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Reproductive biology of the Yellowspotted Puffer *Torquigener flavimaculosus* (Osteichthyes: *Tetraodontidae*) from Gulf of Suez, Egypt.

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ABSTRACT
The present study assesses reproductive biology of Yellowspotted Puffer *Torquigener flavimaculosus*, were collected seasonally from commercial catches at the Attaka fishing harbor in Suez from winter 2017 until autumn 2018. The sex ratio was found 1:1.08 for male and female, respectively. The fish length at first sexual maturity (L_{50}) was 8.2 cm for males and 9.5 cm for females. In addition, the allometric pattern of gonadal growth was studied to validate the use of the gonado-somatic index (GSI) in assessments of the reproductive cycle. The highest peak of GSI (10.5 ± 1.012%) and (4.3 ± 0.084%) for female and male were recorded in summer, respectively. Values for hepato-somatic index (HSI) is very high and strong inverse relationship with gonado-somatic index (GSI) we inferred that lipid reserves in the liver play an important role in gonad maturation and spawning. Somatic condition factor (Kr) also varied, albeit less so, throughout the year, suggesting that body fat and muscle play lesser roles in providing energy for reproduction. Seasonality of liver lipid content and different spawning seasons have important implications for designing sampling strategies using these fish, especially when monitoring lipophilic contaminants. According to the present results of seasonal variations in maturity stages gonado-somatic index (GSI), hepato-somatic index (HSI) and somatic condition factor (Kr) indicate that the reproductive season of *Torquigener flavimaculosus* collected from Gulf of Suez represented in summer season.

INTRODUCTION

The puffer fishes (Family: *Tetraodontidae*) are commonly known of all type of fish poisoning and has been recognized from ancient times. Pufferfishs are common in tropical, subtropical and temperate estuaries (Bell *et al*., 1984 and Hindell and Jenkins, 2004), There are as many as 120 species of puffer fish that live mostly in tropical seas. All belong to the order Tetraodontiformes. They also called blowfish, toadfish, swellfish, globefish and balloon fish Torda *et al*., (1973). They are named after their habit of inflating themselves with water or air when threatened, making it difficult for a predator to swallow them. Pufferfish are among the most poisonous animals on earth. Tetrodotoxin (TTX) is known to be the substance of puffer fish poison. TTX is 100 times more toxic than cyanide and it has been suggested that TTX in pufferfish is a result of accumulation through food chain, which starts from marine bacteria *Vibrio alginolyticus, Shewanella* sp., *S. putrefaciens, Alteromonas tetraodonis* by Nouguchi and Arakawa (2008).

Yellow-spotted puffer fish (*Torquigener flavimaculosus*) belongs to the *Tetraodontidae* family. The species belonging to the tetraodontidae contain high amount of TTX (Hardy, 1983; Ha and Sato, 2013 and Azman *et al*., 2014). *T. flavimaculosus* is distributed in tropical and temperate regions of the east Africa, India, and Persian Gulf. Recently, it has been recorded in Turkey (Hardy and Randall, 1983; Bilecenoğlu, 2003 and 2005; Ergüden *et al*., 2015 and Engin and Seyhan, 2017), in the northern red sea by Golani and Lerner, (2007), in the eastern Mediterranean Egyptian coast by Farrag *et al*. (2016), in the Aegean Sea (Corsini-Foka *et al*., 2006). Studies on the *T. flavimaculosus* are very limited and most of the studies are related to the distribution of it (Golani, 1987; Bilecenoglu, 2003 and 2005; Ergüden and Gürlek, 2010; Turan, 2010; Froese and Pauly, 2013; Sabour *et al*., 2014 and Farrag *et al*., 2016).

Reproductive processes may influence the storage, mobilization and transfer of lipids (Merayo, 1996 and Alonso-Fernandez and Saborido-Rey, 2012) and hence the partitioning of lipophilic contaminants among different tissues (Fletcher and King, 1978), so the reproductive cycles and changes in condition of any species to be used as a biomonitor should therefore be well understood. A species on the west coast of Australia, *Torquigener pleurogramma*, is a broadcast spawner during summer and sexual maturity is reached at two years of age (Potter *et al*., 1988). Habib (1979) found a similar species in New Zealand *Contusus richei* also matures at two years old and spawns in summer whereas the smooth puffer *Tetractenos glaber* in the Sydney region spawns in winter reported by Booth and Schultz (1999). The reproductive biology of other tetraodontids, other than the few large pelagic species used commercially for human consumption (Sabrah *et al*., 2006) has not been documented.

The aim of this work is to increases the knowledge on the reproductive biology of the puffer fish *T. flavimaculosus* from Gulf of by studying sex ratio, seasonally maturity stages and length at first sexual maturity. In addition, to study seasonal changes in Gonado- Somatic Index (GSI), condition factor (Kr) and hepato-somatic index HIS.

MATERIALS AND METHODS

The collection of samples was collected seasonally from commercial catches at the Attaka fishing harbor in Suez from winter 2017 until autumn 2018. In this study, a total of 164 *T. flavimaculosus* was examined. The total lengths (TL) of all fish were measured to the nearest mm, whereas the weights were recorded with an electronic balance at the nearest 0.01 g. Sex and maturity were determined macroscopically and were classified as either I, immature; II, developing; III, spawning-capable; IV, spent/resting (regressing/regenerating) (modified from Brown-Peterson *et al*. (2011) and the gonad weights (GW) were recorded to the nearest 0.01 g. The spawning season was determined following the seasonal changes of the gonado-somatic index (GSI), and calculated after Anderson and Gutreuter (1983); GSI = 100 x (GW/TW) where GW is the gonad weight and TW is the total fish weight. Length at maturity was determined as the length at which 50% of individuals were at maturity stages III or IV, during the spawning season. The length at 50% sexual maturity (L_{50}) was estimated by fitting a logistic model to the combined percentage of fish with maturity stages III and IV in each 1 cm size class as Rogers *et al*. (2009). Condition factor index, Kr and hepato-somatic index (HSI) were calculated as indirect indices of energy status. The two parameters were estimated as follows according to McPherson *et al*. (2011): HSI = 100 x LW (g) / SW (g) and Kr = 100 x SW (g) / FL^3 (cm).

RESULTS AND DISCUSSION

Sex Ratio:

The present study showed that the sex ratio of *T. flavimaculosus* was 48.2% male and 51.8% female (ratio 1 male: 1.08 female) which is not significantly different from 1:1 ($P > 0.05$). Chi- square test showed significance difference between males and females numbers ($X^2 = 0.185$, $P < 0.05$). Çek *et al.* (2017) reported that the sex ratio of *T. flavimaculosus* was 62.5% female and 37.5% male collected from the Iskenderun Bay southeast coast of Turkey. While, Sabrah *et al*. (2006) investigated sex ratio in Silver-cheeked toadfish, *Lagocephalus sceleratus* was found to be 1:1.3, which was not significantly different from 1:1 (P>0.05). Ali *et al*. (2015) showed sex ratio (males: females) for *L. sceleratus* through the study period were female biased by 1:1.07 and 1: 1.29 for Gulf of Suez and Mediterranean populations, respectively. This difference may be related to the variance in the availability of both sexes for the fishery, and to the spatial segregation of sexes or a feeding behavior (Korpelainen, 1991 and Mendonca *et al*., 2006).

Length at maturity:

Fifty percent of male *T. flavimaculosus* reached sexual maturity at 8.2 cm and for females, 50% reached sexual maturity at 9.5 cm (Figs. 1 & 2). At the total length of 12 cm, all females were sexually mature while all males above 13 cm were mature.

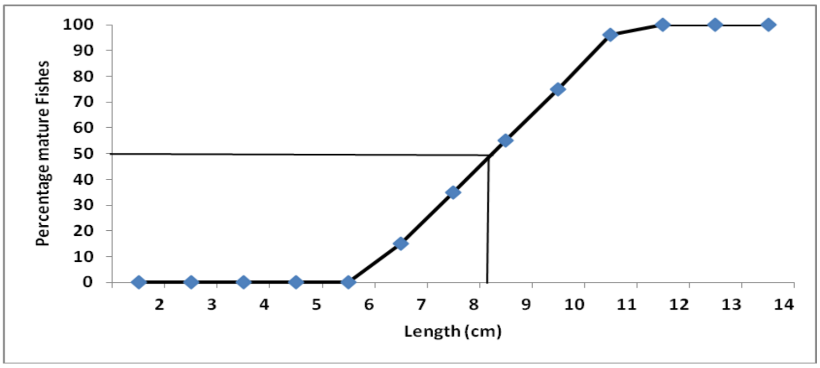

Fig. 1: Length at first sexual maturity (L_{50}) of male puffer fish *T. flavimaculosus* from Gulf of Suez during the period from winter 2017 to autumn 2018.

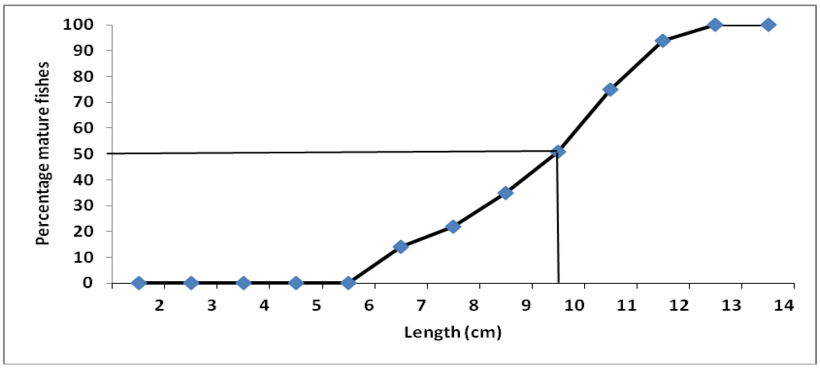

Fig. 2: Length at first sexual maturity (L_{50}) of female puffer fish *T. flavimaculosus* from Gulf of Suez during the period from winter 2017 to autumn 2018.

Although gonad development and subsequent spawning may depend on various environmental stimuli, individuals must reach a certain age or size before they are capable of spawning as reported by King (2001), and dependency of maturation to the age or length is strongly linked to growth and is also regulated by the water temperature and feeding success (Yoneda *et al*., 2001 and Takemura *et al*., 2004). The length at maturity in pufferfish in this study was similar to that of *U. richei* that matures at 7.5 to 11.6 cm as demonstrated by Habib (1979) and *Marilyna pleurosticta* female reached sexual maturity at 8.9 cm and for males at 9.2 cm (Mat Piah and Bucher, 2014) while Ali *et al.* (2015) determined that the large species *Lagocephalus sceleratus* in the Gulf of Suez reached maturity at a length of 32.5 cm for males and 36.3 cm for females.

Maturity stages:

The seasonal variations of the different maturity stages and their distribution throughout the study period, reflects the time of spawning. Both testes and ovaries of *T. flavimaculosus* showed a seasonal progression of developmental stages, culminating in a switch from a majority of fully mature fish in summer (60 % for male and 70 % for female) to a majority of spent and resting individuals in autumn (67 % for male and 75 % for female) (Fig. 3).

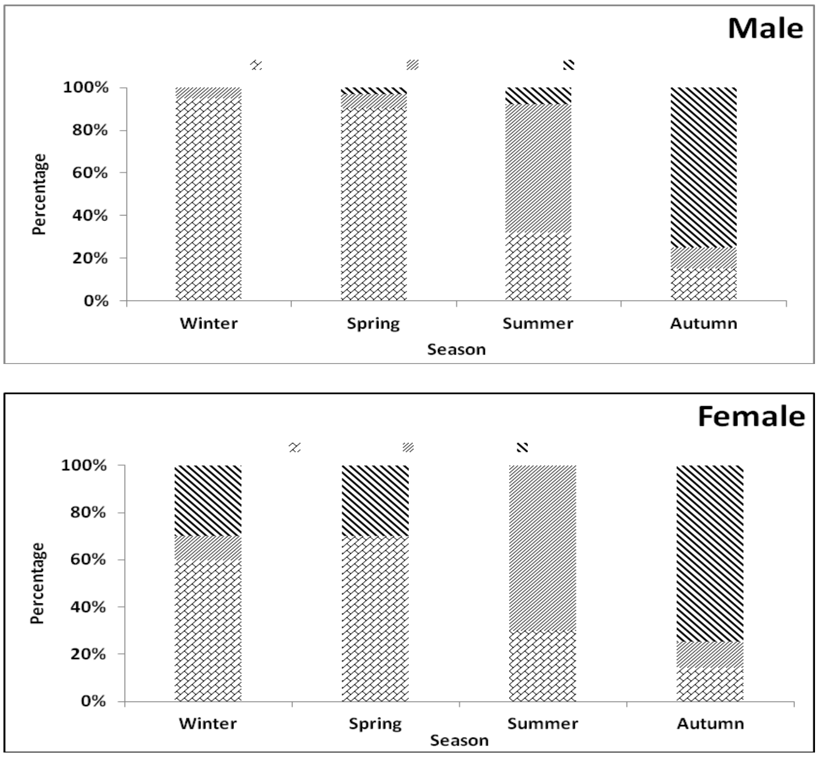

Fig. 3: Seasonal variations of maturity stages for both sexes of *T. flavimaculosus* collected from Gulf of Suez, during the period from winter 2017 to autumn 2018.

Gonado somatic index (GSI):

Monthly variations in GSI are shown in Figure 4. The data indicated increase during winter and spring. The highest peak of GSI (10.5 ± 1.012%) and (4.3 ± 0.084%) for female and male were recorded in summer, respectively. While, the lowest GSI value (1.05 ± 0.012 and 0.4 ± 0.003%) for female and male in autumn, respectively.

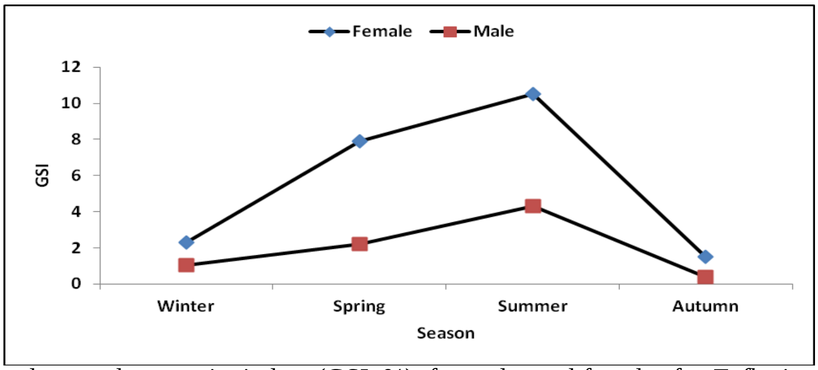

Fig. 4: Seasonaly gonado-somatic index (GSI, %) for males and females for *T. flavimaculosus* from the Gulf of Suez during the period from winter 2017 to autumn 2018.

Gonado-somatic index (GSI) has been widely used as an indicator of reproductive condition and spawning time in fishes (Plaza *et al.*, 2007). In the present study female GSI value is highest than male GSI value where the ovaries of teleost fishes are larger several times than the testes as reported by Moyle and Cech (2004). So, the GSI is almost higher for females than males, particularly during spawning season in summer season. The reproductive cycle of *T. flavimaculosus* in this study was coincides to other tetraodontid species reported, most of which spawn in summer (Habib, 1979; Potter *et al*., 1988; Sabrah *et al*., 2006; Peristeraki *et al*., 2010; Aydin, 2011; Kalogirou, 2013 and Rousou *et al*., 2014).

Seasonal changes in GSI, Kr and HIS:

The ranges of seasonal mean condition factor indices Kr were very similar for both sexes. The seasonal patterns of average HSI were similar for the two sexes and have been pooled for analyses (Fig.5). The seasonal mean hepato-somatic and gonado-somatic indices have an inverse relationship to each other. Many studies calculate K using total weight including the gonads. In the case of puffers that have relatively light bodies with reduced skeletal components and large gonads and liver, such formulae would have been strongly influenced by individual organ development and variable gut fullness, potentially masking changes in muscle and fat body mass. For this reason, Kr was calculated by excluding visceral weight from the numerator. The cyclical variation in hepato-somatic index while condition changed much less suggests a central role for the liver a source of lipid and metabolic energy fuelling gamete production.

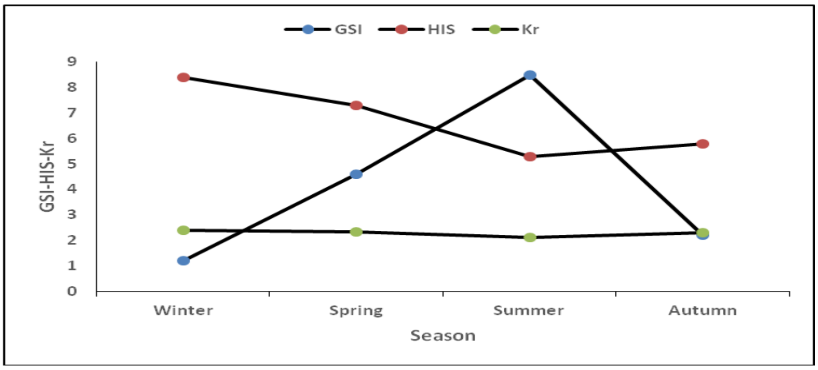

Fig. 5: Seasonal relationships between mean hepato-somatic Index (HIS, %), condition factor (Kr) and gonado-somatic index (GSI, %) of *T. flavimaculosus* (sexes combined).

There is usually a direct correlation between hepato-somatic index and body condition index, and an inverse correlation of these factors to gonado-somatic index

(Htun-han, 1978). In this study, HSI displayed a strong inverse correlation with GSI in *T. flavimaculosus*, but showed no correlation with other two indices. However, in *T. hamiltoni* Kr showed a positive relationship with HSI. The increase in GSI during the period of gonad maturation is mainly due to the deposition of large amounts of proteins and lipids in the developing eggs and spermatozoa (Htun-han, 1978). Part of this material comes directly from ingested food but a major proportion comes from reserves of food deposited during the active feeding season in organs such as liver and muscles (Larson, 1974). It is therefore reasonable to expect that the weight of liver and muscle would reflect the cycle of accumulation and utilization of these energy reserves.

HSI and Kr declined in *T. flavimaculosus* during pre-spawning presumably for gonad maturation. This pattern is similar to that of smooth puffer fish *Tetractenos glaber* in the Sydney region by Booth and Schultz (1999). In a study of liver weights of brook trout by Larson (1974), suggested that the decrease in liver weight during pre-spawning season was due to the passage of materials from the liver to the gonads and concluded that weight changes of the liver plays an important role in gonad maturation. High values for hepato-somatic indices and their strong inverse relationship with gonado-somatic indices demonstrate that mobilization of lipid stores in the large liver is important for fuelling gametogenesis and low feeding success therefore, potentially severely affect spawning success.

There was not only a change in weight but also a change in color and texture of the liver with different stages of the gametogenic cycle. The pre-spawning liver of *T. flavimaculosus* was firm and pale while the post-spawning liver was soft, dark and flaccid. This supports the concept that that lipid in the liver has been used for the spawning process. Rossouw (1987) reported that the liver color was in synchrony with the variation in the total liver lipid content in both sexes of sand sharks. He found that the higher liver lipid concentration in the liver, the lighter the livers become in appearance.

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ARABIC SUMMARY

بيولوجيا التكاثر لسمكة القراض ذى النقط الصفراء *Torquigener flavimaculosus* (Osteichthyes: *Tetraodontidae*) من خليج السويس

أمل م. رمضان و مجدى م. الحلفاوى

معمل تناسل الأسماك، قسم الاستزراع، المعهد القومى لعلوم البحار والمصايد، مصر.

تم دراسة بيولوجيا التكاثر لسمكة القراض ذى النقط الصفراء Yellowspotted Puffer *Torquigener flavimaculosus*، وقد تم جمعها موسميا من خليج السويس في ميناء صيد الأتكة في الفترة من شتاء ٢٠١٧م حتى خريف ٢٠١٨م. تم حساب النسبة الجنسية ١: ١.٠٨ ذكور: إناث، على التوالي. كان طول السمك عند النضج الجنسي الأول (L_{50}) ٨.٢ سم للذكور و ٩.٥ سم للإناث. بالإضافة إلى ذلك، تمت دراسة دليل المناسل الجسمى (GSI)، حيث كانت أعلى قيمة لـ GSI هى ١٠.٥ ±١.٠١٢ % و ٤.٣± ٠.٠٨٤% للإناث والذكور في الصيف، على التوالي. قيم مؤشر دليل الكبد الجسمى (HSI) كان لها علاقة عكسية عالية جدا وقوية مع مؤشر GSI. لقد استنتجنا أن احتياطيات الدهون في الكبد تلعب دورا هاما في نُضج الغدد التناسلية والبيض. يختلف معامل الحالة الجسدية (Kr) أيضًا، وإن كان أقل من ذلك، على مدار العام، مما يشير إلى أن دهون الجسم والعضلات تلعبان أدوارًا أقل في توفير الطاقة للتكاثر. يكون لموسم محتوى الدهون في الكبد ومواسم التفريخ المختلفة آثار مهمة في تصميم استراتيجيات أخذ العينات باستخدام هذه الأسماك، وخاصة عند مراقبة الملوثات الدهنية. وفقًا للنتائج الحالية للتغيرات الموسمية في مراحل النضج، فإن دليل الكبد الجسمى (HSI) ومعامل الحالة الجسدية (Kr) يشيران إلى أن موسم التكاثر في *Torquigener flavimaculosus* الذي تم جمعه من خليج السويس يمثل في موسم الصيف.