Vitellogenesis, oocyte maturation pattern, spawning rhythm and spawning frequency in *Otolithes ruber* (Schneider, 1801) (Sciaenidae) in the Kuwaiti waters of the Arabian Gulf

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SUMMARY: Vitellogenesis, oocyte maturation pattern, spawning rhythm, spawning frequency, batch fecundity and oocyte diameter-frequency distribution of the tigertooth croaker, *Otolithes ruber* (Schneider, 1801) in Kuwaiti waters were investigated from March 1999 to February 2000 and from January to May 2005, using histological and morphological methods. Oogenesis is described in four phases: vitellogenic, mature, spent and regressed. Vitellogenesis, in turn, is described in three classes: early vitellogenic, mid-vitellogenic and late vitellogenic. Development of the yolky oocyte is an asynchronous process resulting, by the time of oocyte maturation, in a clear differentiation between a ready batch of oocytes (ready for spawning) and a reserve pool. Consequently, *O. ruber* is capable of spawning multiple times during the reproductive season. Spawning frequency estimates, based on the final oocyte maturation (FOM) method indicated that the species spawns once every 2.8 days, while the estimates based on the post-ovulatory follicle (POF) method indicated a spawning every 2.2 days, during a 5-month spawning season lasting from January to May. Batch fecundity (BF) was significantly positively correlated with both ovary-free body weight (OFBW) (p<0.05) and standard length (SL) (p<0.05), but SL was a better predictor of BF (r² = 33%) than was OFBW (r² = 19.9%). Batch fecundity was also significantly different between March and April (p<0.05) and March and May (p<0.05), but not between April and May (p>0.05). Relative batch fecundity was 716 eggs/g OFBW; thus, estimates for potential annual relative batch fecundity were 10024 eggs/g OFBW using the FOM method for spawning frequency estimation, and 7876 eggs/g OFBW using the POF method. The oocyte diameter-frequency distribution analysis revealed a multimodal distribution, confirming the evidence of multiple spawning.

Keywords: *Otolithes ruber*, Kuwait, reproduction, multiple spawning, spawning frequency.

RESUMEN: VITELLOGÉNESIS, PATRÓN DE MADURACIÓN DE LOS OOCITOS Y RITMO Y FRECUENCIA DE PUESTA EN *OTOLITHES RUBER* (SCHNEIDER, 1801) (SCIAENIDAE) EN LAS AGUAS KUWAITÍES DEL GOLFO DE ARABIA. – La vitellogénese, el patrón de maduración de los oocitos, el ritmo y la frecuencia de puesta, fecundidad parcial y la distribución de frecuencias del diámetro de los oocitos de *Otolithes ruber* (Schneider, 1801) en aguas de Kuwait fueron investigados de marzo de 1999 a febrero de 2000 y de enero a mayo de 2005 usando métodos histológicos y morfológicos. Se describe la oogénesis en cuatro fases: vitelognética, medio-vitelognética y vitelognética avanzada. El desarrollo del vitelo de los oocitos es un proceso asincrónico que, en el momento de la maduración del oocito, resulta en una clara diferenciación entre los oocitos hiratados (listos para la puesta) y los oocitos de reserva. Consecuentemente, *O. ruber* es capaz de poner múltiples veces a lo largo de la estación de puesta. Las estimas de frecuencia de puesta, basadas en el método de maduración final de los oocitos (FOM) indicaban que la especie pone una vez cada 2.8 días, mientras que la estimada en base al método de los foliculos post-ovulatorios (POF) indicaba una puesta cada 2.2 días, durante una estación de puesta de 5 meses, que se extendía de enero a mayo. La fecundidad parcial (BF) estuvo positiva y significativamente correlacionada tanto con el peso del cuerpo (sin ovario) (OFBW) (p<0.05) como con la longitud estándar (SL) (p<0.05), sin embargo SL fue un mejor predictor de BF (r² = 33%) que OFBW (r² = 19.9%). La fecundidad parcial fue también significativamente diferente entre marzo y abril (p<0.05) y marzo y mayo (p<0.05), pero no entre abril y mayo (p>0.05). La fecundidad parcial relativa fue 716 huevos/g OFBW; por tanto, la estima de la fecundidad parcial relativa anual potencial fue 10024 huevos/g OFBW, usando el método FOM para la estimación de la frecuencia de puesta y 7876 huevos/g OFBW, usando el método POF. El análisis de la distribución de frecuencias del diámetro del oocito revela una distribución multimodal, confirmando la evidencia de múltiple puesta.

Palabras clave: *Otolithes ruber*, Kuwait, reproducción, puesta múltiple, frecuencia de puesta.
INTRODUCTION

Representatives of the family Sciaenidae are widely distributed in the tropical and subtropical waters of the Indian, Atlantic and Pacific oceans (Trewavas, 1977; Longhurst and Pauly, 1987). In the Kuwaiti waters of the Arabian Gulf, the sciaenid species, *Otolithes ruber*, plays an important role in the multispecies commercial fishery. Although the reproductive biology of many sciaenid species has been documented across the Gulf of Mexico (Merriner, 1976; DeMartini and Fountain, 1981; Lowerre-Barbieri et al., 1996; Brown-Peterson and Warren, 2001; Brown-Peterson et al., 2002), in South African waters (Griffiths, 1996, 1997; Fennessy, 2000) and in the West African coast of Benin (Pillai, 1983), only scant information is available from the Arabian Gulf and the Indo-West Pacific (Hussain and Abdullah, 1977; Abu-Hakima et al., 1983; Dadzie and Abou-Seedo, 2004). In Kuwaiti waters, Hussain and Abdullah (1977) described the monthly changes in the gonadosomatic index, while Abu-Hakima et al. (1983), in addition, reported on the histological changes during the seasonal maturation of the gonads. Dadzie and Abou-Seedo (2004) described the testicular structure and spawning only in the males. Apart from these reports, no other studies have been carried out on *O. ruber* either locally or regionally.

In view of the continued importance of *O. ruber* to the commercial fishery in Kuwait, a comprehensive study of the reproductive biology of the species is warranted. Thus, the purpose of this study was to investigate aspects of the reproductive biology of the species. Specifically, the following tasks were undertaken: (1) histological investigation of the vitellogenic process during oogenesis, (2) description of oocyte maturation from the final oocyte maturation stage to the spawning stage, (3) description of the spawning rhythm, (4) estimation of the spawning frequency, (5) estimation of batch fecundity and (6) analysis of the oocyte diameter-frequency distribution during the spawning season.

MATERIALS AND METHODS

Fresh samples of *Otolithes ruber* were collected monthly from commercial gill-net catches in the northern part of the Kuwaiti waters of the Arabian Gulf (Fig. 1) during a 12-month sampling period, from March 1999 to February 2000. Supplementary samples were taken from the same area from January to May 2005, a period coinciding with spawning in the species in Kuwaiti waters. Salinity and water temperature values, which affect the reproductive patterns of sciaenids in other regions (Brown-Peterson and Warren, 2001; Brown-Peterson et al., 2002), were compared for the two periods using the ANOVA test. The results indicated no differences between the two data sets for salinity (p>0.05) or for water temperature (p>0.05). The data were therefore merged to create one annual composite data-set to overcome low sample size. The nets used, measuring 1000-2500 m long, were laid at depths of 6-12 m. They were set at dawn between 0300 and 0500 h, and raised for collection of the fishes between 1300 and 1400 h. The vessels with *O. ruber* samples docked by 1530 h, and study samples were obtained within 2 h and kept on ice. The total length (cm), standard length (cm) and body weight (g) of each fish were recorded upon arrival in the laboratory. A total of 395 females were sampled during the study. All of them were dissected, and then the gonads were removed, weighed (to the nearest 0.001 g) and fixed in Bouin’s fixative for a minimum of 48 h. Large ovaries were cut into pieces to facilitate penetration of the fixative. They were then dehydrated in alcohol, cleared in toluene and infiltrated with and embedded in paraffin wax. Sections of 5 μm were stained in hematoxylin and
counterstained with eosin for studies of the histological changes during the maturation of the oocytes, with particular attention to vitellogenesis, final oocyte maturation and the spawning rhythm.

The developing oocytes were classified into different maturation classes and the monthly percentage frequency of occurrence of females in each class was determined (Table 1). Cell and nuclear sizes were measured during the process of development, using a Reichert research microscope. For this exercise, ovaries of five fish from each maturation class were examined, and in each ovary, oocyte and nuclear diameters were measured for 100 randomly-selected cells and the means determined. Only those oocytes which had been sectioned through the nucleus were measured. If the cell and nucleus were spherical, the diameters were measured, but if they were oval or irregular in shape, the mean of the longest and shortest axes were taken. This procedure has been shown to be representative of the true oocyte diameter (Foucher and Beamish, 1980).

Monthly spawning frequency was determined by calculating the percentage of females with postovulatory follicles (POF) and oocytes undergoing final oocyte maturation (FOM) from histological examination of ovarian tissues, following the procedures described by Hunter and Macewicz (1985). These provide estimates of fish in the population that will spawn within the next 12 h (FOM) or have just spawned in the past 12-24 h (POF). The potential annual RBF was estimated, following Karlou-Riga and Economidis (1997), by multiplying the RBF by the potential annual spawning frequency.

Ova diameter-frequency distributions from Gilson’s fluid-preserved ovaries collected from the peak spawning period in March, through to May, were determined with the view to further confirming the spawning rhythm and the spawning type. Ripe females, judged visually by the yellowish or pinkish-pale colour of the ovaries and relatively larger, strippable-size oocytes, were used for this study. Five sub-samples were taken from each ovary, and approximately 100 oocytes from each sub-sample were measured using an eyepiece graticule fitted on a stereomicroscope (Cambridge Instruments). Care was taken to ensure that only viable cells were measured. Percentage frequency of occurrence of oocytes in different size classes in monthly samples were plotted as histograms. Since all oocytes <100 μm belonged to the reserve pool, they were eliminated from the counts.

RESULTS

The present report on oogenesis in *O. ruber* is restricted to a brief characterisation of four phases: vitellogenesis, mature, spent and regressed. The per-
Vitellogenesis

The vitellogenic phase of oocyte development of *O. ruber* is divided into three classes: (i) early vitellogenic, (ii) mid-vitellogenic and (iii) late vitellogenic. 

**Early vitellogenic class**

The first signs of vitellogenesis are noticed in December when tiny oil vesicles, recognised on histological slides as empty vacuoles, are observed within the cytoplasm of the oocytes. The latter measure $114.7 \pm 17.5 \mu m$, and $57.3 \pm 8.9 \mu m$ for the nucleus (Fig. 2a). The oocytes enter a period of growth during which the oil vesicles increase in size and quantity.
Mid-vitellogenic class

The primary characteristic of the mid-vitellogenic class oocytes is the appearance in them of small (5.6 ± 2 μm), eosinophilic yolk granules in the outer layer of the cytoplasm, which then spread centripetally. The oil vesicles continue to increase in quantity and size (12.6 ± 0.9 μm), and take up the entire cytoplasm except for the peripheral layer which is taken up by the yolk granules. A rapid increase in size of the oocytes is observed and the latter are now surrounded by a layer of connective tissue cells with spindle-shaped nuclei. Yolk granules increase in quantity and size (10.8 ± 0.9 μm), and become globular. Oil vesicles further increase in size (20.8 ± 1.5 μm) and begin to coalesce around the nucleus which still lies at the centre of the oocyte. Yolk and oil are now intermingled throughout the cytoplasm. The chorion, measuring 7.6 ± 0.5 μm thick, with clear, visible striations, makes its appearance around the oocyte. The latter increases in size to 352.9 ± 5.8 μm, and 99.2 ± 2.4 μm for the nucleus (Fig. 2b). Apart from the mid-vitellogenic class oocytes, early vitellogenic ones continue to be recruited from immature class oocytes.

Late vitellogenic class

The cytoplasm is packed with numerous eosinophilic yolk globules. As the yolk globules increase in size (12.8 ± 0.74 μm), a further substantial coalescence of the oil vesicles takes place, resulting in the formation of considerably larger oil droplets, measuring 49.5 ± 4.3 μm, surrounding the nucleus and pressing tightly to it, tending to displace it from the centre. Oocyte and nuclear sizes increase further to 396.5 ± 3.4 μm, and 111.9 ± 2 μm, respectively, and the nucleus now attains an irregular shape. The zona radiata, now differentiated into an outer eosinophilic layer, the zona radiata externa, with faint radial striations, and an inner basophilic layer, the zona radiata interna, with more prominent striations, makes its appearance around the oocyte. The latter increases in size to 352.9 ± 5.8 μm, and 99.2 ± 2.4 μm for the nucleus (Fig. 2b). Apart from the mid-vitellogenic class oocytes, early vitellogenic ones continue to be recruited from immature class oocytes.

Mature phase

During oocyte maturation, oil drops coalesce into a single globule, measuring 135.9 ± 2.38 μm in diameter, situated either at the vegetative pole or at the centre of the ovary, thus completely displacing the nucleus which migrates from the centre of the cell to the animal pole where it elongates and finally dissolves (Fig. 2d). Yolk globules also coalesce into a homogeneous mass and the oocytes become transparent. After completion of homogenisation, hydration of the oocytes begins, leading to ovulation. Mature oocytes in migratory-nuclear and yolk homogenisation stages were first observed in January, indicating imminent spawning.

Spent phase

During the spent phase, the ovary is characterised by the presence of different generations of POF, their number being a rough reflection of the number of times the female has spawned. Different classes of vitellogenic oocytes are also present (Fig. 3a).

Regressed phase

The regressed ovary presents many oocytes and follicles undergoing resorption, as well as immature, non-yolky oocytes broadcast within the organ.

Spawning rhythm

The main spawning in O. ruber begins in January and ends in April, although 4% of the specimens were spawning in May, as evidenced by the presence of FOM oocytes (Table 2). Histological evidence showed that females in the late vitellogenic phase sampled in January contained oocytes in different developmental classes. This represents a typical asynchronous type of ovarian development. The ovaries of partially-spent females sampled in January and February contained POF and a wide variety of vitellogenic classes continue to be elaborated in the ovaries and undergo vitellogenesis asynchronously. These observations were made in the ovaries of the January samples, indicating that oocyte maturation in O. ruber is an accelerated process lasting only one month from the start of vitellogenesis.
oocytes (Fig. 3a), indicating a recent spawning event and the potential for additional batches of eggs to be released, confirming that O. ruber is a typical multiple spawner. Sequential ovulation of the FOM from the additional batches of late vitellogenic class oocytes occurs synchronously and they form the subsequent batches of eggs which are spawned later. After repeated spawnings, POF from distinctly different generations, indicating successive spawnings, are present in the ovaries. The height of spawning occurred in March, as the majority of the specimens (68.2%) exhibited ovaries packed with FOM (Fig. 3b). The histological features of the April samples suggested strongly that spawning was drawing to a close after the peak spawning the month before. This is evidenced by the presence of different generations of POF, including large ones, suggesting recent release of another batch of eggs, and oocytes undergoing post mortem degeneration (Fig. 3c). In addition, rela-
tively few of the ovaries of the April samples (15%) revealed the presence of FOM (Table 2). Reorganisation in the regressed ovary was fairly rapid at this time as, apart from the very few remnants of late stage atretic oocytes, the overwhelming majority of the samples contained numerous immature, non-yolky oocytes (Fig. 3d).

**Spawning frequency**

During the spawning period, (January-May), a total of 205 ovaries were sectioned, of which 69 were in the FOM stage and 105 in the POF stage (Table 2). The mean monthly spawning frequency was 35.5% using the FOM method and 45.3% using the POF method, indicating spawning frequencies of 2.8 days and 2.2 days respectively, which were not significantly different from each other (ANOVA, p<0.05). These figures give a potential number of spawns during the year of 14 and 11, using the FOM and POF methods, respectively.

**Batch fecundity**

A total of 50 mature females ranging from 37 to 55 cm SL, with corresponding weights of 739.8 and 210 g, respectively, were used for the batch fecundity analysis. Batch fecundity ranged from a minimum of 190000 eggs for a 40 cm SL fish weighing 977.6 g, to a maximum of 1753247 eggs for a 50 cm SL fish weighing 1677.8 g, with a mean of 779737 eggs. Batch fecundity was significantly positively correlated with both OFBW (p<0.05) (Fig. 4a) and SL (p<0.05) (Fig. 4b). SL was a better predictor of BF ($r^2 = 33\%$) than was OFBW ($r^2 = 19.9\%$). A significant difference was found in BF between March, the height of the spawning season, and April (p<0.05) and between March and May (p<0.05), but not between April and May (p>0.05) (Fig. 5). The relative batch fecundity was 716 eggs/g OFBW. Therefore, the potential annual relative fecundity is 10024 eggs/g OFBW using the FOM method of spawning frequency estimation, and 7876 using the POF method.
Oocyte diameter-frequency distribution

In female *O. ruber* sampled at the height of spawning in March, the oocytes of the mature females exhibited three modes in diameter-frequency distribution, as revealed by examination of Gilson’s fluid-preserved whole eggs, as follows: the most advanced group measured 700-800 μm, the intermediate mode measured 400-500 μm, and the remaining mode measured 100-300 μm (Fig. 6). The most advanced size class of eggs was clearly distinguishable and did not overlap in size with the other two size classes.

Examination of the eggs using a stereomicroscope revealed three egg-types that correspond, more-or-less, with the modes found in the frequency distributions of oocyte diameters. The most advanced size class of cells was that of ripe eggs and consisted of two types: (a) spherical, hydrated, hyaline and golden, with gelatinous threads coiled around them, characteristic of hydration stage oocytes and (b) golden and translucent at the animal pole, characteristic of migratory-nuclear stage oocytes. The intermediate group, made up of cells with dense, dark and compact yolk, were maturing, vitellogenic oocytes, while the smaller mode did not differ much in appearance from the former, except by size.

In April, a similar modal pattern, except for a considerable overlap in modes between the intermediate and smallest size classes, was evident. However, the most advanced group continued to be distinct, but at a reduced size class ~ 500-700 μm. By May, most of the large size class eggs are shed, and the oocyte diameter-frequency distribution reveals only a single mode, at 100-400 μm. Generally, there is a reduction in the size of eggs spawned as spawning progresses, from 800 μm in March, to 400 μm in May.

DISCUSSION

Nearly two decades after the pioneering studies on reproduction in *Otolithes ruber* (Hussain and Abdullah, 1977; Abu-Hakima et al., 1983), evidence is presented in the present study to support the earlier findings that the main spawning season of the species in Kuwaiti waters is from January to April. However, this study has also revealed that a small proportion of the stocks continue spawning into May. The present findings are also in conformity with the gonadosomatic index profiles depicted in Abu-Hakima et al. (1983), in terms of the acceleration of the process of vitellogenesis. Once initiated in December, vitellogenesis proceeds very rapidly, leading to maturation and the onset of spawning in January. Accelerated vitellogenesis and extended spawning durations could be common features of the sciaenids from a latitudinally similar (sub-tropical) but geographically discrete range (Fennessy, 2000; Brown-Peterson et al., 2002).

Vitellogenesis in *O. ruber* is an asynchronous process. During the first part of the process, development of oocytes is markedly asynchronous, so oocytes of different sizes, corresponding to different vitellogenic classes, are present in the ovaries at the same time. By the end of the vitellogenic phase, due to the asynchrony in development, a clear differentiation between oocytes meant for the first spawning
(FOM, or the ready batch) and those for subsequent spawnings (late vitellogenic, mid-vitellogenic and early vitellogenic classes, or the reserve pool), becomes discernible. Discussed together with the data on oocyte diameter-frequency distribution, as the oocytes pass into the maturation phase, the ready batch becomes increasingly differentiated into two types of cells. One includes oocytes in the migratory-nuclear stage, and the second includes oocytes in the yolk homogenisation and hydration stage. These two maturation phase oocytes are probably shed together during spawning as one batch, since transformation of migratory-nuclear oocytes into yolk homogenisation and hydration ones during oocyte maturation in teleosts is usually quite rapid and is accomplished within 24 h (Hunter and Macewicz, 1985; Selman and Wallace, 1989; Karlou-Riga and Economidis, 1997).

The oocyte diameter-frequency distribution data revealed no distinct hiatus between the maturing and immature size-class ova (the reserve pool) due to their asynchronous development. However, by the end of vitellogenesis, as the asynchrony evens out, and the ova constituting the ready batch are ovulated, the cells forming the intermediate mode apparently become distinctly differentiated into a new ready batch for the spawning of the next batch of eggs. Meanwhile, part of the smaller size class, as a result of asynchronous development, recruits into the intermediate pool which, in turn, transforms into the ready batch for the spawning of the next batch of eggs. These sequential or rhythmic events continue until the last batch of eggs is shed. These observations typify multiple spawning reported in a number of teleosts (Bagenal and Braun, 1971; Jones, 1978; Hampell, 1979; DeMartini and Fountain, 1981; Hunter and Leong, 1981; Conover, 1985; Hunter et al., 1986, 1992; Lowerre-Barbieri et al., 1996; Brown-Peterson and Warren, 2001; Almatar et al., 2004).

The FOM and POF methods for estimating spawning frequencies have been used as a tool for comparing dynamics of egg production in species with a wide latitudinal distribution, in species inhabiting different regions of the same water-bodies, in species with different reproductive seasons, and in year-to-year differences within a species (Hunter and Goldberg, 1980; Conover, 1985; Hunter and Macewicz, 1985; Hunter et al., 1992; Brown-Peterson and Thomas, 1988; Karlou-Riga and Economidis, 1997; Brown-Peterson and Warren, 2001; Brown-Peterson et al., 2002). These studies were conducted mainly on fishes inhabiting North American waters. Apart from Almatar et al. (2004) who reported on the spawning frequency of *Pampus argenteus* in Kuwait waters, no other study has been undertaken either locally or regionally. From the present study, with spawning occurring in females on average every 2.8 days according to the FOM method of estimation, and every 2.2 days as revealed by the POF method, it may be concluded that the potential annual number of spawns in *O. ruber* is 11 and 14, according to the FOM and POF methods, respectively.

From the results of this study, it may be concluded that vitellogenesis in *O. ruber* is an asynchronous process resulting, at the time of oocyte maturation, in the formation of different classes of oocytes. Spawning is a rhythmic activity lasting five months during which eggs are liberated once every 2.2 to 2.8 days, reflecting the multiple spawning habits of the species. Oocyte diameter-frequency distribution revealed a multimodal pattern, in further confirmation of multiple spawning. Finally, batch fecundity was a function of both ovary-free body weight and fish standard length.

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