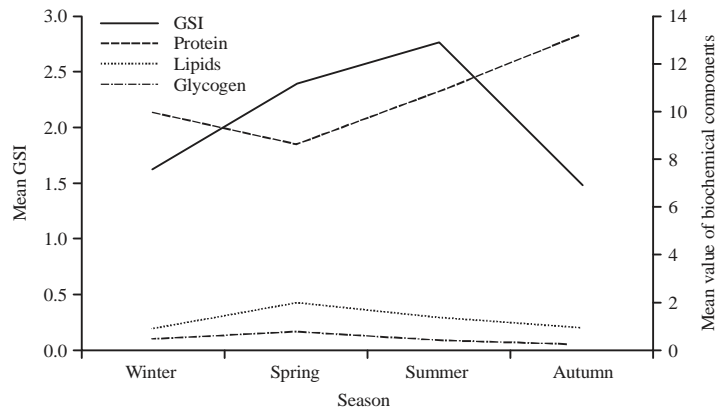


Fig. 7: Mean seasonal variation in gonado-somatic index in relation to the mean values of biochemical components in *T. puta* along the Suez Canal

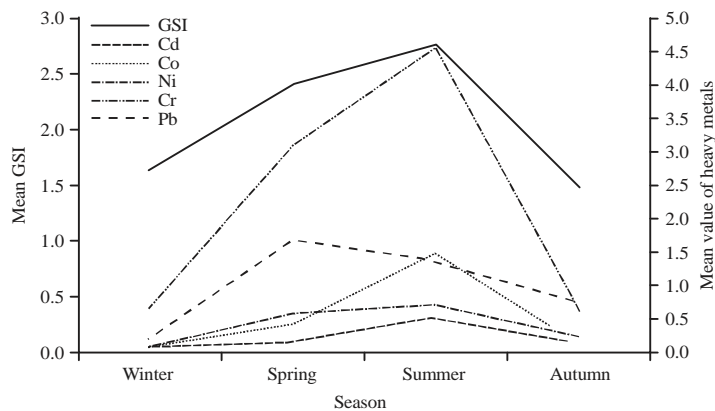


Fig. 8: Mean seasonal variation in gonado-somatic index in relation to the mean values of heavy metals in *T. puta* along the Suez Canal

Table 8: Comparison between concentrations of metals in *Terapon puta* samples of present study and other studies

Locations	Species	Metal concentration ( $\mu\text{g g}^{-1}$ )								References
		Cu	Zn	Fe	Pb	Cd	Ni	Co	Cr	
Suez Bay	<i>Terapon puta</i>	0.40-1.53	2.58-5.33	4.36-5.47	0.29-1.91	0.08-0.39	0.08-0.79	0.06-1.55	0.42-5.08	Present study
Bitter Lake	<i>Terapon puta</i>	0.38-2.48	2.51-6.51	3.24-5.57	0.06-1.79	0.08-0.34	0.13-0.69	0.07-1.03	0.62-3.74	
Lake Timsah	<i>Terapon puta</i>	0.37-0.90	3.14-5.53	4.42-5.79	0.23-1.37	0.04-0.19	0.07-0.65	0.09-1.82	0.72-4.84	
Bitter Lake	<i>Rhabdosargus haffara</i>	0.431	3.139	4.54	0.119	0.055	0.03	0.104	0.041	Hamed <i>et al.</i> (2012)
Lake Timsah	<i>Rhabdosargus haffara</i>	1.009	4.496	5.59	0.129	0.067	0.037	0.154	0.051	
Caspian Sea	<i>Rutilus frisii kutum</i>	2.76	15.45	-	0.270	0.000	-	-	-	Monsefrad <i>et al.</i> (2012)
Bengal Bay, India	<i>Pomadays hasta</i>	1.245	13.02	-	-	0.006	-	-	1.56	Shanthi and Ramanibai (2009)
Bengal Bay, India	<i>Therapon puta</i>	1.448	16.14	-	-	0.005	-	-	1.05	Shanthi and Ramanibai (2009)
Lake Edku	<i>Oreochromis niloticus</i>	2.80	27.6	-	0.59	-	-	-	-	Saeed and Shaker (2008)
Lake Borollus	<i>Oreochromis niloticus</i>	1.77	9.88	-	0.016	-	-	-	-	
Lake Manzala	<i>Oreochromis niloticus</i>	44.84	212.44	-	10.10	-	-	-	-	
El Khoms area	<i>Pagellus acarne</i>	0.515	5.557	-	0.036	0.174	-	-	-	Metwally and Fouad (2008)
Lake Qarun	<i>Oreochromis niloticus</i>	2.59	0.09-0.18	-	0.35-0.63	-	-	-	-	Authman and Abbas (2007)
Bitter lake	<i>Siganus rivulatus</i>	0.68	3.87	0.11	0.37	0.26	7.32	0.14	0.20	El-halfawy <i>et al.</i> (2006)
Lake Timsah	<i>Siganus rivulatus</i>	0.48	2.61	0.07	0.30	0.11	4.87	0.13	0.19	
Lake Balaton	<i>Abrams brama</i>	1.99	12.70	-	1.04	0.52	-	-	-	Farkas <i>et al.</i> (2003)
Permissible limits		30	30		0.5	0.5				FAO (1983)
Permissible limits							70-80		12-13	FDA (1993)

(Yilmaz and Dogan, 2008). Also it can be notice that the water take the same pattern of concentration in the fish muscles which mean that the fish accumulated the elements from the surrounding environment. The present study showed that the *Terapon puta* recorded approximately higher concentrations than other fishes recorded in the same sites and other sites except *Rutilus frisii kutum* from the Caspian Sea and *Oreochromis niloticus* from Lake Borollus recorded higher concentrations than *Terapon puta*. These results indicate the tolerance of the studied fish towards the surrounding area (Table 8). Fish are often considered as an important bio indicator for aquatic ecosystems because they obtain a high trophic level and are an important source of balanced protein in the human diet and muscles are the main edible part of fish and can directly influence human health (Pintaeva *et al.*, 2011). According to FAO (1983), it can be noticed that the level of Pb exceed the permissible limits in summer in all studied sites, also Cd in Timsah in the same season although all the other studied metals are within the permissible limits (Table 8).

### CONCLUSION

Fish are often considered as an important bio indicator for aquatic ecosystems because they obtain a high trophic level and are an important source of balanced protein in the human diet and muscles are the main edible part of fish and can directly influence human health. The present study revealed

that levels of heavy metals and biochemical contents are influenced by the reproductive activity of *T. puta*. According to the obtained results, this species should be prohibiting in the spring and summer seasons as their muscles contained high level of heavy metals that directly influence human health at the long run of eating. Also, the lower level of protein and glycogen lessen the nutritional value of the fish in these seasons.

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