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BIOLOGY AND TOXICITY OF THE PUFFERFISH *LAGOCEPHALUS SCELERATUS* (GMELIN, 1789) FROM THE GULF OF SUEZ

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ABSTRACT

Some biological aspects of the pufferfish *Lagocephalus sceleratus* were studied and correlated with the toxicity of the fish. A sample of 176 fish with total lengths ranging from 18.5 to 78.5 cm were collected from commercial catches at the Attaka fishing harbor between October 2002 and June 2003. Length weight regression parameters for males, females and all individuals were estimated. Modal progression analysis output indicates ten distinct age groups. The parameters of the von Bertalanffy's growth model were L_∞ = 81.1 cm and K = 0.26 per year. The overall sex ratio of males to females was 1: 1.3. The spawning takes place during summer and the size at which 50% of fishes are mature is 42.1 cm for males and 43.3 cm for females. Analysis of the diet composition showed that the fish is carnivorous and the maximum feeding intensity was attained in April. The toxicity of the gonads of twenty five specimens in different sexual maturity stages was assayed. The results showed that the highest toxicity scores were recorded during April, May and June for both sexes during the spawning season. The highest toxicity scores were recorded at the spawning stage of maturity (3500 MU/g for males and 3950 MU/g for females). While most of the specimens in maturity stage II (developing stage) were found non toxic.

1. INTRODUCTION

The pufferfishes are commonly known in the Red Sea and Gulf of Suez. There are as many as 120 species of puffer fish that live mostly in tropical seas. They also called blowfish and globefish, they are named after their habit of inflating themselves with water or air when threatened, making it difficult for a predator to swallow them. Puffer fishes

intestines, and skin of these fish contain tetrodotoxin that can cause death in

has only occasionally been detected in the muscles of these fishes. If cleaned and dressed properly, the puffer flesh or musculature is edible and considered a delicacy by some persons in Japan (Torado *et al.*, 1973).

contain a powerful and complex neurotoxin called tetrodotoxin (TTX). Tetrodotoxin (TTX) is a non-protein organic compound (aminoperhydroquinazoline) and one of the strongest marine paralytic toxins known today. TTX named after the order of fish from which it is most commonly associated, the Tetradontiformes (*tetras*-four and *odontos*-tooth) or the tetraodon puffer fish (Halstead, 1978). The liver, gonads, approximately 60% of persons who ingest it (Ellenhorn and Barceloux, 1988). The toxin

Occasional accidental intoxications including numerous deaths have been reported from allover the world due to eating puffer fish. The majority of reported cases have occurred in southeastern Asia including Malaysia, Taiwan, Hong Kong, and Korea (Kan, 1987; Yang *et al.,* 1996;

Kanchanapongku, 2001). In Egypt, although landing of these fishes is forbidden as a commercial species they are illegally landed and consumed as food in the Red Sea and Gulf of Suez areas. Although both the ministries of Health and Purveyance prevent handling these types of fish, it stills present in fish market after some special treatment. The pufferfish, *Lagocephalus scleratus,* is considered as one of the most delicious seafood in Suez city. Zaki (2004) described the clinical manifestations, age, sex and time elapsed between the ingestion and the death of eight cases from 11 cases of puffer fishes poisoning in Suez City.

There is no studies on the biology of the pufferfishes particularly *Lagocephalus scleratus* in the Gulf of Suez, however, the toxicity of them have investigated by many authors (Sherif, Alli, Abbas and Mohamed, 1994; Ali, Sherif, Abbas and Mohamed, 1995; Kotb, 1998; Youssef, 1999; Mohamed, 2003; and Zaki, 2004).

The present work is the first attempt to study the biology of the pufferfish *Lagocephalus sceleratus* in the Gulf of Suez with regard to toxicity level and its correlation with the biological activity of the fish.

2. MATERIAL AND METHODS

Specimens of the pufferfish *lagocephalus scleretus* were collected from the commercial catches at the Attaka fishing harbor between October 2002 and June 2003. For each fish, the total length (TL) was measured to the nearest mm and total weight (TW) to the 0.1 g. Sex and maturity stages were determined macroscopically and weight of the gonads (GW) was recorded to the nearest 0.01 g. Stages of maturity were classified as follows: I, immature; II, developing; III, mature; IV, ripe; V, running; and VI, spent. Accordingly gonads were separated for toxicity tests.

The sex ratio of the sampled population was analyzed monthly and according to the size intervals. The spawning season was determined following the monthly changes of the gonadosomatic index (GSI), calculated as follows (Anderson and Gutreuter, 1983);

$$GSI = 100\ GW/TW$$

Where GW is the gonad weight and TW is the total fish weight.

Size at maturity was determined as the length at which 50% of individuals were in maturity stage III and above.

The length weight relationship was calculated according to the exponential regression equation $TW = a\ TL^b$ (Ricker 1975) where a and b are the constant parameters to be estimated. The von Bertalanffy (1938) growth model was used to fit growth curve to the length frequency data. The asymptotic length (L_∞) and the growth coefficient (K) were obtained using ELEFAN I program (Pauly, 1984) and Wetherall's (1986) method.

The growth performance index ($\Phi = \log_{10} K + 2 \log_{10} L_\infty$) of Pauly & Munro (1984) was calculated to allow comparison of growth parameters. The modal progression analysis (MPA) (Bhattacharya, 1967) was used to separate length groups from modal distribution curves. Differences in mean lengths at the age obtained from the different models were tested using Tukey's multiple comparison test (Zar, 1984).

The relative volume of food in each fish stomach was determined irrespective of fish size. The degree of stomach fullness was estimated by an arbitrary 0-4 point scale as follows: 4 for full, 3 for 3/4 full, 2 for 1/2 full, 1 for traces of food and 0 for empty stomachs. The fullness index was calculated as the number of empty stomachs divided by the total number of stomachs multiplied by 100. Stomach contents were sorted into groups and analyzed for relative frequency (Hureau 1969).

The mouse assay method for Tetrodotoxin TTX (Kawabata, 1987) was used for assessing toxicity. Toxicity was calculated from the dose-death time relation ship for TTX. The lethal potency of the test solution was determined in mouse units. One mouse

(MU) is defined as the amount of toxin required to kill the mice after 30 min.

3. RESULTS

3.1. Length weight relationship

The pufferfish individuals used in this study consisted of 77 (43.8%) males and 99 (56.2%) females. Total length of males ranged from 18.5 to 78.5 cm with a mean TL of 45.90 ± 14.66 cm. The corresponding weight ranged from 82.9 to 5100 g with a mean weight of 1393.8 ± 1123.52 g. Lengths of females ranged between 19.1 and 69.5 cm with a mean length of 45.20 ± 14.73 cm and weight ranged between 115 and 4445 with a mean weight of 1368.4 ± 1063.67 g. The mean lengths and weights of males and females were not significantly different (*P* = 0.7547 and 0. 8802 respectively).

Length weight regression constants for males, females and sexes combined are given in Table (1). There was no significant difference in the coefficient of regression between males and females (*P* = 0.0924). Positive allometric growth was observed for all individuals (Fig. 1).

3.2. Age and Growth

The modal progression analysis (MPA) output indicates 11 distinct modes or length/age groups (Fig. 2). The first mode at mean length of 18.5 cm could be considered as age group (0). The rate of growth is rapid during the first four years of life then it slows down (Table 2).

Estimates of L_∞ and K obtained from ELEFAN were 82.3 cm and 0.191 yr^{-1}, respectively. Wetherall's (1986) method put the asymptotic length L_∞ at 81.74 cm with a Z/K of 3.048. The growth performance index Φ estimated in this study was 3.23. The predicted lengths for age were estimated from MPA analysis and compared with the output from ELEFAN and the von Bertalanffy's growth model with input parameters of L_∞ (81.1 cm), K (0.26) as estimated by the least squares method (Table 3). There was no significant difference at 95% confidence level in mean lengths at age for the different growth models (Tukey's multiple comparison test, $P > 0.05$).

3.3. Sex ratio

The overall sex ratio of males to females was 1: 1.3, which is not significantly different from 1:1 ($P > 0.05$). The females predominated the catch in late spring (April, May and June), while males predominated during February and March (Fig. 3). Sex ratio between males and females by size intervals (Fig. 4) showed that males dominated in the small (15-20 cm) and large (70-80 cm) size groups.

3.4. Spawning season

Analysis of the seasonal variation in the GSI (Fig. 5) shows that the average values of gonadosomatic index increase substantially from winter to summer. The maximum values were recorded during summer months; this indicates that the puffer fish *L. sceleratus* has an extensive spawning season in summer. Males and Females in running stage were found during the months of summer (Fig. 5), while post spawning stages occurred during autumn winter and spring.

3.5. Size at first maturity

Analysis of the percentage of mature and immature fish in each length class (Figs. 6, 7) showed that size at which 50% of fishes get mature is 42.1 cm for males and 43.3 cm for females. There was no significant difference in length at 50% maturity between sexes ($P > 0.05$). These results indicate that all individuals over three years of age are sexually mature.

3.6. Stomach analysis

For the determination of the feeding intensity, all the examined individuals were classified whether their stomachs were empty or not. Out of 174 fish were examined, 88 (50.57%) had full stomachs, and 86 fish

(49.42%) had empty stomachs. During autumn and winter months more than 60% of the examined stomachs were empty (Fig. 8). Thus the maximum feeding intensity was attained in April where most of the examined fishes (88.5%) were found to be feeding while the minimum feeding intensity was observed in autumn, particularly during November, where 72.7% of the examined stomachs were found empty.

Analysis of the diet composition of the puffer fish *L. sceleratus* showed that the fish is carnivorous where the diet was composed mainly of 70% cephalopods (squids and cuttle fishes), 25% crustaceans (particularly crabs) and 5% fishes.

3.7. Toxicity

The toxicity of the gonads of twenty five specimens of the puffer fish *L. sceleratus* (14 males and 11 females) in different sexual maturity stages was assayed. The scores of the toxicity were classified as: Non toxic < 10 MU/g, weakly toxic ≥ 10-99 MU/g, moderately toxic ≥ 100-999 MU/g and strongly toxic ≥ 1000 MU/g.

The results showed that the toxicity values ranged between 5 MU/g and 3950 MU/g. About 24% of the examined fishes were found non toxic, 32% were weakly toxic, 20% were found moderately toxic, while the strongly toxic specimens represented about 24% of the studied specimens. The highest toxicity scores were recorded during April, May and June for both sexes (Fig. 9). The average toxicity scores were not significantly different between males (659.79 MU/g) and females (636.09 MU/g) (P = 0.9608).

The study of toxicity in different maturity stages revealed that the toxicity values increased with the development of gonads for both sexes (Fig. 10). The highest toxicity scores were recorded in the spawning stage of maturity (3500 MU/g for males and 3950 MU/g for females). While most of the specimens in maturity stage II (developing stage) were found non toxic.

Table (1) Parameters of the length weight relationship for the pufferfish *L. sceleratus* collected from the Gulf of Suez.

	a	b	n	r2
Males	0.0160099	2.904471132	77	0.988325408
Females	0.0209024	2.841876861	99	0.980383539
Combined sexes	0.0187120	2.86761230	176	0.983504101

Table (2) Mean length, standard devistion, population number and separation index (S.I.) for each age group for L. seceleratus as estimated from Bhattacharya method

Group	Mean Length	S.D.	Population number	S.I.
0	18.5	2.6	4	n.a.
1	24.27	0.69	21	3.51
2	34.20	2.5	29	6.23
3	44.20	2.44	12	4.05
4	50.98	1.53	32	3.42
5	56.15	1.79	37	3.11
6	61.11	1.27	29	3.24
7	65.43	1.41	13	3.22
8	69.94	1.28	6	3.35
9	74.4	1.36	4	3.1
10	78.80	1.85	3	0

Table (3) Mean lengths (cm) at age of the puffer fish *L. sceleratus* computed by various methods.

Age	Mean length MPA (Bhattacharya)	Predicted lengths FiSAT	Mean lengths VBGE
1	24.27	18.60	25.84
2	34.20	32.90	34.57
3	44.20	43.90	43.86
4	50.98	52.40	51.15
5	56.15	59.00	56.43
6	61.11	64.10	61.11
7	65.43	68.00	65.43
8	69.94	71.00	69.94
9	74.4	73.30	73.96
10	78.80	75.10	78.26

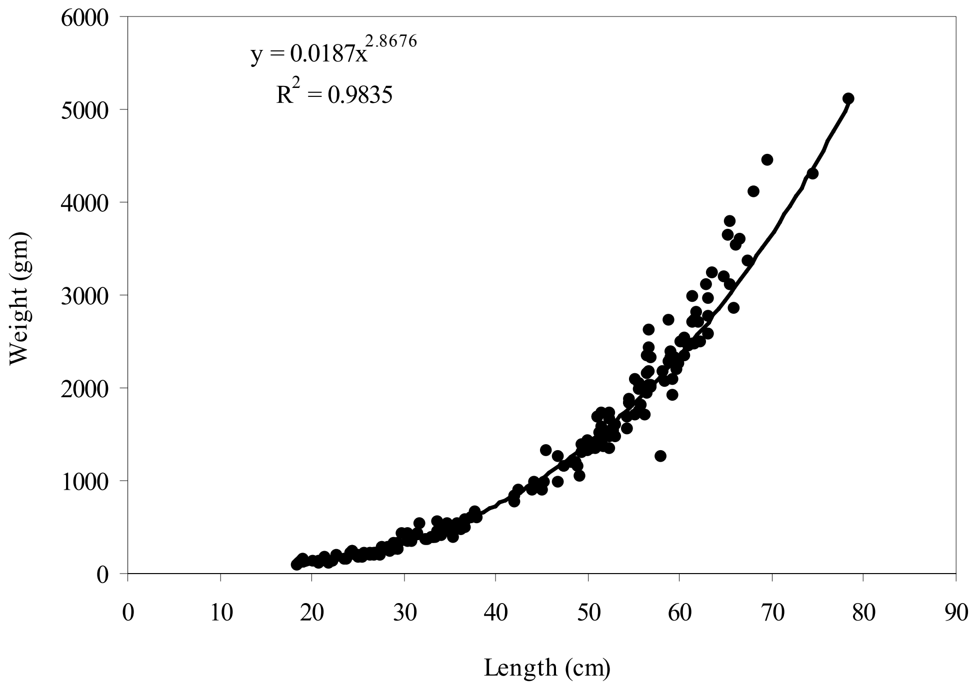


Fig (1) Length weight relationship of the combined sexes of puffer fish L. sceleratus in the Gulf of Suez.

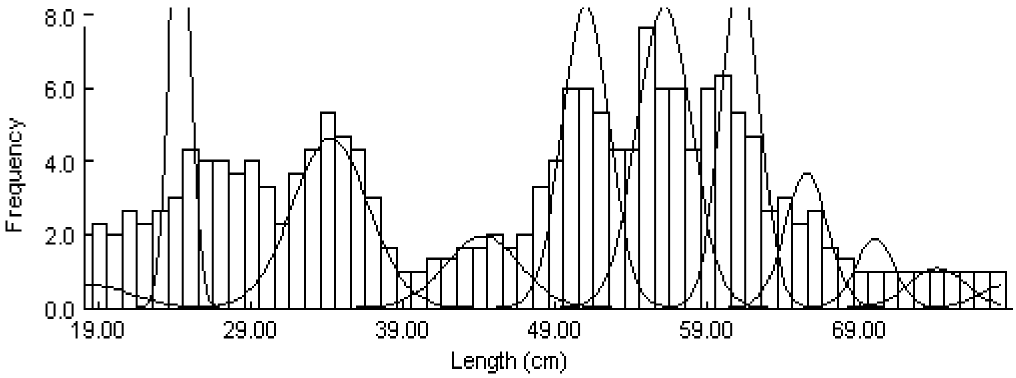


Fig (2) Length frequency distribution and decomposed age groups of L. sceleratus collected from the Gulf of Suez.

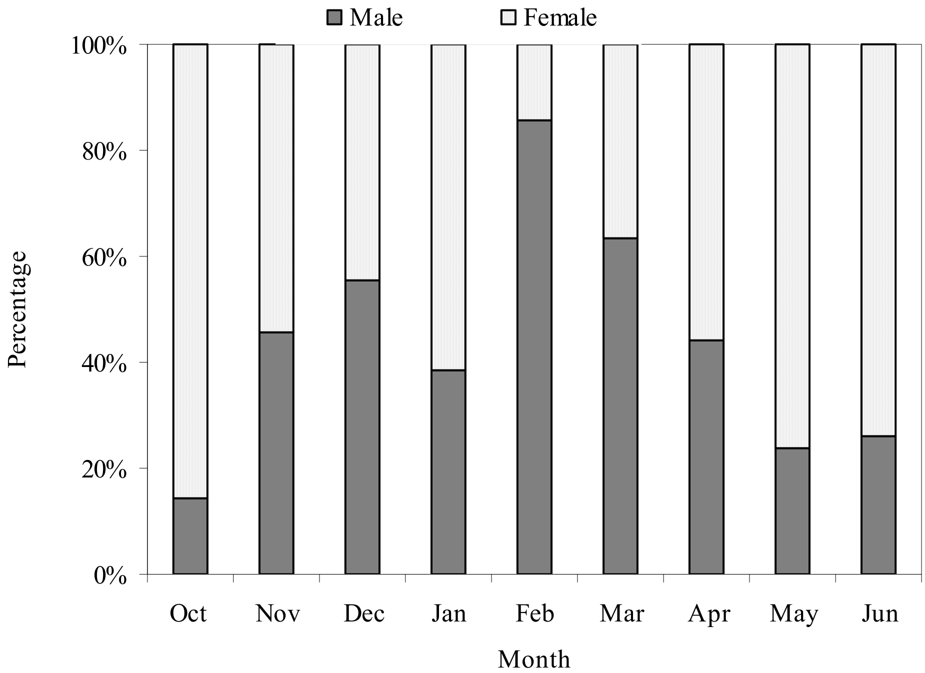


Fig (3) Monthly variations in sex ratio of the puffer fish L. sceleratus in the Gulf of Suez.

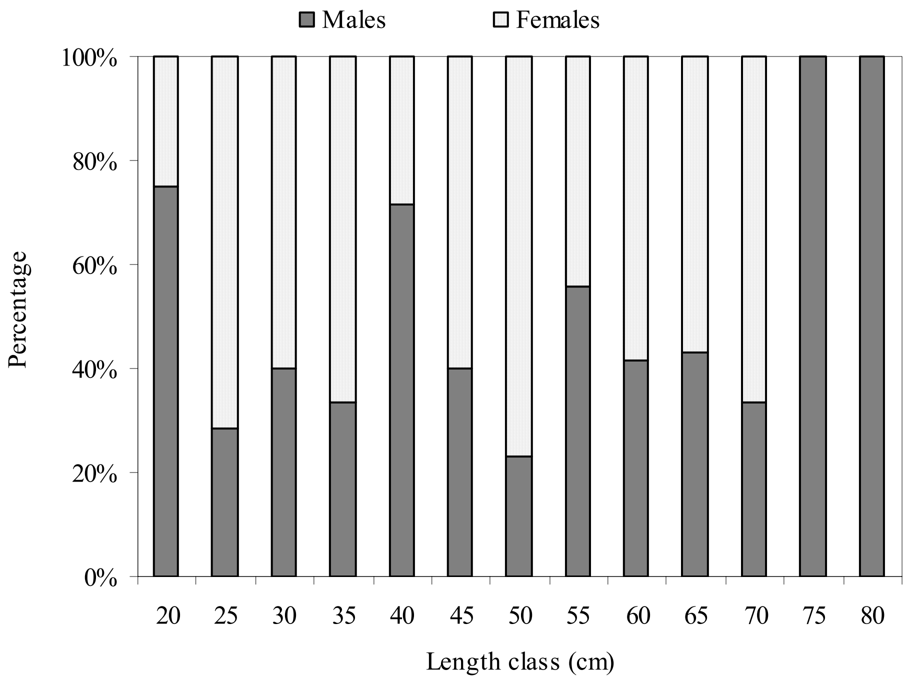


Fig (4) Variation in sex ratio according to size of the puffer fish L. sceleratus in the Gulf of Suez.

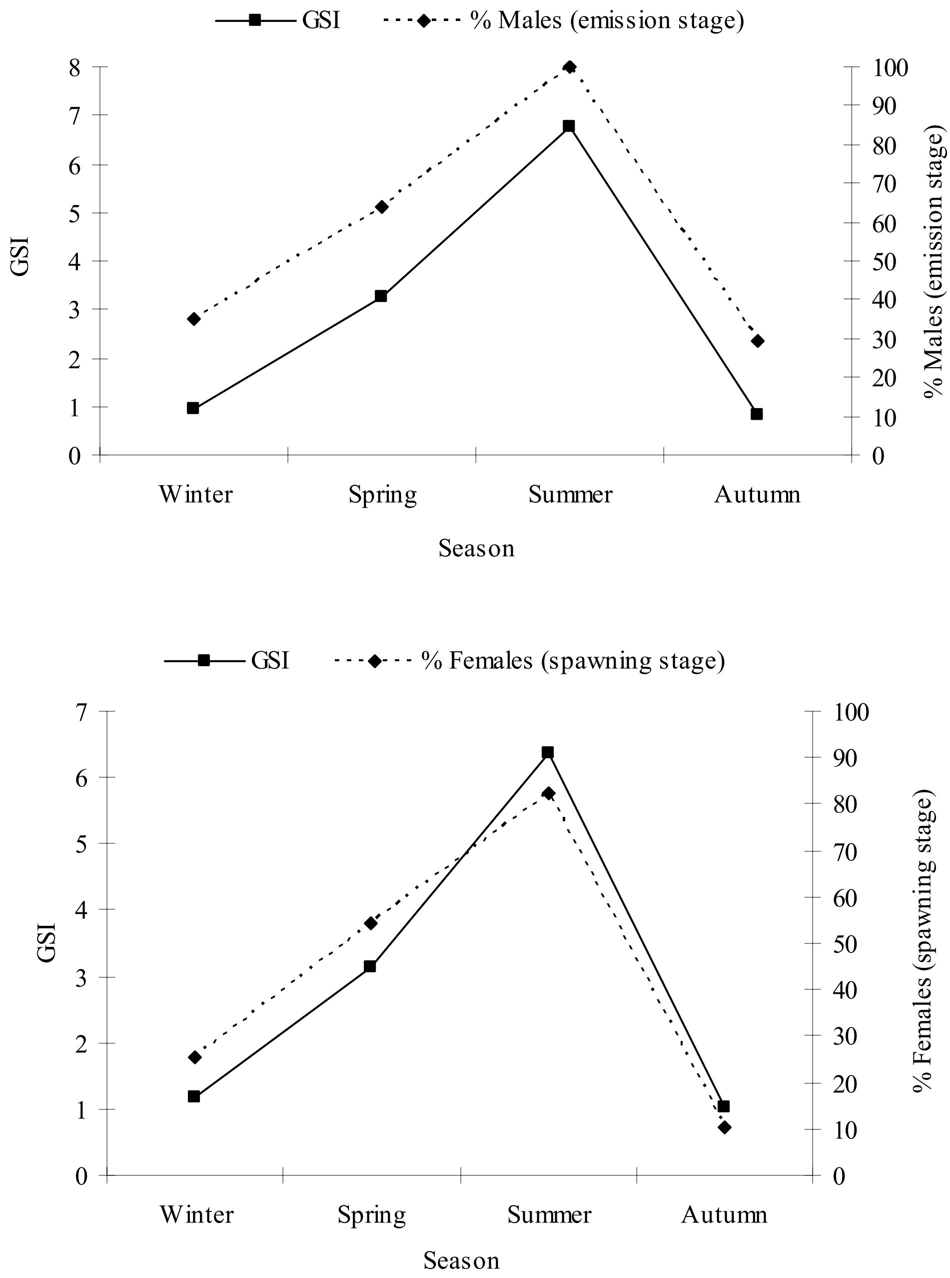


Fig (5) Monthly gonad somatic index (GSI) and percentage of spawning maturity stage for males and females for L. sceleratus from the Gulf of Suez. The error bars represent + standard diviation (SD).

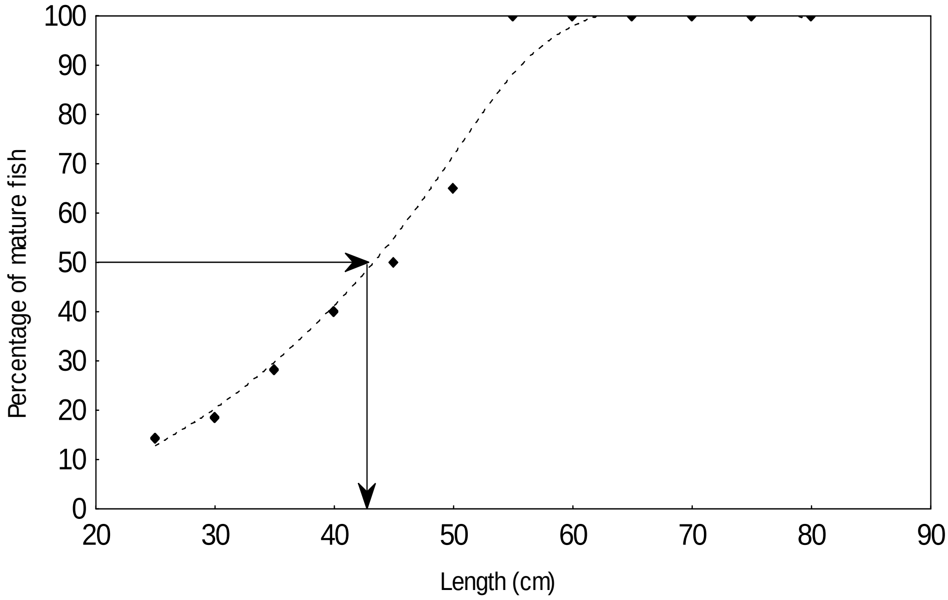
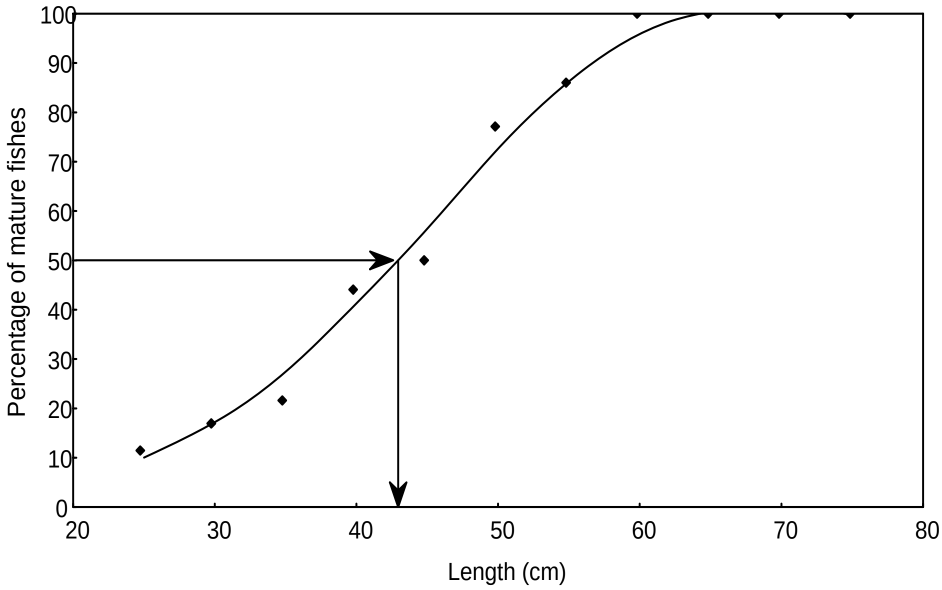


Fig. 6 Length at 50% maturity of males puffer fish *L. sceleratus from theGulf of Suez.*



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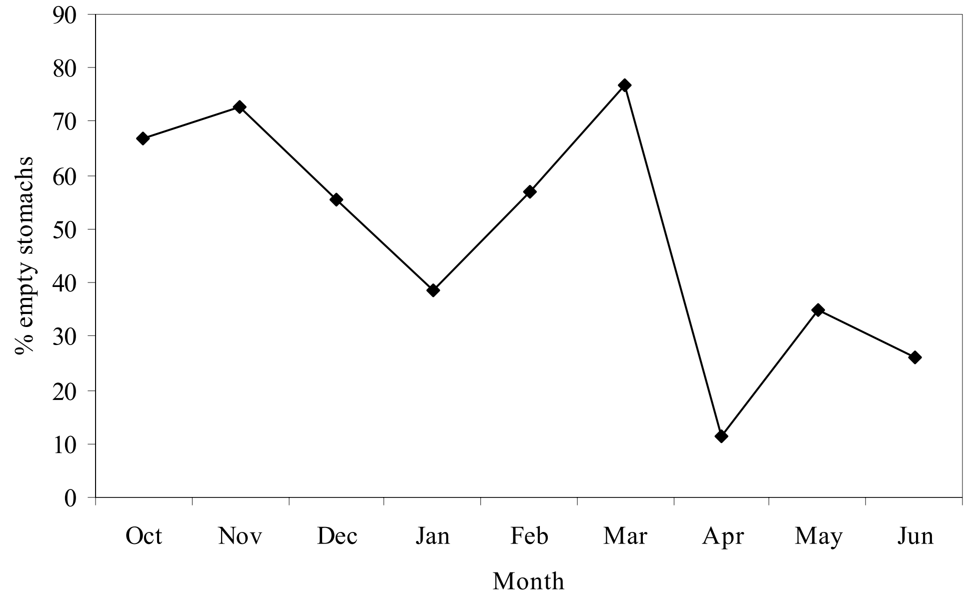

Fig (8) Monthly variations in the percentage of empty stomachs of the puffer fish L. sceleratus in the Gulf of Suez.

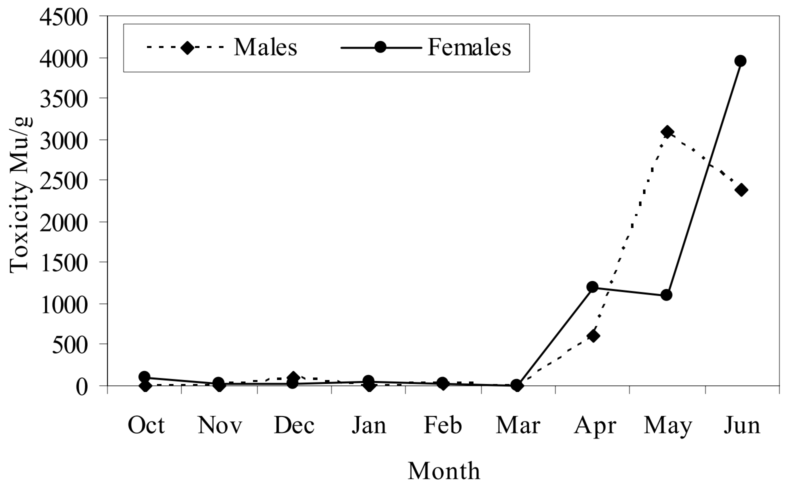

Fig (9) Monthly variations in toxicity of males and females Pufferfish Lagocephalus sceleratus from the Gulf of Suez.

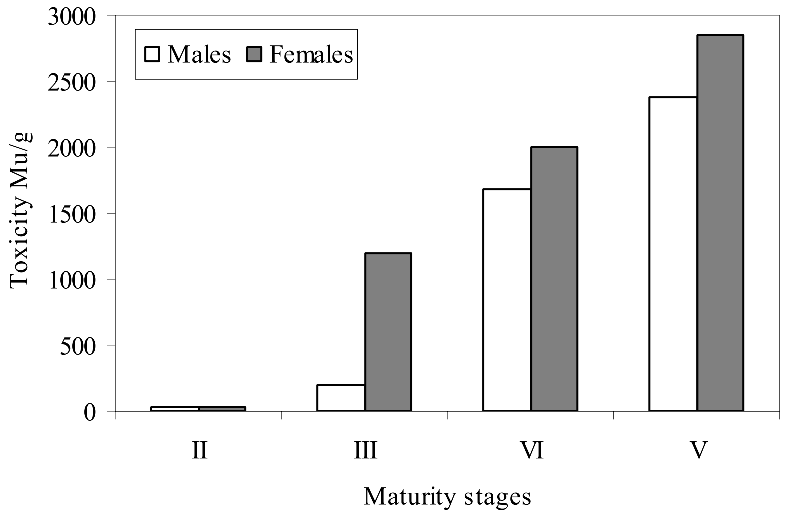

Fig (10) Variations in toxicity of the Pufferfish gocephalus sceleratus according to the gonad maturity stages.

4. DISCUSSION

Although landing of the buffer fish is prohibited in the Red Sea, it is illegally landed and consumed as a good food along the Gulf of Suez and Red Sea coasts. There is no catch statistics for this species, however, its relative abundance (expressed as catch per unit effort) in the Gulf of Suez was estimated from a trawling survey during spring 2003 (El-Ganainy and Yassien, in press). It was found that it is abundant allover the Gulf and it was represented in most of the trawling tows by an average of 4.2 kg/h.

The maximum observed length (78.5 cm) is much higher than that reported by Chan and Liew (1986) (18 cm) in Malaysia, and relatively higher than the one recorded by Assadi and Dehghani (1997) for the relative species *L. inermis* (56 cm) in the Persian Gulf and Sea of Oman. The length weight relationship revealed that weight increases allometrically with length in all individuals of *L. sceleratus* which were reported by Letourneur *et al*., (1998), and that males are heavier than females for a given length.

The theoretical maximum length value (L_∞ = 81.1 cm) was close to the size of the largest fish examined and the growth coefficient value (K = 0.26 $year^{-1}$) indicated relatively slow growth rate. The only available literature for growth parameters of *L. sceleratus* was that of Chan and Liew (1986) who estimated L_∞ = 18.0 cm and K= 1.5 per year, this estimates are very small and can't be compared with ours.

Analysis of the GSI together with maturity stage data suggest that the spawning period takes place during summer. The fish reaches sexual maturity in the third year of life.

Analysis of stomach contents showed that the pufferfish *L. sceleratus* is mainly carnivorous feeding on cephalopods (squids and cuttle fishes), crustaceans (particularly crabs) and fishes. The same stomach contents were reported for *L. laevigatus* (Le Loeuff and Intes, 1973) in Cote d'ivoire, and for *L sceleratus* by Tortonese (1986) in the Mediterranean and Mohsin *et al.,* (1986) in waters of Malaysia.

Many investigators reported that the gonads specially the ovaries have the highest toxicity when compared with the other tested organs (Kanoh *et al.*, 1984; Kanoh, 1989; Fuchi *et al.*, 1988; Ali *et al* 1995; Kotb, 1998;), so in the present study, the variations in toxicity of the gonads were examined monthly and according to the different maturity stages. Seasonal variation of toxicity is a characteristic feature observed in the majority of animals containing tetrodotoxin and other marine toxins (Koyama *et al.*, 1982; Noguchi *et al.*, 1985 and Ali *et al.*, 1995). The highest toxicity scores of the puffer fish *L. sceleratus* were recorded during April, May and June for both sexes which coincide with the spawning season, this fact was reported by Kodama *et al.,* (1983) and Kotb, (1998) who found that the toxicity generally begins to increase just before the spawning season and then decreases sharply after spawning activity.

Since, puffer fishes are relatively abundant in the Gulf of Suez. People may like such fishes because they are thought to be a very delicious, beneficial in potency, analgesic for backbone pain, curiosity and/or due to its low cost. It may be a must to establish a public agency that deal with collection of these fish, monitoring its toxicity and deals with its safe flesh either for local consumption or for exportation. On the other hand, the rest of fish (especially gonads) which contain higher level of TTX can be used as a source of the toxin to be used pharmaceutically.

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