

Oogenesis, Histological Gonadal Cycle, Seasonal Variations and Spawning Season of Female Silver Pomfret (*Pampus argenteus*, Euphrasen) From the Spawning Grounds of Kuwait

KHALID P. LONE*, SALAM AL-ABLANI AND SULAIMAN ALMATAR
Mariculture Fisheries and Marine Environment Department, Food Resources and
Marine Sciences Division, Kuwait Institute for Scientific Research, Kuwait
E-mail: kplone@hotmail.com

Abstract.- A study of the seasonal variations, gross structure and gonadal cycle of wild female silver pomfret, *Pampus argenteus*, was conducted on samples collected during three successive spawning seasons off the coast of Kuwait. The ovary of the Zobaiddy is a typical teleost ovary. Because of the spatial restriction of the visceral cavity (which itself is dependent upon the anatomical form of the fish), it is quite small and curved and is "L" shaped. Grossly, female gonads were classified into eight stages of development. Minimum GSI for females (0.69 ± 0.11) occurred in January when the lowest seawater temperature (13.90°C) was recorded. The maximum GSI occurred in June (7.90 ± 0.04) when the water temperature was 30.60°C . The GSI was related to temperature more than the ovarian weight which showed a continuous decrease (due to spawning) after the June peak till October after which time a precipitous decrease was noted in both ovarian weight and GSI. This was related to shorter days and decreasing water temperatures. Based on ovarian maturity, a maximum GSI of 14.90 was found for Stage 5 (gravid or ripening) fish. The ovaries develop to a peak during the spawning season which starts in Kuwait's coastal waters in late May with a peak in June and continues through September. In October, there may be a second phase of spawning when the temperatures come down again from a peak. This second spawning is not very significant, though. The peak spawning time is in July. From the detailed studies, it seems that a female may spawn many times during the spawning season.

Key words: Butterfish, gonadal cycle, gonadosomatic index; annual reproductive cycle, Zobaiddy,

INTRODUCTION

Reproduction in many fish is cyclic although the length of the cycle is extremely variable (Lam, 1983; Dodd and Sumpter, 1984; Wang *et al.*, 2001; Beros *et al.*, 2004; Brickle *et al.*, 2005; Yanagimoto and Humphreys, 2005). Many fishes in the temperate and tropical regions exhibit a rhythm of reproduction that is correlated with the photoperiod and temperature variations. In the tropics and sub-tropical areas rainy season is also considered an important influence because of the productivity of the water and its importance for the newly hatched larvae (Ganias *et al.*, 2004; Park *et al.*, 2006; Juchno *et al.*, 2007; Narimatsu *et al.*, 2007).

Silver pomfret (Family Stromateidae) is widely spread throughout the Indo-Western Pacific region and supports valuable fisheries along the coast of India (Kagwade, 1988; Pati, 1981), the eastern part of China, the western and south western Korean Peninsula (Cho *et al.*, 1989) and western Asia to the Arabian Gulf (Al-Hussaini, 2003).

Along the coast of western Bengal in India, silver pomfret (Zobaiddy, in local Kuwaiti dialect) undertake spawning migrations to their breeding and nursery grounds in the north during the onset of the spawning season, and migrate to the south in the post-spawning period (Pati, 1981). Most catches of Zobaiddy in the East China Sea and Yellow Sea come from areas where oceanic fronts occur due to mixing of warm and cold currents (Cho *et al.*, 1989). Zobaiddy migrates northward or southward according to the distribution of warm water currents thus, they migrate to the north in summer and to the south in winter. In the Bay of Bengal, two peaks of spawning were observed: February to April and July

* Present address: University of Wah, The Mall, Wah Cantt. Pakistan.

to August (Pati, 1981), while in the eastern China Sea, the spawning peak was from May to July (Lee and Jin, 1989).

In India, Zobaiddy is also caught all along the coasts of Maharashtra (Bombay coast) and Gujrat. In Arabian Sea, silver pomfret spawning grounds have been described in coastal waters of Gulf of Kutch and Gulf of Cambay, areas that border Pakistan's coastal waters. Spawning in Bombay coasts is from October-December. Juveniles are available in inshore areas from January to March. However, on the Gujrat coast, mature and spent fish are caught during the period April to June; however, the spawning season extends from February to August (Gopalan, 1969). This difference in the spawning period may be due to the two different non-homogenous stocks, the water temperature and currents. No report on spawning or biology from Pakistani waters is available.

Silver pomfret is a fish of choice in Kuwait and is preferred by the local population. The value of the local Zobaiddy landed in Kuwait's fish markets in 1993 was US \$ 9,477,000 out of the total fish value of US \$ 19,410,000 of Kuwait's fish production. The catch of the Kuwaiti gillnet fleet has declined to less than 88%, from 1112 tons in 1994 to 120 tons (US \$ 1,570,103) in 2000 (CSO, 2001; Al-Hosaini, 2003). Because of its commercial value, studies were initiated on the biology of wild fish so that work on the aquaculture of pomfret could be started (Almatar *et al.*, 2004; Lone *et al.*, 2008).

In the present study, we are describing a detailed account of the reproductive biology of female silver pomfret captured from the spawning grounds of Kuwait for three years. This stock of pomfret is shared with Iran and Iraq.

MATERIALS AND METHODS

During May 1998 to October 2000 (30 months), more than 600 pomfret (known as Zobaiddy in Kuwait) gonads were collected from their spawning grounds and fixed in 10% buffered formalin in sea water. These samples came from a total of 5795 (1883 female and 3912 male) Zobaiddy collected from the spawning grounds situated in the coastal waters of Kuwait. These fish were captured

by trawl fishing during three successive spawning seasons of the fish. At the time of capture, the fish were examined for eggs and measured onboard the boat. The gonads were immediately removed, checked grossly and anatomically, and classified. The fixed samples were brought to the laboratory, and after a proper fixation time were processed for histological studies (Lone *et al.*, 2008).

The fish were measured for total length (TL), fork length (FL) and standard length (SL) to the nearest millimeter. Total and gutted weights were also recorded. The gonads were removed and were also weighed to the nearest milligram. Females were classified according to the maturity scale both macroscopically and, later, microscopically by inspecting oocyte samples in histological slides. The details of these studies are given elsewhere (Almatar *et al.*, 2004; Lone *et al.*, 2008). The gonado-somatic index (GSI) was calculated as follows:

$$\text{GSI} = \frac{\text{Weight of gonad}}{\text{Gutted weight of fish}} \times 100$$

At the time of processing for the histological studies, the gonads were removed from the jars, and a suitable sample was taken. Generally, three samples were taken from the same ovary. These were from anterior (proximal and cephalic), mid (at the highest width), and posterior (distal or caudal) points of the ovary. These samples were taken in order to determine whether the distribution of oocytes was uniform or zonated inside the ovary. These samples were then transferred to 50% isopropyl alcohol and kept overnight. After this time, the samples were loaded in an automatic tissue processor (Citadel 1000, Shandon) and passed through different grades of isopropyl alcohol, Histo-sol or xylene and embedded in paraffin wax, according to a program. These embedded tissues were molded into paraffin blocks with the help of a Histo-embedder (Leica, Germany) for sectioning. A total of 260 fish at different gonadal stages were processed for this study.

The blocks were trimmed, and transverse sections (3 to 5 μm) were obtained with the help of an automatic rotary microtome (Jung Supercut, Germany). The sections were placed on microscopic slides after stretching, and were stained with

haematoxylin and eosin and studied (Lone and Marzouk, 2000; Lone *et al.*, 2001).

RESULTS AND DISCUSSION

Study of the annual reproductive cycle in wild female Zobaidy

The data regarding photoperiod, water temperature, ovarian weight and GSI are given in Figure 1.

The ovaries of Zobaidy are paired, L-shaped structures. They are attached to the viscera on one end and kidneys on the other. The two lobes are enveloped in a transparent sheath which brings the two lobes in close proximity to each other. Towards the distal end (the end where they are attached with the body at the anus), the two lobes unite and form a very short oviduct that opens to the outside and is used to expel the ripe eggs to the exterior at the time of spawning. Unlike testes, Zobaidy ovaries can easily be classified according to the standard terminology; eight stages were recognized based on the gross structure of the ovaries. The stages are: virgin, resting, developing, maturing, gravid or ripening, running or spawning, partially spawned, and spent. The ovaries were then studied histologically. The Figure 2 describes the relationship between the gross ovarian stage and GSI. A summary of the gross structure of the ovary and its related histological picture has been given elsewhere (Almatar *et al.*, 2004). Each sectioned ovary was studied thoroughly, and the following oocyte characteristics were looked for: oocytes that had not begun depositing yolk (non-vitellogenic), also known as primary oocytes; oocytes in early vitellogenic stages (endogenous vitellogenesis), or secondary oocytes; oocytes with advanced vitellogenesis (external vitellogenesis), or tertiary oocytes; migratory nuclei oocyte (germinal vesicle movement stage; GVM); germinal vesicle breakdown (GVBD); hydrated oocytes; and post-ovulatory follicles (POF). The GVM and GVBD stages are sometimes collectively known as the final oocyte maturation (FOM) stage. The presence of different stages of POF can help in defining the ovarian stage and spawning frequency of the fish in a spawning season. The stained sections were also looked at for atresia. This stage can help in deciding

the time course for the cessation of spawning for the season. Based on these characteristics, the ovarian maturity cycle of the Zobaidy female is described by stages below.

Stage 1 (Virgin)

Very few fish in this stage were found at the spawning ground. The gonad was very thin and thread like. Sometimes it was difficult to sex Zobaidy at this stage, and the actual sex could only be confirmed histologically. Histologically, the tunica albugenia was thick and the stroma was very well developed. Oogonia were very common. The cytoplasm of the oocytes was very basophilic (bluish), and the nucleus-to-cytoplasm ratio was lower than one. All oocytes were in the chromatin nucleolus stage or in the early peri-nucleolus stage (Fig. 3). The size of the oocytes never surpassed the 100 μm mark, but the majority was in the range of 50 to 70 μm and formed one group (peak) The GSI was 0.6 ± 0.2 (n=26).

Stage 2 (Resting)

The GSI for this stage (n=35) was 0.70 ± 0.20 (Mean \pm SD). The thickness of the gonads was slightly higher than the stage 1 ovary. The majorities of the oocytes present in this type of gonads were small and comprised of oogonia and primary oocytes. Some oocytes were in the chromatin nucleolar stage. The biggest oocytes present were in the early peri-nucleolus stage. The size of the oocytes never surpassed 200 μm ; however, the majority was in the range of 75 to 150 μm . The oogonia were basophilic in nature and stained darkly. The nucleus was quite big, while the cytoplasm was in the form of a narrow rim around the nucleus. The stroma of the ovary was well developed, while the tunica albugenia was thick (Fig. 4).

Stage 3 (Developing)

There was an increase in the size of the gonad. The girth of the ovary increased. The color of the gonad was pale yellow and the blood vessels were quite visible on the surface of the ovary. The texture of the ovary was compact and hard. The GSI for this stage was 2.0 ± 0.9 (n=36). Histologically, the primary oocytes dominated, but some early

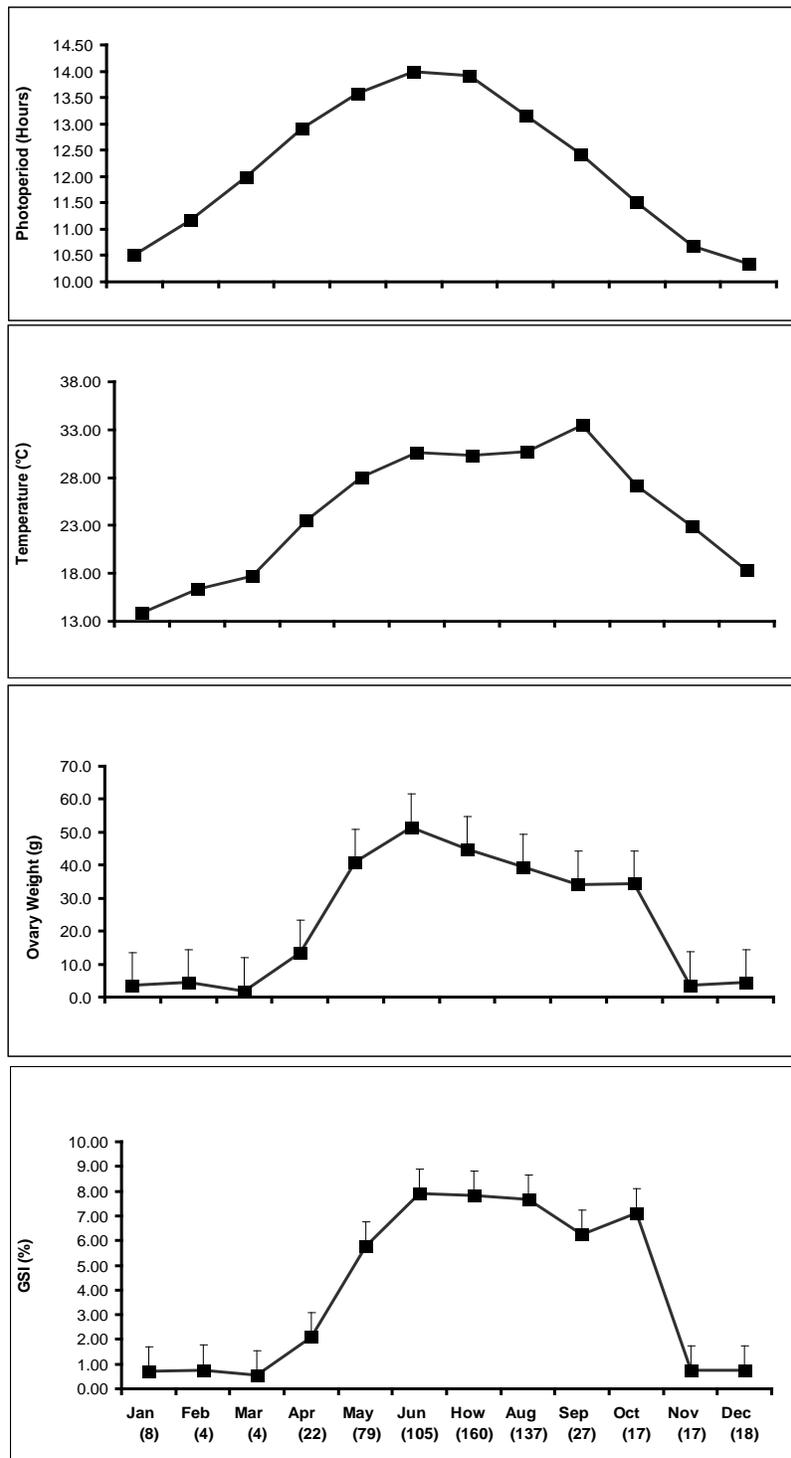


Fig. 1. Monthly variations in photoperiod, temperature, ovary weight (g) and gonado-somatic index (Mean±SE) of wild female pomfret, *Pampus argenteus*, at the Kuwaiti spawning grounds. For other details see Materials and Methods.

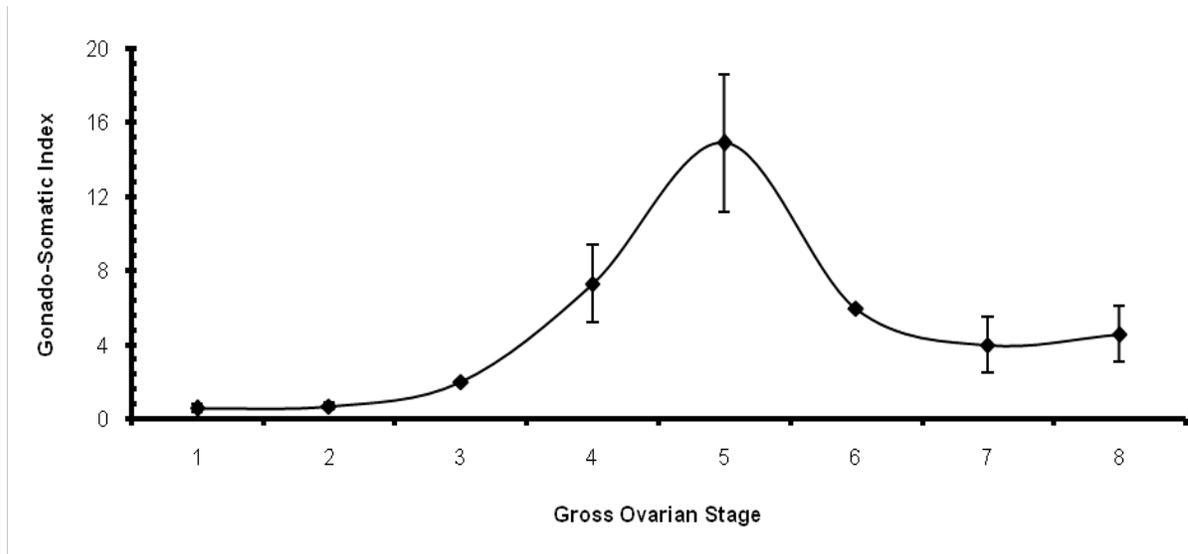


Fig. 2. Relationship between gross ovarian stage and gonado-somatic index of wild female pomfret, *Pampus argenteus*, at the Kuwaiti spawning grounds. The data provided are Mean \pm SEM.

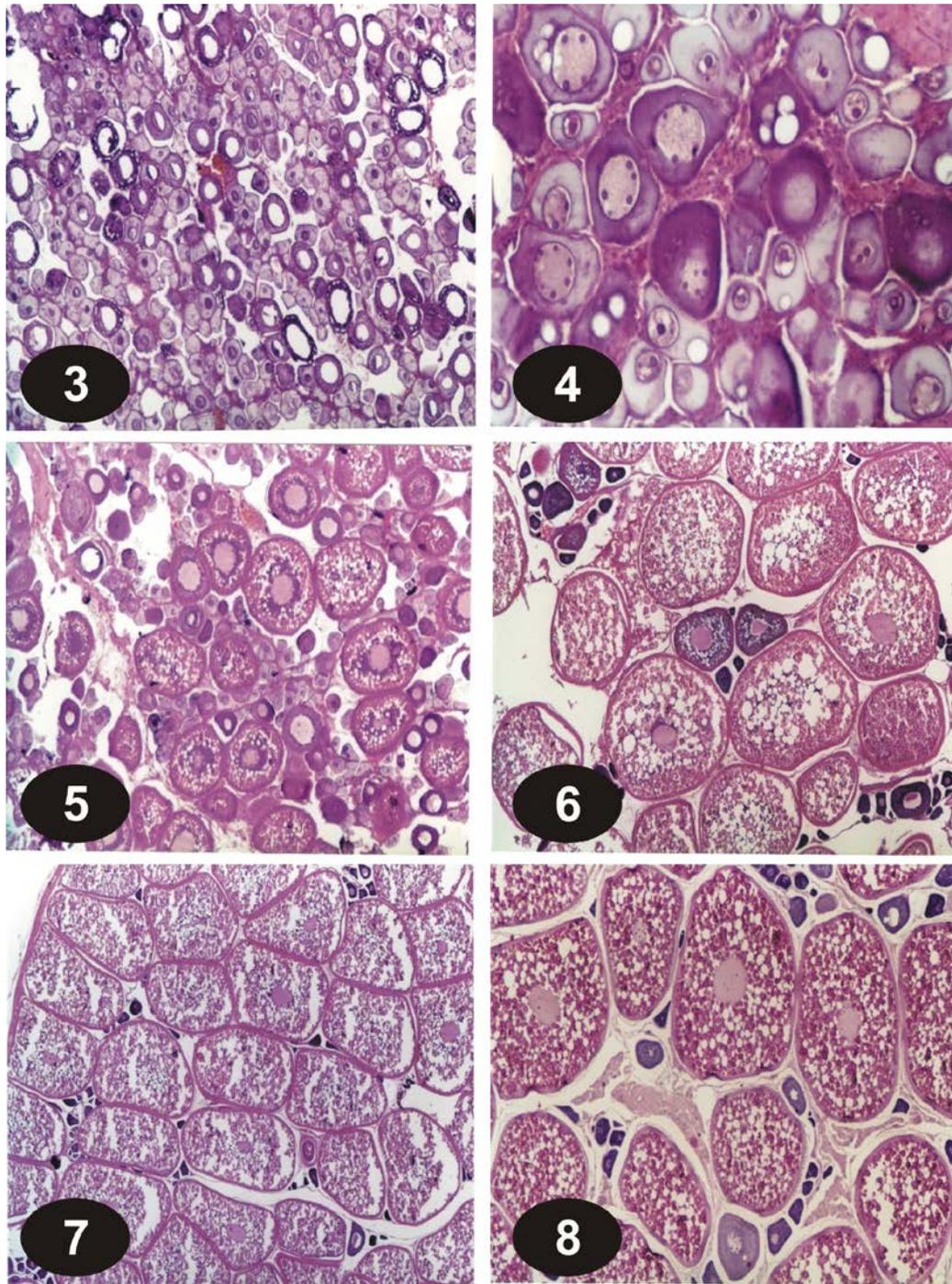
stages of secondary oocytes in the yolk vesicle stage were also seen. The size of the oocytes increased because of yolk vesicle deposition. The nucleus-to-cytoplasm ratio also increased. The cytoplasm became less basophilic and looked pinkish-blue rather than dark blue, as was seen in very early oocytes. In the secondary oocytes, zona radiata was now established. The size distribution of the oocytes at this stage ranged up to a maximum of 600 μm . However, this size distribution was season-dependent. Early in the season, stage-3 ovaries had oocytes in the smaller range, but during the months of May, June and July, the females in this stage had more bigger oocytes than small oocytes. These were arranged generally in two groups (peaks). The first peak was seen around 200 μm , while the second peak was between 450 and 550 μm (Fig. 5).

Stage 4 (Maturing)

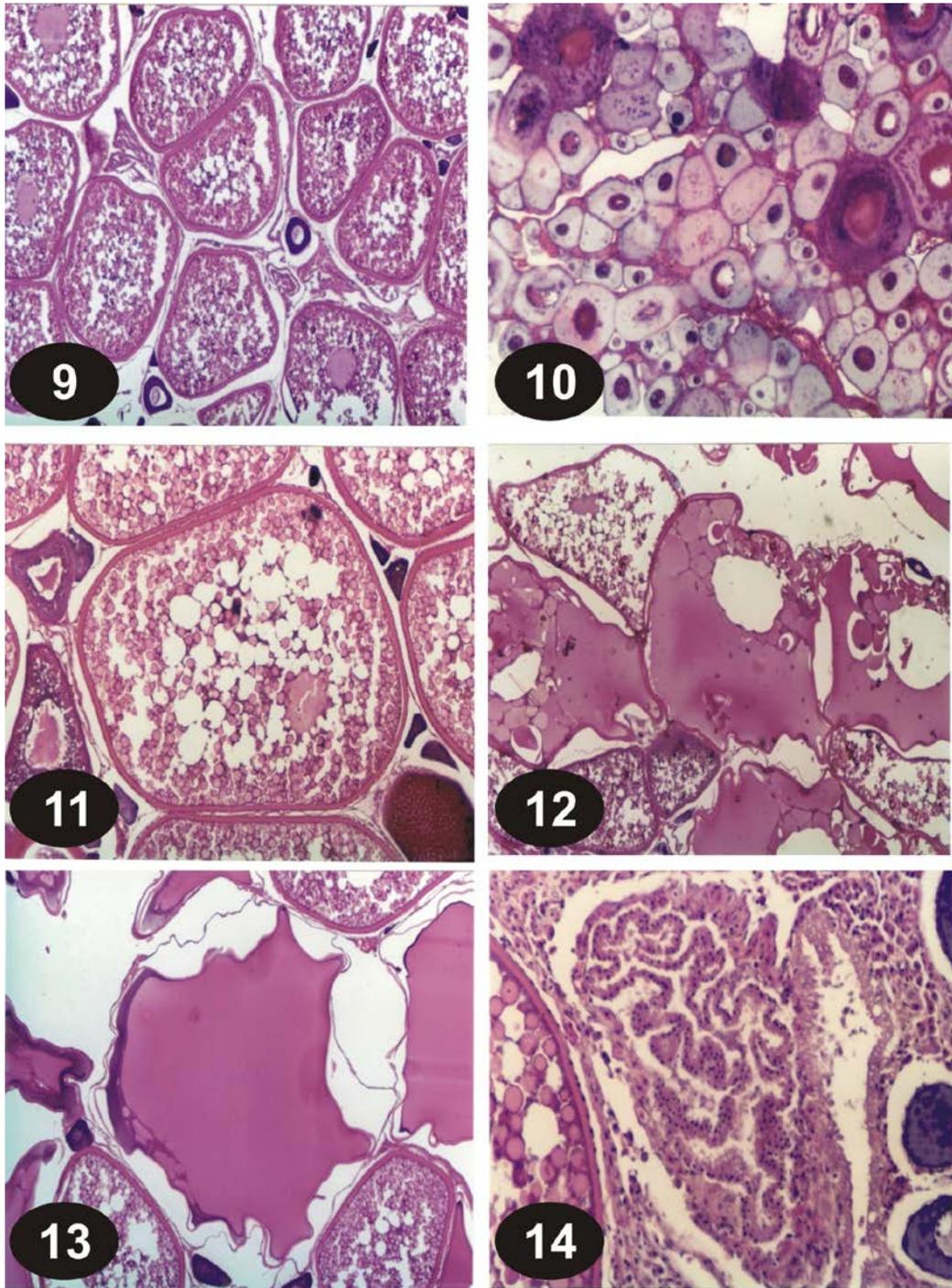
The weight of the ovaries increased linearly since stage-1 and by now there was an increase of up to 12 times in the weight of the ovaries from that of stage-2 ovaries (Fig. 1). The GSI for this stage was 7.3 ± 2.1 ($n=75$). The ovarian texture was still solid but blood circulation had increased. The entire surface of the ovary was covered with big and small arteries. The colour of the ovary became dark yellow because of the yolky oocytes. Histologically,

three types of oocytes could be seen. A very small group of primary oocytes was present between the crevices made by the growing oocytes. Some were in the secondary stage, but by far, the largest group was of tertiary-stage oocytes. These oocytes were characterized by a well developed zona radiata. This was further divided into zona externa and zona interna. The theca and granulosa cells were seen around the zona externa. The tertiary-stage oocytes had well developed, true yolk granules and some fat droplets. The nucleus or germinal vesicle stained pink and had nucleoli in it. The stroma was thinner and all stages of atresia (α , β , γ and δ) of the tertiary yolked oocytes were commonly seen. The tunica was thinner than in the previous stages, and the yolked oocytes could be seen through the tunica (Fig. 6).

The histological picture can be slightly different in different parts of the year and on the spawning stage. Since *Zobaidy* is a multiple-spawner, the oocyte development is asynchronous type (all stages of oocyte development can be seen all the time) but the final maturation is group synchronous type. This point will be elaborated upon later in Stage 5 and Stage 6 fishes. If the fish is caught in the late spawning season and is preparing for the second or subsequent spawning, then the texture will be slightly softer, and there will be



Figs. 3-8. Histological sections of wild Zobaidy ovaries. The sections have been arranged according to the maturity stage of the ovary based on the gross structure of the gonad. The ovaries were fixed in 10% buffered formalin and three parts that are proximal, middle and distal portions were cut horizontally at 3-5 μ m. The sections were stained with Haematoxylin-Eosin. For details, see Results and Discussion. 3, gross stage 1 (December, 1999; 150X); 4, gross stage 2 (September, 1998; 300X); 5, gross stage 3 (October, 1998; 60X); 6, gross stage 4 (May, 1999; 40X); 7, gross stage 5 (June, 1999; 40X); 8, gross stage 6 (June, 1999; 60X).



Figs. 9-14. Histological sections of wild Zobaidy ovaries. The sections have been arranged according to the maturity stage of the ovary based on the gross structure of the gonad. 9, gross stage 7 (June, 1998; 60X); 10, gross stage 8 (December, 1998; 150X); 11, an oocyte showing germinal vesicle movement (GVM). The lipid yolk granules (white globules) are sequestering to form bigger drops. Zona radiata is well developed. Protein yolk (red granules) is on the periphery (May 1998; stage 5; 100X); 12 and 13, oocytes in the final maturation stages (July, 1999; 60X); 14, a high powered micrograph of post-ovulatory follicle (POF) (July, 2000; stage 7; 300X).

spaces in the ovaries caused by the release of the mature and hydrated oocytes during the previous spawning. Also, the atretic oocytes will be more prevalent in this type of fish. This is because the fish is removing non-expelled or residual oocytes, and making space for the new wave of mature oocytes for subsequent spawnings. The maximum size of the oocytes never surpasses 800 μm . Two groups clearly established, but in late Stage 4 fish, a third group of bigger, tertiary oocytes ($>800 \mu\text{m}$) may be present. Some old POFs might also be seen in such Zobaiddy. This is most true of those fish that have spawned earlier.

Stage 5 (Ripening)

The weight of the gonads continued to increase and the maximum weight of the ovary was observed during this stage. The GSI was 14.9 ± 3.7 ($n=96$). This means that from Stage 2, the GSI of the fish increased 21 times. The ovaries at this stage exhibited a softer, speckled appearance when seen through the surface because the tunica was thin, completely stretched and transparent. This speckled nature was due to the appearance of hydrated oocytes in the ovaries. The overall color was light yellow with a spotted appearance of yellow (yolked oocytes) and white (hydrated oocytes). The blood supply was at its peak, and the surface was completely covered with the thick arteries and their capillaries.

Histologically, only two types of oocytes could be seen: The primary oocytes present in the crevasses, and the tertiary stage oocytes and oocytes entering the FOM stage (Fig. 7). This stage was characterized by the movement of the (nucleus) germinal vesicle (GVM). Along with this there is sequestration of the oil droplets and the yolk granules. In the later stages, the nuclear membrane of the germinal vesicle breaks down and the chromosomes are free in the cytoplasm (GVBD). This stage is a clear-cut indication of the final maturity of the oocyte. By this time, the yolk is completely liquefied and nuclear elements are completely masked by this (Figs. 12-13). These stages are very much interrelated and occur in quick succession, and may take 8 to 12 h before ovulation and actual spawning. The oocytes of this type were

tightly packed and were at the same stage of development, *i.e.*, group synchronous development. The hydrated oocytes were filled with liquefied yolk, and the tunica was very thin. Size wise, three groups of oocytes could be seen in such ovaries. One group, or peak, is around 200 μm for primary oocytes, the second peak is around 500 to 600 μm for tertiary oocytes ready to enter the FOM stage, and the third peak is between 900 and 1200 μm for hydrated oocytes and those oocytes that are at different stages of FOM.

Stage 6 (Spawning or running)

This stage is characterized by the free flow of ripe eggs ovulated from the ovaries when the fish are handled slightly. Because of this activity, there is a decrease in the weight of the ovary. It was difficult to estimate the GSI for running-ripe females because eggs oozed out freely as the fish were taken out of the water. The value 6.0 ± 0.5 ($n=41$) is based on weight of ovary that was encountered after the fish were sacrificed in the lab or from the fish which were stripped on board the capture boat. When the data from these fish were taken into account, the values reached up to 16-17% of the body weight. The gross appearance of the ovary was whitish and jelly-like because of the overwhelming presence of hydrated oocytes. The tunica was very thin, and oocytes could be clearly seen through it. The oviduct was well developed and full of mature, ovulated oocytes or eggs. Blood supply to the ovaries was similar to that described for Stage 5 fish. Some ovaries were bloodshot because of the bleeding that ensued at the time of ovulation.

Histologically, the ovarian picture is more or less similar to that of stage-5 fish in that only two types of oocytes, *i.e.*, primary and tertiary oocytes, are present. Occasionally, a secondary oocyte can also be seen. The major difference between Stage 5 and Stage 6 ovaries is the absence or presence of POFs. The POFs are absent in Stage 5 fish but are an integral part of the Stage 6 ovary (Fig. 8). These structures are formed by the expulsion of the oocyte (egg) from its follicle. POF is a cell mass that surrounds the oocyte and is composed of granulosa and theca cells. These cells are very important in the

growth of oocyte in that they provide the food for the growing oocyte and also synthesize sex hormones needed for the final maturation of the oocyte and secondary sexual characteristics. POFs are important in ascertaining the multiple-spawning characteristics of the ovary (Fig. 14).

The oocytes in the stage-6 ovary generally form two groups, but in some fish, a third peak of mature eggs is also present. The first peak of primary cells is around 200 μm , the second peak of tertiary oocytes is around 600 μm , and third one is around 900 to 1300 μm . This third peak is due to residual oocytes or eggs that could not be spawned and are present in the ovarian lumen. These eggs will be involuted and recycled by the process of atresia, and the energy and space salvaged will be used for the next wave of maturing oocytes (coming from the 600 μm group).

Stage 7 (Partially spawned)

The weight of the ovary further decreases in this stage. This stage was generally seen in the mid-spawning season when at least one batch of eggs had already been spawned out. Grossly, the ovarian outline was saggy and flaccid, and sometimes bloodshot. The tunica is wavy, and oocytes could be seen through them. However, in bloodshot ovaries, this was somewhat difficult. The GSI was 4.0 ± 1.5 (n=41).

Histologically, the ovarian structure is very similar to that of Stage 6 ovaries except that different types of POFs may be seen. They point to different times or frequencies of spawning. The presence of at least one type of POF is a must in order to classify a gonad as a stage-7 ovary (Fig. 9). The sizes of different groups of oocytes are also similar to those described in the stage-6 ovary (Fig. 8). Nearly all stages of atresia of tertiary and hydrated oocytes were seen. Atresia is more common in ovulated eggs that were not spawned during previous spawning.

Stage 8 (Spent)

Normally, the weight of the ovary decreases further as this type of ovary is found in the last part of the spawning season or in those Zobaidy that have stopped spawning for the season. The ovarian outline is loose and baggy, and the tunica is thick.

Nothing can be seen through it. This may be because of involution of the ovarian parts and the diminishing blood supply. Sometimes a fish with a mature-looking ovary is also encountered, but because of the season, the oocyte bypasses FOM to enter the atretic cycle. This particular type of ovary is quite prevalent during October, when the water temperature once again becomes favorable after reaching a peak in September. In fact, some Zobaidy may spawn during this month and readily enter this stage instead of Stage 7, which is normally the course in the middle of the spawning season (Figs. 5 and 6). This fact is seen in the GSI for this fish, which is similar to or slightly higher than that for the Stage 7 fish. The GSI for this stage was 4.6 ± 1.5 (n=15).

Histologically, only the primary cells predominated in the ovarian cavity. These were at the peri-nucleolar or chromatin-nucleolar stage. In fishes where some development occurred because of favorable temperatures, secondary and tertiary oocytes were also seen. The majority of these were also undergoing atresia. There were a lot of empty spaces present in the ovary and between the ovigerous folds. Stroma was developing again, and occasionally, old POFs could also be seen. Size wise, only one peak of oocytes between 150 and 300 μm could be seen (Fig. 10).

Seasonal variations in the ovary and vitellogenic cycle

In the section above the ovarian cycle was described according to the gross stage of the ovary. As the fish were collected from the wild for three consecutive years, the histological structure related to the ovarian recruitment of the oocytes in the ovary of the Zobaidy was also analyzed. In the following account, this cycle is reported. The ovaries in January were very thin and in Stage 2. Their gross structure and histological stages can be seen in the above description. The GSI during this month was 0.69 ± 0.11 (n=8). The GSI remained more or less at the same level till the month of March when the GSI was 0.55 ± 0.14 (n=4). After March, when the water temperature and photoperiod started increasing, there was an increase in the weight of the ovaries, and in April, the GSI reached 2.10 ± 0.43 (n=22). This GSI was similar to that of

the Stage 3 ovaries. This means that real changes in the development of the ovaries started in the last days of March and the early days of April. This was the time when endogenous vitellogenesis had already started and early phase of exogenous vitellogenesis could also be seen. Endogenous vitellogenesis means that the oocyte's cytoplasm is directly involved in the synthesis and deposition of the yolk nucleus, yolk vesicles and related materials in the cortical cytoplasm of the developing oocyte. In this stage, the oocytes' own nuclear genes, are actually taking part in the transcription and translation of the genetic material into yolk proteins. This is actually seen in the oocyte cytoplasm in the form of nuclear material rich in polyadenylic acid (poly-A RNA) oozing out from the nuclear membrane into the cytoplasm. This material stains very deeply with basic stains. This blue-colored material is sometimes referred to as Balbiani bodies. The term exogenous vitellogenesis means that the precursors for the vitellogenic material, i.e., phosphitin and lipovitellin, are synthesized in the liver of the fish under the influence of the female sex hormone (estradiol-17 β), and are transported to the ovaries through blood to be finally assembled and deposited as yolk proteins and lipid yolk in the cytoplasm of the developing oocytes. This initiation of the whole process is dependent to a large extent on the water temperature and, to a lesser extent, on the longer days (long photoperiod). The increase in the water temperature also causes the abundance in food and an increase in the foraging behavior of the fish.

The GSI increase further and reach 5.76 ± 0.55 (n=79) in May. During this month, Zobaigy of stage-3, -4 and -5 were encountered in increasing numbers. In June, a peak (Fig. 1) in the GSI was observed, and the values reached 7.90 ± 0.40 (n=105). Here, fish of Stages 3 to 6 were found, and some spawning was observed. The GSI showed a plateau during June, July and August. During these three months, a total of 407 fish were actually observed to record the stage of their gonads (ovaries). The actual number that was caught in the spawning ground was much higher. Although there was no statistical difference among the GSIs of the months June, July and August, there was a clear trend towards decrease of the GSIs. The reason for

this was that spawning was at its best, and the highest spawning figures were observed in the month of July. Spawning activities continued in August and September although it was less than July. When the water temperature declined in October, a second increase in the GSI (7.12 ± 1.01 ; n=17) was noted. This caused another, albeit minor, peak in spawning activity. This second peak in the GSI was the reason that the earlier researcher described the two spawning peaks in Kuwait's waters. However, the study of gonadal weights, GSI, histology and actual observations on *in situ* spawning in the wild has shown that the Zobaigy spawning season is from late May to October (Almatar *et al.*, 2004) with a peak in July and a minor spawning event in October (Fig. 1). Also, it was observed that in October, the smaller fish predominated in the spawners. After October, there was precipitative decrease in the GSI of Zobaigy. This was a signal for the end of the year's spawning season. The GSI during November (0.74 ± 0.03 ; n=17) and December (0.74 ± 0.04 ; n=18) remained the same. This trend actually continued until March of the next year, and was probably dependent upon the lowering water temperatures and shorter photoperiods (Fig. 1).

The present detailed study on the reproductive biology of pomfret from the natural spawning grounds of Kuwait is interesting from viewpoint that it describes an integrated account of the gross structure of the ovary, seasonal cycle and the oogenesis cycle for the benefit of the fishery biologists and aquaculturists. Based on the presence of various stages of POF in the histological studies, it was recognized that the fish is a multiple spawner and may spawn up to at least 4 times in a spawning season extending from June to October. However, in a previous study, based on spawning frequency and fecundity data, we estimated that this fish is capable of spawning up to six times in a spawning season (Almatar *et al.*, 2004). However, this data needs further verification from the same and other areas of its distribution.

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