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Ovarian Maturation and Spawning Season of Por's Goatfish *Upeneus pori* (Mullidae) from Mediterranean Sea, Egypt[1]

A. M. Ramadan and M. M. El-Halfawy

National Institute of Oceanography and Fisheries, Suez, 43511 Egypt

e-mail: elhalfawy2006@yahoo.com

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Abstract—The body length of Por's goatfish (*Upeneus pori*) at 50% sexual maturity is estimated and the results indicate that L_{50} is 10.6 cm. Monthly variations of the gonadosomatic index indicate that the spawning occurs mainly in May. Mean hepatosomatic index significantly increases from February to May, peaking in March (2%) and April (1.91%) during vitellogenesis and spawning. Mean condition factor values vary slightly from 0.98% in December to 1.17% in November. Based on histological investigation of female's oocyte development, five maturity stages are demonstrated. Ovarian maturity is defined by the maturation stage of the most advanced oocytes, and it is divided into four phases. By using the monthly changes in the percentage frequency of ovarian phases, the ovarian cycle of *U. pori* is divided into three periods: a long period of early oogenesis (November to February), a short period of vitellogenesis (February to April) and a spawning period from April through June with peak activity in May.

Keywords: Por's goatfish, *Upeneus pori*, Mullidae, Mediterranean Sea, oocyte histology, gonadosomatic index, hepatosomatic index

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INTRODUCTION

The Por's goatfish *Upeneus pori* is a Lessepsian migrant species which penetrates into the Mediterranean Sea through the Suez Channel (Golani and Ben-Tuvia, 1995). The Por's goatfish is a subtropical species distributed along the Western Indian Ocean from the Red Sea to Southern Oman (Ben-Tuvia and Golani, 1989). It is a commercially important demersal species living mostly in sandy and muddy substrate to 50 m depth and caught in large quantities by trawl in shallow waters of 10–40 m (Cicek and Avsar, 2011). Recently El-Drawany (2013) showed that, the Lessepsian fish species such as *U. pori* and *U. moluccensis* represent an important species in the commercial fishing along the Southern Mediterranean Coast.

Basusta et al. (2002) reported that Por's goatfish, which was previously misidentified as *U. asymmetricus* (Torcu and Mater, 2000), was first observed in the Northeastern Mediterranean coast of Turkey at the end of the 1940 (Kosswig, 1950). After that date, there has been a noticeable increase in the catch of *U. pori* in the fishery along the coastal zone of the Mediterranean. Sabrah (2006) and Sabrah and El-Ganainy (2009) studied goatfish species in the Gulf of Suez, Egypt. However, there are no precise data for the annual catch of Por's goatfish because the catch of mullet always occurs without separation of species in Mediterranean costal zone.

Knowledge of several components of a stock's reproductive biology, such as spawning season, maturity stage, and spawning-stock biomass, are essential for fisheries management. These data form the basis of the biological reference points on the maximum fishing mortality that a population could sustain and the minimum biomass required for average recruitment. A review of reproductive characteristics relies on the accurate estimation of gonadal development and determination of maturity stages in individual fish. Determination is based either on a macroscopic (visual) examination or on a more accurate histological analysis of the gonad.

Macroscopic examination is a quick and inexpensive method of estimating the reproductive state of many species in situ (Tomkiewicz et al., 2003). However, classifying individuals based on the method of visual examination of gonads has important limitations. The stage of oocyte development cannot be examined (needed to distinguish sexually active from sexually inactive females), the number of oocytes ready to be released remains unknown, and the presence of postovulatory follicles or atretic stages cannot be assessed (Costa, 2009; Nŭňez and Duponchelle, 2009). These limitations could hinder development of an effective management scheme, especially for multiple-spawning species, because the incorrect classifi-

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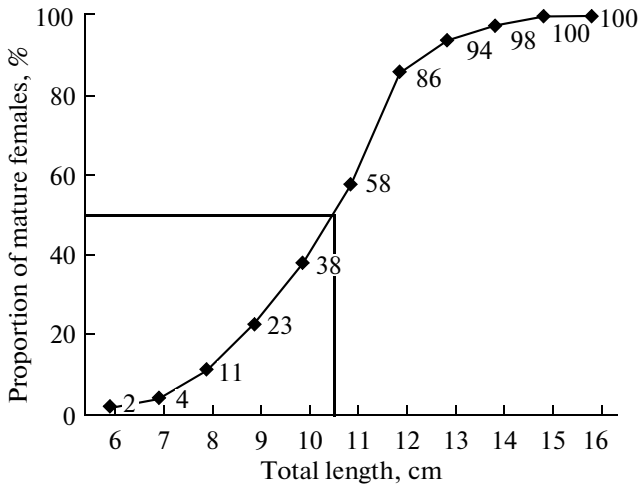

Fig. 1. Body length at first maturity of *Upeneus pori* females; the numbers of exemplars are indicated, and the length at 50% maturity is shown.

cation of maturity can lead to crucial over- or underestimations of fundamental reproductive parameters (Costa, 2009; Nŭňnez and Duponchelle, 2009). Several studies reveal discrepancies between the macroscopic and histological examination of the gonads and emphasize the lack of accuracy using macroscopic inspection (García-Díaz et al., 1997; La Mesa et al., 2003; Vacchi et al., 2007; Costa, 2009; Pavlov et al., 2011; McBride et al., 2013). Clearly, histological examination is important to verify visual observations.

There are only very few attempts to describe the comprehensive biology and ecology of Por's goatfish. Most of the available information is on the distribution of the species except for a few studies of Golani and Galil (1991) that provided some information on its feeding habits. Emel'yanova et al. (2013) described gonadal differentiation and development of sex cells in the tropical representative of the family Mullidae, manybar goatfish *Parupeneus multifasciatus*, using histological methods and observations of oocytes in vivo.

There are several attempts to describe the comprehensive biology and ecology of the Por's goatfish (Ben-Tuvia and Golani, 1989; Golani, 1990, 1994, 1996, 1998; Golani and Galil, 1991; Galil, 1993; Gucu et al., 1994; Golani and Ben-Tuvia, 1995; Mater et al., 1995; Basusta, 1997; Taskavak and Bilecenoglu, 2001; Cicek et al., 2002). Golani (1990) and Golani and Galil (1991) provided some information on the feeding habits of the species.

The aims of this study are the description of the occurrence of oocytes in Por's goatfish (mainly in the females) at different developmental phases in sexually mature individuals during the reproductive season and description of the changes of oocyte morphology during the final period of oogenesis (maturation).

MATERIALS AND METHODS

Pori goatfish individuals (672 females and 581 males) were randomly sampled on a monthly basis between October 2011 and September 2012. All fish were caught by commercial bottom trawl in Port Said fish port. For each specimen, total length (*TL*), total and gutted weight, and gonad and liver weights were measured. The following parameters were assessed:

1—length at first sexual maturity;

2—gonadosomatic index (GSI), (GSI = 100 × gonad weight/gutted weight);

3—hepatosomatic index (HSI), (HSI = 100 × liver weight/gutted weight;

4—condition factor (K), ($K = W/L_3 \times 100$, where W = total weight, g and L = total length, cm).

For histological study, at least five ovaries for each developmental stage were fixed in Bouin solution and then transferred to 70% ethanol followed by dehydration in increasing concentrations of ethanol and clarified in xylene and then embedded in paraffin wax. The standard paraffin sections of 6 μm thickness were stained with haematoxylin and eosin and observed under a Leica microscope. Ovarian maturity stages were determined from microscopic observations of the ovarian histological slides. The oocyte development classification of Grier et al. (2009) and Uribe et al. (2012) was used to define stages of oogenesis.

The maturity stages are divided into steps designed to identify the morphological changes in oocytes during growth and maturation. Ovarian maturity was defined by the maturation stage of the most advanced oocytes, and divided into four phases, following the conceptual models and the standardized terminology proposed by Nunez and Duponchelle (2009) and Brown-Peterson et al. (2011).

The significance of difference (at the 5% level) in GSI, HSI and K between monthly samplings and maturity stages was tested by analysis of variance (ANOVA) using the statistical package STATISTICA 9.

RESULTS

Length at first sexual maturity. Frequencies of immature and mature females during the period of gametogenesis (March to June) were divided into groups according to different total length (*TL*). The total length at which 50% of females attain sexual maturity (L_{50}) was 10.6 cm (Fig. 1).

Gonadosomatic index. Monthly variations in GSI revealed that fish mass spawning occurs in May, when the GSI for female reached its highest level (7.5%) (Fig. 2a, $p < 0.001$). A considerable drop of GSI occured in June and reached 0.24% in August. Thereafter, the indices remained almost constant until December (0.04%). From January, the values of GSI increased gradually reaching their maximum level in May.

Hepatosomatic index. The monthly variations in HSI increased from February to May, peaking in March (2%) and April (1.91%) during vitellogenesis and spawning (Fig. 2b, $p < 0.001$).

Condition factor. The mean K values varied slightly from 0.98 (December) to 1.17% (November) tending to be lower from December to May during vitellogenesis and spawning (Fig. 2c).

Monthly variation of maturity stages. Reproductively inactive ovaries (immature or regenerating stage ovaries (Stage I)) were present almost all year round but with decreasing rates between November and May (Fig. 3). Early developing ovaries (Stage II) indicating the initiation of ovarian growth and oocyte development were initially seen from November to December but mainly from January onwards. The ovaries entered the late developing stage (Stage III) (vitellogenesis) from February onwards. Spawning-capable females (Stage IV) were observed between April and May, peaking in May (100% spawning, Stage V). Females probably finished spawning in June, as none capable of spawning and many fish with regressing spent ovaries (Stage VI) were observed between June and August.

Reproductively inactive ovaries. Reproductively inactive ovaries were divided into sexually immature (immature subphase) and mature but reproductively inactive ovaries (regenerating subphase). Ovaries at both subphases contained oogonia and primary growth phase oocytes (mainly at the perinucleolus step) possessing a spherical or ovoid germinal vesicle (Fig. 4a). The diameter of primary growth oocytes reached on average 56 μm. Regenerating subphase ovaries had more space between follicles, higher presence of interstitial tissue, and a thicker ovarian wall.

Developing ovarian phases. Developing phase ovaries were divided into early and late developing subphase ovaries. Early developing ovaries were characterized by the appearance of oocytes in the yolk vesicles phase, in which spherical inclusions (yolk vesicles) were dispersed throughout the ooplasm. Yolk vesicles were delimited by a membrane and could be clearly observed using light microscopy (Fig. 4b). The follicle cell layer was visible, but the zona radiata was not yet stained. Primary growth oocytes reached an average diameter of 122 μm. Late developing phase ovaries included secondary growth stage oocytes, which in turn enclosed spherical acidophilic yolk granules that progressively increased in number and dispersed within the ooplasm (Figs. 4c and 4d). Additionally, spherical oil droplets progressively appeared around the germinal vesicle, increased in size and accumulated throughout the ooplasm (Figs. 4c and 4d). The zona radiata remained wide, and it was densely stained by eosin. Secondary growth stage oocytes are of various sizes and development stages, with few yolk globules (secondary growth stage oocytes at early step) to moderate and many yolk globules (secondary growth stage oocytes at the late phase). The average diameter of secondary growth oocytes can be up to 173 μm, whilst secondary growth stage oocytes reach on average 261 μm (the nucleus migrates to the animal pole of the oocyte).

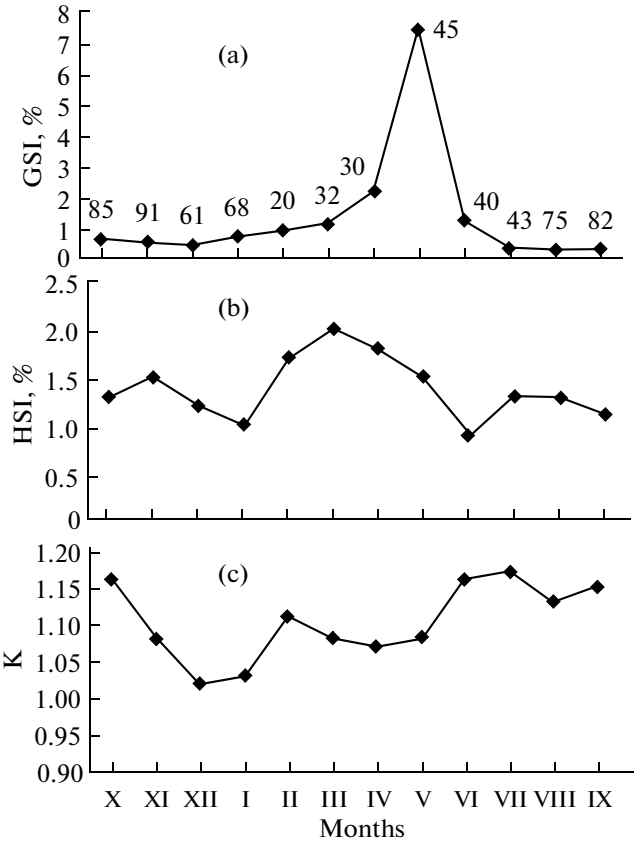

Fig. 2. Monthly variation of mean: (a) gonadosomatic index (GSI), (b) hepatosomatic index (HSI), (c) condition factor (K), of *Upeneus pori* females; the numbers of exemplars collected during each month are indicated.

Spawning capable ovaries. Ovaries capable of spawning were divided into two subphases: ovaries capable of spawning (spawning capable) and actively spawning (active spawning). Spawning capable ovaries contained full-grown vitellogenic oocytes, which were the most advanced oocytes. These oocytes were distinguished by the formation of large regions of fluid yolk, formed by the progressive fusion of yolk granules. As a consequence, the ooplasm was displaced into the peripheral rim surrounding the yolk mass (Fig. 4e). During this step, the oil droplets concentrate around the nucleus, and then fuse into one or a few oil droplets. The germinal vesicle has an irregular shape, and its envelope becomes progressively folded. Numerous small and large nucleoli can also be seen, and the zona radiata reaches its maximum length. Some full-grown vitellogenic oocytes were shrunken, and the zona radiata, follicle cells, and theca were separated from the oocyte. Such shrinkage was likely due to the histological preparation protocol. Full-grown vitellogenic

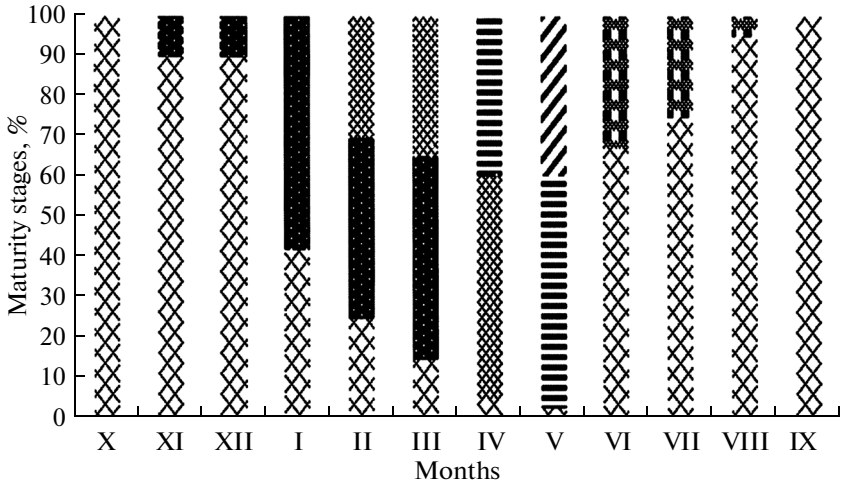

Fig. 3. Monthly variation of ovarian maturity stages of *Upeneus pori* females: (▨) stage I, reproductively inactive ovaries; (■) stage II, early developing ovaries; (▩) stage III, late developing ovaries; (▬)stage IV, spawning capable ovaries; (◪) stage V, spawning active ovaries; (▩)stage VI, regressing phase ovaries.

oocytes reached on average 359 μm. During the final maturation phase, spawning active ovaries are characterized by the presence of hydrated oocytes; the yolk material is completely fused and, a few (or one) oil droplets are formed (Fig. 4f). The appearance of the oocyte is homogenous, finely granular, whilst the germinal vesicle is invisible due to disintegration and dispersion of the nuclear membrane. The ooplasm is restricted to a narrow rim located near the zona radiata, the latter zone has become thinner. In some spawning-capable ovaries, empty ovarian follicles (post ovulatory follicles) were present, indicating that ovulation had occurred.

DISCUSSION

The passage of Lessepsian fish species to the Mediterranean has affected the ecosystem balance of the species in the Mediterranean. Even though there are no sufficient studies conducted, it is observed that the Lessepsian species which entered and rapidly colonized the Mediterranean competed with the indigenous species in terms of sharing the food and the habitat (Ismen, 2006; El-Drawany, 2013).

To our knowledge this is the first time histological analysis is being used to describe the reproductive pattern of Por's goatfish, an important target species for Mediterranean fisheries. Oocyte development during oogenesis shows similar characteristics to those described for other marine teleosts (Tyler and Sumpter, 1996; Nunez and Duponchelle, 2009). The maturation scale defined in this study is well in line with the universal need for standardized and harmonized terminology on reproductive classification of fish, accompanied by manual-like features that can be used by researchers beyond the local area to allow for a better comparability of results.

Early developing phase ovaries, indicating the initiation of gonadal growth and gamete development, were initially seen from November to December. The early oogenesis coincides well with the seasonal drop in water temperature and minimum range of day length. Photoperiod and temperature are among the key factors that control sexual maturity and spawning in fish, and they could be the environmental correlatives to the initiation of ovarian growth, functioning as proximate cues in the case of Por's goatfish. Similarly to *M. surmuletus* (N'Da and Deniel, 1993), the period of early oogenesis is long (November to February), but the period of vitellogenesis is short, from February to April.

In the beginning of the period of vitellogenesis of *U. pori*, first vacuoles, which (most likely) contain lipids, appear in the cytoplasm. Similar feature is registered in the majority of marine fishes distributed at low latitudes (Oven, 2004). In some other fish species (*Mesogobius batrachocephalus, Neogobius melanostomus*, *Scomber scombrus*, and *Symphodus rostratus*), vacuolization begins from the periphery of the cytoplasm (Oven, 2004).

The oocytes of *U. pori* that completed vitellogenesis and showed maturational competence were approximately 350 μm in average diameter. The oocyte contains the nucleus located in the center and surrounded by small lipid droplets. In the beginning of the period of maturation, the nucleus is slightly displaced toward the animal pole, and lipid droplets become larger due to their partial fusion.

Based on subsequent gradual increase of oocyte diameter, the processes of subsequent fusion of lipid droplets and hydration of the cell occur synchronously reaching the largest intensity during final homogenization of yolk. Nevertheless, in several marine fish species, oocyte diameter is stable during increase of the size of lipid droplets, and this diameter increases

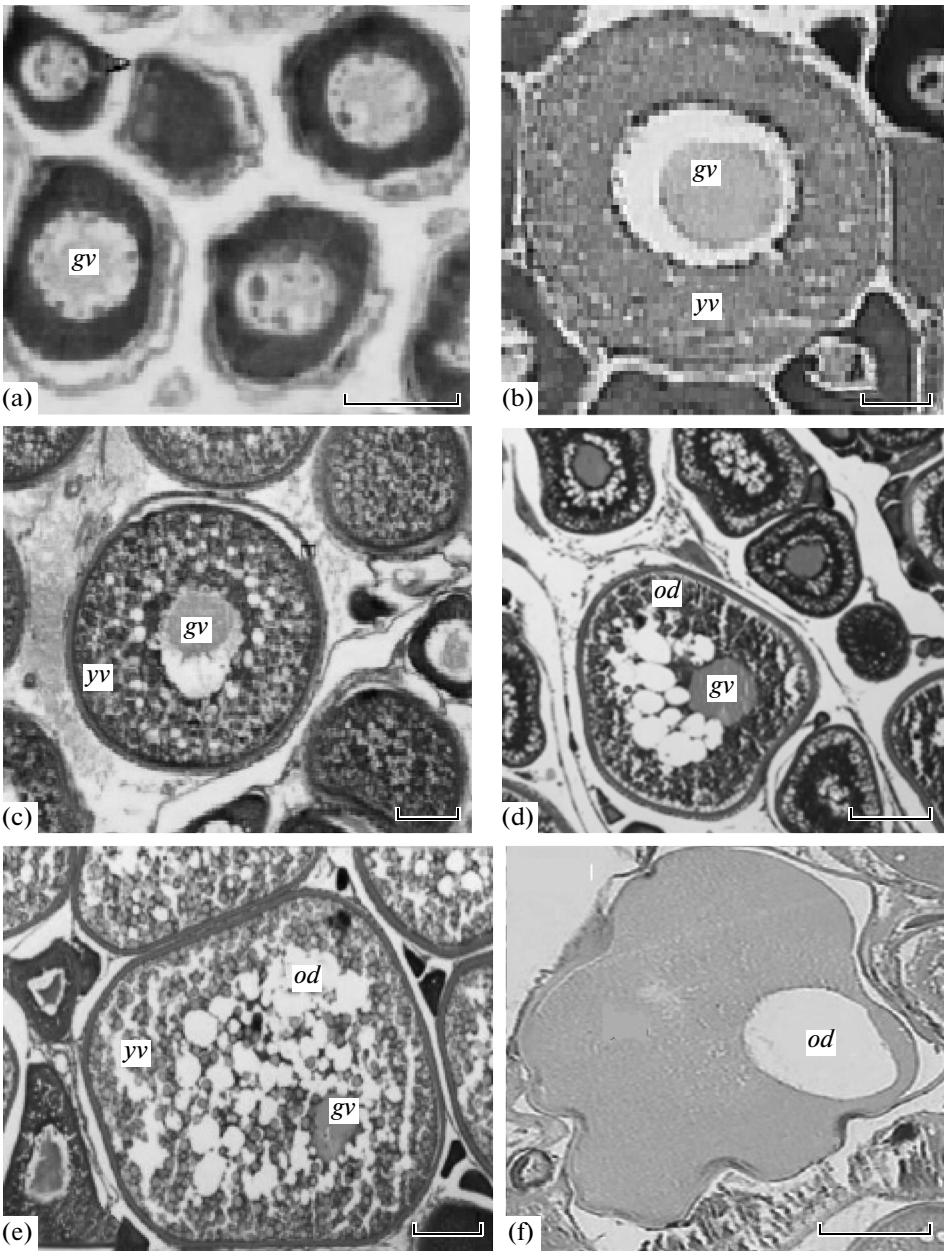

Fig. 4. Ovarian sections of *Upeneus pori*: (a) primary growth phase oocyte at the perinucleolus step possessing an ovoid germinal vesicle (*gv*); (b) primary growth phase oocyte at yolk vesicles phase with yolk vesicles (*yv*) dispersed throughout the ooplasm; (c, d) secondary growth stage oocytes with spherical acidophilic yolk granules (*yg*) and spherical oil droplets (*od*); (e) full-grown vitellogenic oocyte with large regions of fluid yolk and a few oil droplets; (f) oocyte at the final maturation phase with yolk material completely fused and an oil droplet. Scale bars: (a–e) 20, (f) 100 μm.

only during fusion of yolk granules accompanied by hydration of the cell (Alekseev and Alekseeva, 1996).

Results of this study clearly indicate that the spawning in the Mediterranean Sea extends from April to June, with a peak in May. Similar to data on most Mediterranean fish stocks (Tsikliras et al., 2010), the Por's goatfish is a late spring–early summer spawner.

Among the fishes from the family Mullidae, continuous type of oogenesis is registered in *Pseudupeneus grandisquamis* from the Pacific coast of Mexico. In this species, two spawning peaks (in the summer and winter) are reported (Lucano-Ramírez et al., 2006). Another representative of the family, surmullet *Mullus surmuletus*, distributed at higher latitudes along southern coast of Britain spawns from May to June, and it is characterized by discontinuous type of oogenesis and determinate fecundity (N'Da and Deniel, 1993). Thus, in lower latitudes, a prolonged spawning season is accompanied by continuous formation of new batches of mature oocytes. At this reproductive mode,

a probability of survival of the progeny in the conditions of local variation of abiotic and biotic environmental factors increases.

The fertility of Por's goatfish is synchronized with the summer peak of zooplankton abundance (Fernandez de Puelles et al., 2003), a condition that ensures optimum conditions for fish larval growth and survival (Winemiller and Rose, 1992). Warmer summer waters and stability of the water column maintain food patches, thus enhancing larval growth (Sabatés et al., 2007). Summer variation of environmental parameters and the accompanying changes in oceanographic factors (e.g. low level of offshore transport and turbulent mixing) may favor larval retention in spawning grounds (Sabatés et al., 2007) and survival of juveniles during their migration towards the nursery areas (Levi et al., 2003). Spawning capable ovaries contained oocytes at different stages of development and post ovulatory follicles. The existence of several oocyte stages indicates ovarian development typical for multiple spawning species.

The hepatosomatic index increases during the spawning period in a manner positively associated with the gonadosomatic index, supporting the assumption that HSI variations are related to energy storage for reproduction. Increasing hepatocyte numbers and size are linked to vitellogenesis in liver because the precursors of the yolk and proteins of the egg chorion are synthesized in that organ (Hoar et al., 1983; N'Da and Deniel, 1993). Both indices (the GSI and HSI) can be used together to predict the spawning period of *U. pori*. However, the fact that the K index is not associated with the GSI may suggest that reproduction does not influence the condition of the fish (i.e., oocyte maturation is not reached at the expense of body muscle or lipids). Some species may compensate inadequate energy deposits during gonadal development with the energy derived by feeding (Aristizabal, 2007). This is likely the case for female Por's goatfish, which feed throughout their entire spawning period (Vassilopoulou and Papaconstantinou, 1993). Monthly variation in GSI values revealed that this species spawn in May. Ismen (2006) reported that spawning of *U. pori* occurred after April while Cicek et al. (2002) stated that the spawning of the studied fish in the northeastern Mediterranean extends from March to August, and the GSI values reached the maximum level in April. Our results coincide with El-Drawany (2013) who reports that GSI of Por's goatfish reaches the highest values in May.

In the present study the average length of Por's goatfish at first sexual maturity was 10.6 cm. The length at the first maturity of other species of goatfish such as *U. vittatus* and *U. tragula* is reported between (*TL*) 11 and 12 cm for males and females (Sabrah and El-Ganainy, 2009), which corresponds to the end of the first year of their life. Nevertheless, according to El-Drawany (2013), the length at maturity of *U. pori* in the Mediterranean is 10.2 cm for females. Since 1996, the European Union fisheries management policy for Mullidae spp. (European Commission, 1994; Council Regulation no. 1626/94) in the Mediterranean has stipulated a minimum landing size (MLS) of 11 cm *TL*. It should be noted, that this value coincides with the L_{50} value for *U. pori* from Mediterranean coast of Egypt registered in this study. Regarding the Greek mixed fisheries, illegal sizes below MLS (Machias and Labropoulou, 2002; Tserpes et al., 2002; Stergiou et al., 2004; Tzanatos et al., 2008) and extensive removal of immature red mullet have been reported, indicating the ecological inefficiency of the existing MLS value for sustainable management (Stergiou et al., 2009).

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