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# Reproductive biology of Caspian goby, *Neogobius caspius* (Eichwald, 1831) in the southern Caspian Sea (Noor beach)

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**Abstract:**
This study provides fundamental information on some key aspects of the reproductive biology of *Neogobius caspius*, a Caspian Sea endemic, poorly studied Gobiidae fish species. In total, 222 specimens were captured monthly from Noor coastal waters in southern part of the Caspian Sea from April to October 2016, while water parameters (temperature, salinity and pH) were monitored weekly. Gonadosomatic and hepatosomatic indices suggested that the reproductive period of this species was in April to May. Peak of Fulton and Clark's condition factors appeared in April and May for female and male specimens, respectively, then a declining trend appeared until June that remained constant until the end of October. Absolute fecundity ranged from 418 to 1798 oocytes per individual (mean: 1013±357 (SD) oocytes, n=41), which was highly related to the length and weight of fish and weight of gonads. Oocyte mean diameter ranged 35.85±11.80 µm in immature stage to 1103.86±172.01 µm in mature stage. Five stages of maturity for female and four stages for male specimens were described based on macroscopic features and histological description. *N. caspius* appeared as an iteroparous and a batch spawner species, producing more than one oocyte clutch in a single reproductive season.

**Keywords:** Gobiidae, Gonadosomatic Index, Hepatosomatic Index, Caspian Sea

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## Introduction

The endemic Caspian goby, *Neogobius caspius* (Eichwald, 1831), is widely distributed in the Caspian Sea (Berg, 1965; Miller, 2003). It is a large goby species reaching up to 200 mm in the southern Caspian Sea (Iran). The primarily feed items of this fish are polychaetes, crustaceans and small bivalve mollusks (SarpanahSarkohi *et al*., 2010). Like the other gobies in this area, the Caspian goby tends to remain inshore until mid-autumn and migrates to the deep offshore waters during the winter (Miller, 2003). Despite several reports on invasion of *Neogobius* spp. from the Ponto-Caspian area to Europe and North American freshwater habitats (Jude *et al*., 1992; Polacik *et al*., 2009), there are no such reports for *N. caspius*, likely because this species does not enter to the freshwaters in its native range (Berg, 1965).

The Caspian goby has been caught commercially in the former Soviet Union but has no economic importance in Iran. However, it constitutes part of the diet of valuable and commercial fishes such as sturgeons (Miller, 2003). Despite its commercial exploitation and its importance as potential prey for sturgeons, the reproductive biology of this goby is poorly known (Miller, 2003).

The only available study related to reproduction of this species is carried out by Sarpanah Sarkohi *et al*. (2008), which reported gonadosomatic index variations, sex ratio and fecundity of this species in Guilan Province waters. Other studies are about the closely related *Neogobius* species such as *N. fluviatilis* (Abdoli *et al*., 2002; Plachá *et al*., 2010), *N. kessleri* (Kovac *et al*., 2009), *N. melanostomus* (Corkum *et al*., 2004; Hôrková and Kováč, 2014; Macun, 2018) and *N. pallasi* (Alavi-Yeganeh and Kalbassi, 2005; Patimar *et al*., 2008). This study presents the first detailed description of the reproductive biology of *N. caspius* based on a population from the coastal waters of Noor (southern Caspian Sea). The main objective is to provide information on the gonadosomatic and hepatosomatic indices variations, sex ratio, spawning period, fecundity, oocyte diameter and histological characteristics of gonads in relation to several abiotic parameters in this fish.

## Materials and methods

*Sampling and study site*

The sampling site is located in the coastal water of Noor, Mazandaran Province, Iran, at the southern point of the Caspian Sea (36°35′02″ N, 52°02′33″ E). A total of 222 specimens (115 females and 89 males) collected by a beach seine (2×10 m, 10 mm mesh size) during monthly sampling from April to October 2016. During the peak in gonadal changes (April and May), sampling was carried out twice per month. Fish specimens were anaesthetized using clove oil and preserved in an icebox until further examination in laboratory. Water temperature, salinity and pH were monitored weekly (at 12:00 AM by

Multiparameter Water Quality Meter, HANNA HI9829) during sampling periods. Specimens were not available from November to March in coastal area because of their migration to deep offshore waters during the cold months (Miller, 2003).

*Fish biometry, macroscopic and histological study of gonads*

Specimens were weighed to the nearest 0.01 g and measured for total length (TL) and standard length (SL) to the nearest 0.01 mm using a digital caliper. The sex was identified macroscopically and the gonads were assigned a gross maturity stage based on their macroscopic appearance (Biswas, 1993). Specimens with absent or indistinguishable gonads were considered as juveniles and have omitted from the data. The gonads and liver weights were recorded to the nearest 0.001 g for estimating gonadosomatic (GSI: gonad mass/body mass × 100%) and hepatosomatic (HSI: liver mass/body mass × 100%) indices (Bagenal, 1978; West, 1990). Fulton and Clark condition coefficients (Kf and Kc) were calculated to evaluate body condition (Clark, 1928; Le Cren, 1951). Equation for Fulton condition coefficient is $K_f = 100000\ W_T.\ TL^3$ which $W_T$: Total weight (g) and Clark equation is $Kc = 100000\ W_{ev}.\ TL^{-3}$ which $W_{ev}$: Eviscerated fish weight (g). Absolute fecundity was calculated by counting a subsample of ovaries (1-2 g) and the following formula; F: N × (G/g) (Grimes and Huntsman, 1980), where, F: fecundity, N: number of eggs in sub-sample of ovary, G: total weight of ovary and g: weight of the sub-sample (sub-samples weight >0.5 g). Relative fecundity was estimated by dividing absolute number of eggs by total weight of fish, including gonads ($W_{T,}$ wet mass).

Three gonads from each macroscopic stage transferred to Bouin's solution for 48 h and then immersed in 70% ethanol until examination. They were embedded in paraffin and 7 μm sections were stained with haematoxylin-eosin and examined at 40-400X magnification (Nikon 3200). Different stages of gonads development were determined for both sexes (Brown-Paterson *et al.,* 2011; Kagawa, 2013). The diameter of oocytes at each maturity stage was calculated using the Image Tools Ver. 3 to estimate the mean oocyte diameter per ovarian stage.

*Statistical analysis*

The chi-squared test was performed to evaluate the sex ratio. The relationship between absolute fecundity and total length, total weight and gonad weight were analyzed with Pearson correlation test. Comparing mean values of condition coefficient, GSI and HSI in different months carried out by One Way ANOVA, Duncan multiple range test. The difference of condition factors between sexes were statically estimated by independent samples t-test which set at 95% of the significant value and performed using SPSS software Ver. 16.

## Results

*Water parameters*

Monthly variations of temperature, salinity and pH in coastal water of Noor (southern Caspian Sea) are illustrated in Fig. 1. The water temperature ranged between 17.1±1.9 °C in April to 29.0±1.0 °C in August, the salinity between 10.8±0.3 ppt in April to 11.5±0.1 ppt in September and pH between 7.9±0.1 in July to 8.7±0.1 in May (average±SD).

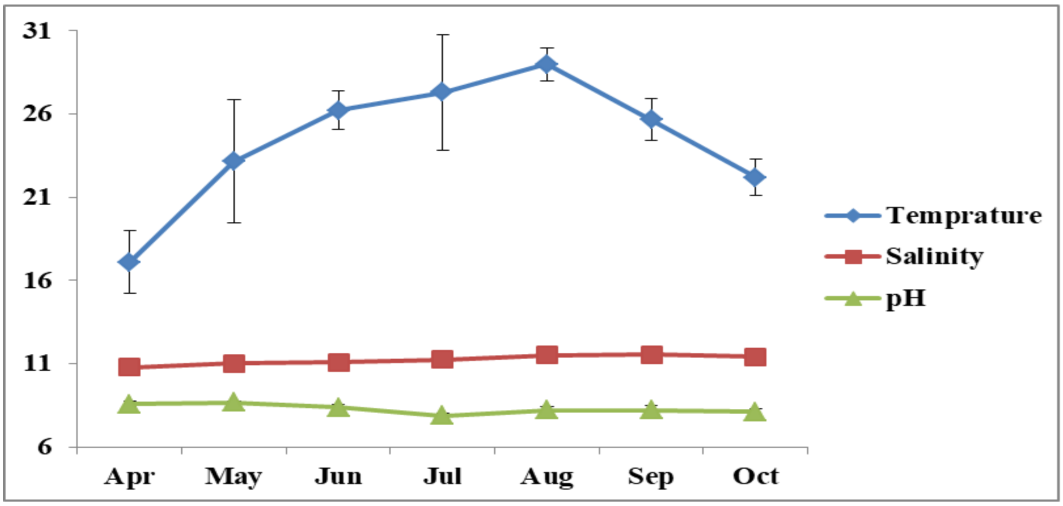



**Figure 1: Water parameters at sampling site (Noor beach, southern Capian Sea, 2016); temperature (ºC), salinity (ppt) and pH. Average and standard deviation of four weekly measurements for each month are presented.**

*Sex ratio and size distribution*

Amongst the 222 collected specimens, 115 were females and 89 males. The overall sex ratio (F:M = 1.3) did not significantly deviate from the hypothetical distribution of 1:1 ($X^2$=3.314, df=1, *p*=0.69). Length-frequency analysis by sex showed a higher proportion of males in the bigger size classes. Male and female specimens ranged from 61.3 to 175.0 mm TL (mean: 95.2±20.9 mm) and from 62.3 to 120.56 mm TL (mean: 94.4±12.9 mm), respectively (Fig. 2). Both sexes exhibited unimodal distributions, with the greatest numbers at class 90-100 mm (36%) for female and 100-110 mm (27%) for males (Fig. 2).

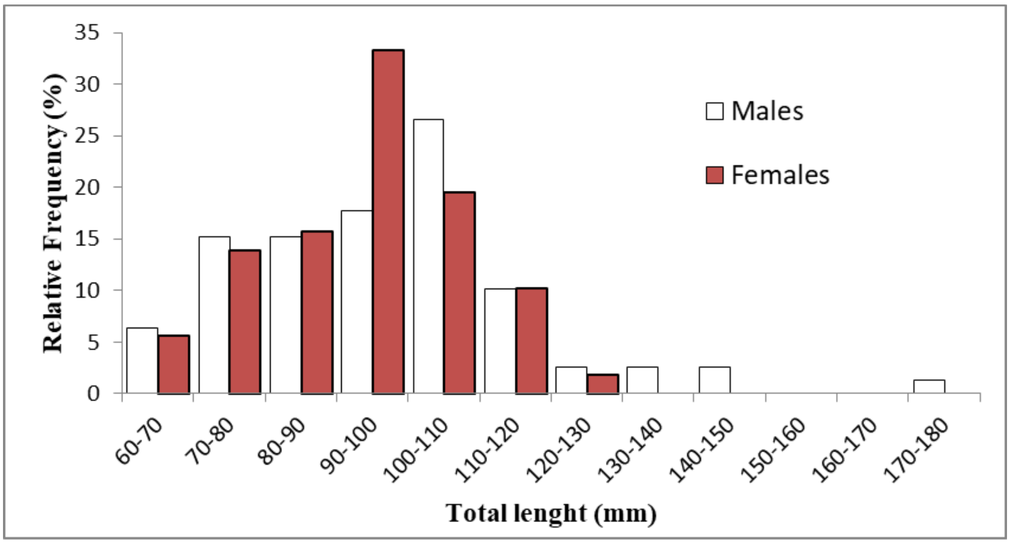
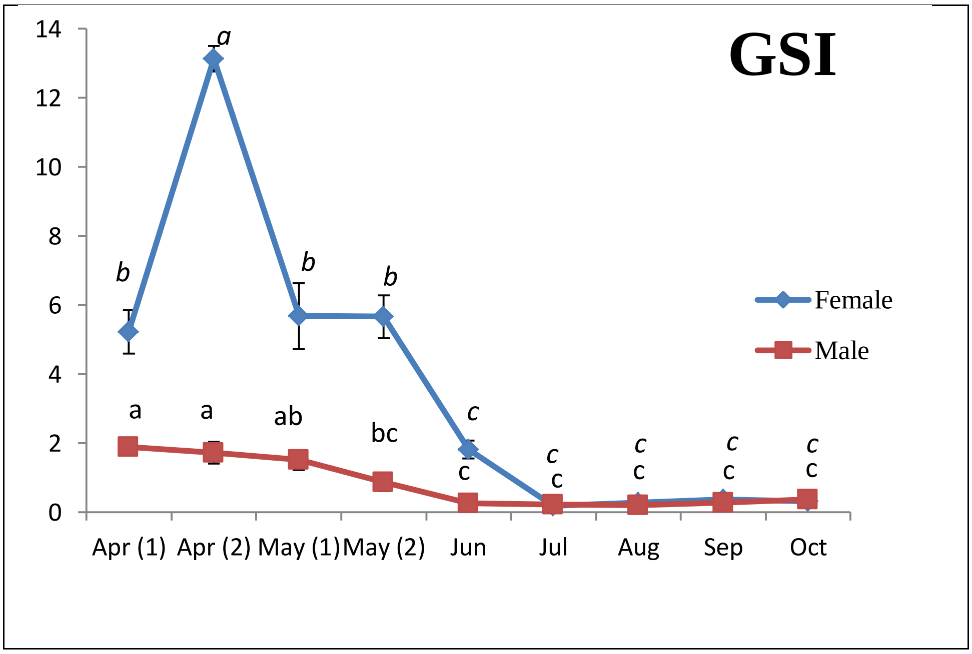



**Figure 2: Female and male length frequency distribution of Caspian goby, *Neogobious caspius* in the southern Caspian Sea (n = 222).**

In parallel, the body weight of females ranged between 2.11 and 17.61 g (mean: 7.69±3.17 g), whereas it was 2.05-62.59 g (mean: 9.40±9.43 g) for males.

*Gonadosomatic and hepatosomatic indexes (GSI and HSI)*

Monthly variations of GSI revealed that females acquired higher values of GSI than the males and for both sexes, unimodal distributions appeared with a peak in April (Fig. 3). For females, this peak postponed to second half of April. Monthly variation in HSI revealed minimum values in April and May for females and males, respectively (Fig. 3).



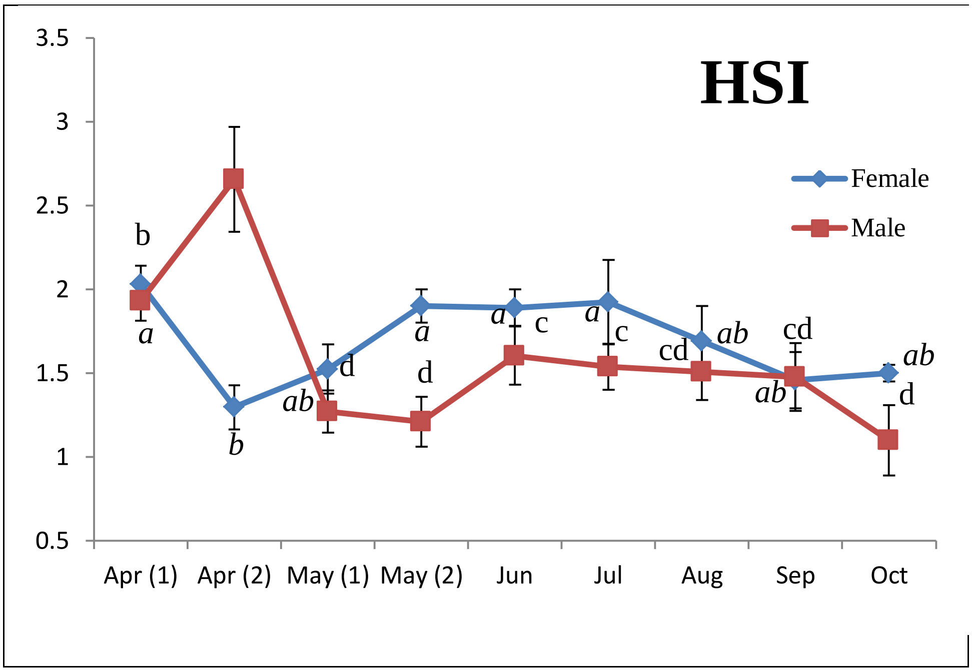



**Figure 3: Monthly variation of gonadosomatic and hepatosomatic indices for female and male specimens of the Caspian goby (*Neogobious caspius*), southern Caspian Sea, 2016**

*Condition factor*

Overall, $K_F$ coefficient peaks appeared in the first half of May and first half of April for males and females, respectively. Female's $K_F$ coefficient stayed relatively high and stable until the first half of May, then a trend was observed in the second half of May that remained constant until the end of October. Similarly, peak value of $K_c$ was seen in the first half of May for males and for females and it was relatively high and stable from April to the first half of May following a decline in the second half of May and a constant rate until October. Although $K_c$ and $K_f$ in males were higher in comparison with females, there was no significant differences between them except in May (the second half), which $K_c$ appeared significantly ($p$<0.05) higher in males (Figs. 4 and 5).

*Fecundity*

The absolute fecundity of *N. caspius* ranged from 400 to 1800 oocytes per fish (total lengths of fish specimens were 83.3 and 120.6 mm, respectively) based on assessment of 41 females, with a mean value of 1000±360. The range of relative fecundity was 50 to 190 oocytes per gram of fish weight (100±30). Results indicate that absolute fecundity is moderately related to fish length (r = 0.66, $p$<0.01), fish weight (r = 0.66, $p$<0.01) and ovary weight (r=0.77, $p$<0.01).

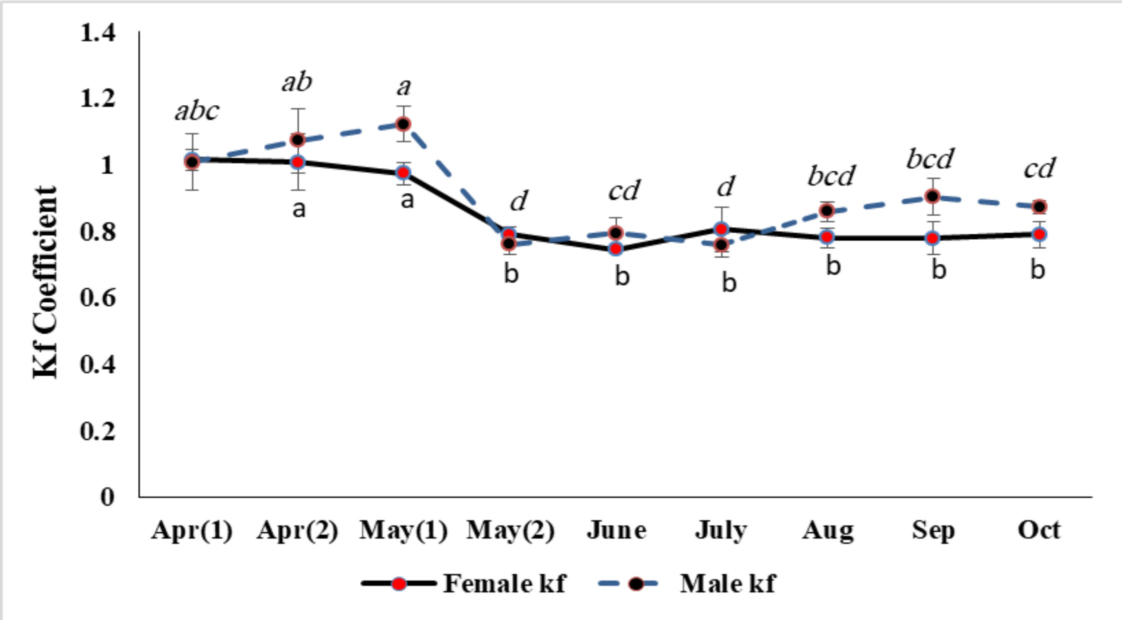



**Figure 4: Variation in the Fulton's condition coefficient (Kf) for female and male specimens of the Caspian goby (*Neogobious caspius*) in the southern Caspian Sea, from April to October 2016.**

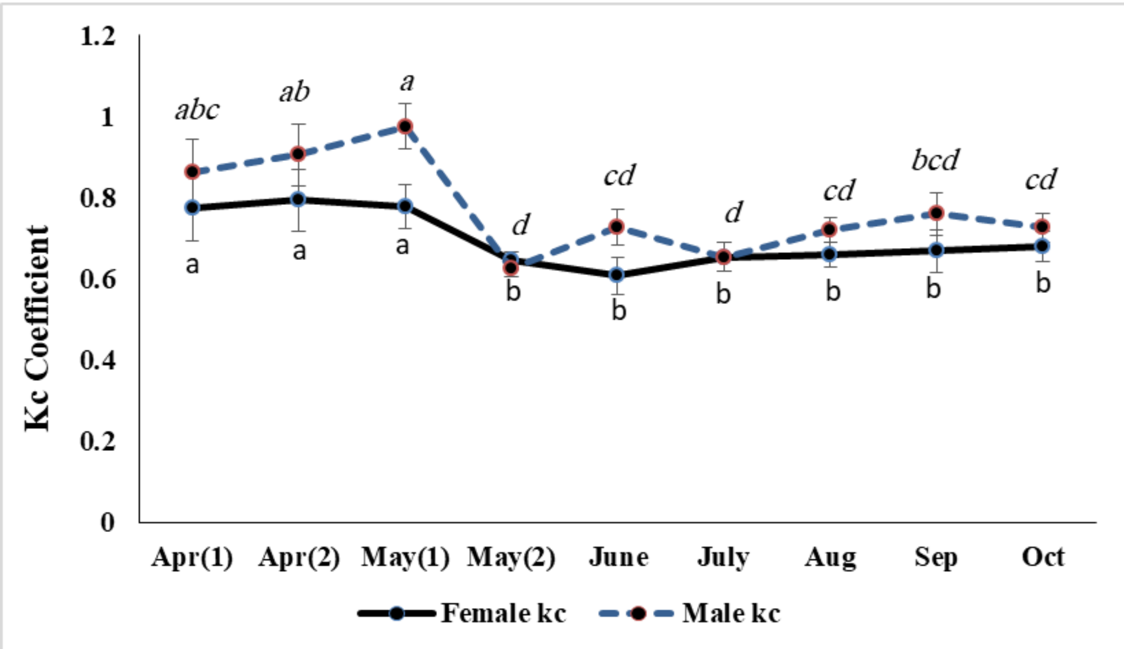



**Figure 5: Variation in the Clark's condition coefficient (Kc) for female and male specimens of the Caspian goby (*Neogobious caspius*) in the southern Caspian Sea, from April to October 2016.**

*Gonadal development*

Five and four stages of maturity were described based on macroscopic features and histological description for collected female and male specimens, respectively (Tables 1 and 2; Figs. 6 and 7). Specimens without sexual organs were assumed as immature and histological inspections were not carried out.

**Table 1: Macroscopic and histological description of the maturity stages of ovaries in Caspian goby, *Neogobious caspius* (Brown-Peterson *et al*., 2011 was followed to classify stages).**

| Stage | Macroscopic and histological features |
|---|---|
| **Cortical alveolar** | Small yellowish ovaries occupy about 50% of abdominal cavity. Eggs are almost visible to the naked eyes. Only primary growth (PG) and cortical alveolar Oocytes (CA) are present. |
| **Developing** | Enlarge yellowish ovaries occupy about 70% of abdominal cavity. Eggs are visible to the naked eyes. PG, CA and vitellogenic oocytes (early vitellogenic oocytes (Vtg1) and secondary vitellogenic oocytes (Vtg2)) are present. There is no evidence of tertiary vitellogenic oocytes (Vtg 3). Small yolk granules slightly filling the cytoplasm of vitellogenic oocytes. |
| **Spawning Capable** | Large orange-yellow colored ovaries, individual eggs were macroscopically distinct. Vtg3 oocytes, germinal vesicle migration (GVM) and germinal vesicle breakdown (GVBD) are appeared. Yolk granules joined together and formed yolk globules throughout oocytes. |
| **Mature** | Very large orange ovaries, eggs were clearly distinct. Yolk globules fused in cytoplasm of mature oocytes. |
| **Spent** | Small flaccid ovaries with no granulation, sporadic eggs were present. Much space within oocytes appears. Oogonia (O), primary growth (PG) and postovulatory follicle oocytes (POFs) are present. |

**Table 2: Macroscopic and histological description of the testis maturation stages in Caspian goby, *Neogobious caspius* (Brown-Peterson et al., 2011 was followed to classify stages).**

| Stage | Macroscopic and histological features |
|---|---|
| **Early developing** | Elongated and threadlike testes are in white color. Only primary and secondary spermatogonia (Sg1 & Sg2) and primary and secondary spermatocyte (Sc1 & Sc2) are present. |
| **Developing** | Slightly broader and fatter testes with cream color. Testes occupied more space of abdominal cavity comparing with early developing stage. All previous spermatogenesis cells (Sg1, Sg2, Sc1, Sc2) in addition of spermatozoa are present but spermatozoa not released into the lumen from lobular sperm duct. |
| **Spawning capable** | Very broad and large testes with pitch cream color which occupy whole abdominal cavity. Spermatozoa are present in lumen of lobule, Spermatocyte are throughout of testes. Sub phases: (a) Continuous germinal epithelium in testes (CGE) and (b) evidence of discontinuous germinal epithelium (DGE) appear throughout testes. |
| **Regressing or spent** | Testes reduce in size and discharge sexual products. There are no spermatozoa in the lumen and only few lobules contain spermatogonia. |

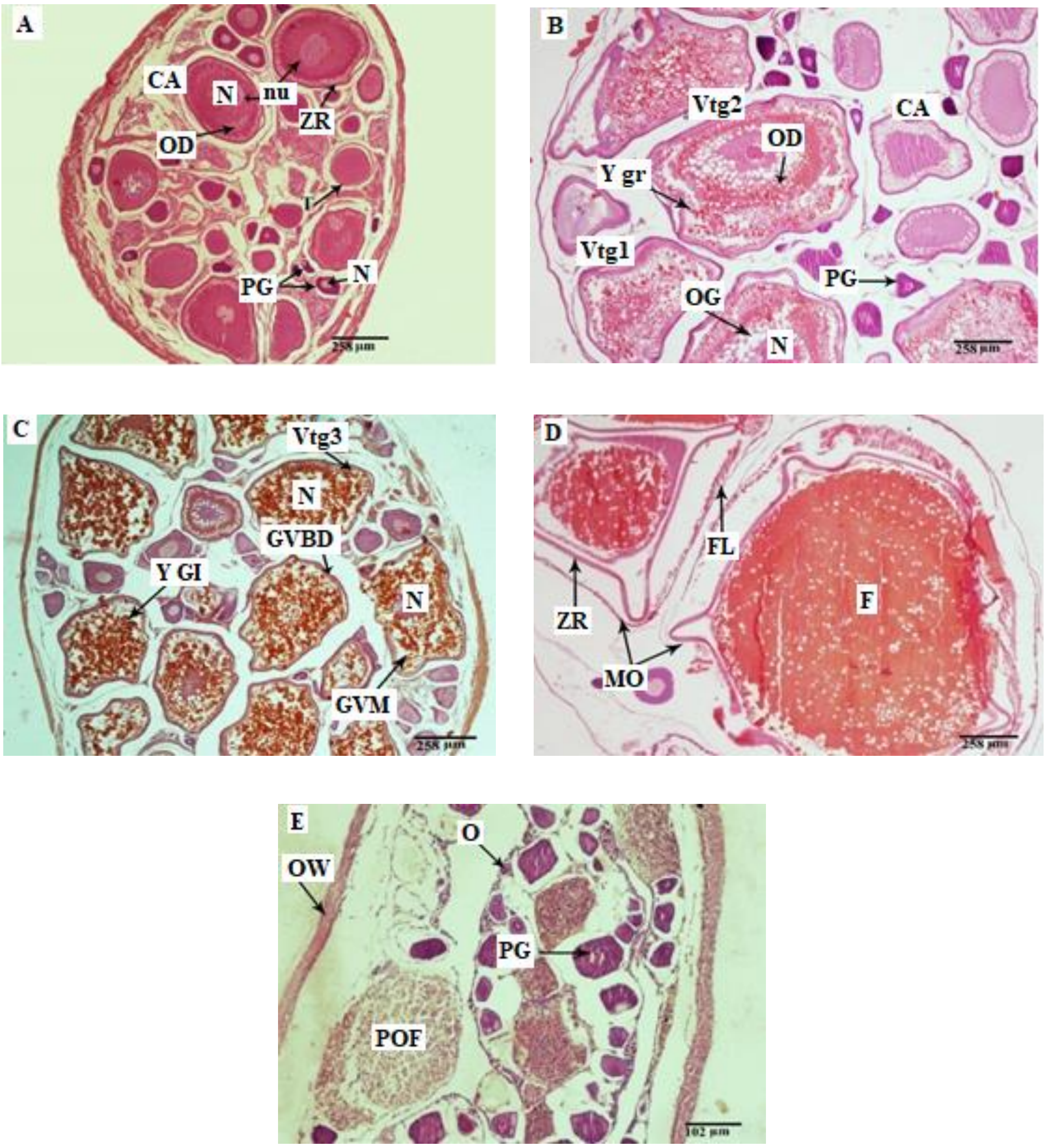



**Figure 6: Ovaries maturation stages in the Caspian goby (*Neogobius caspius*): A) Cortical alveolar stage, B) Developing stage, C) Spawning Capable stage, D) Mature stage and E) Spent stage. CA: Cortical alveolar Oocyte, F: Fused yolk globules, FL: Follicular Layer, GVBD: Germinal Vesicle Breakdown, GVM: Germinal Vesicle Migration, MO: Mature Oocytes, N: Nucleus, nu: nucleoli, O: Oogonia, OD: Oil Droplet, OG: Oil Globules, OW: Ovarian Wall, PG: Primary Growth Oocyte, POF: Postovulatory Follicle Oocyte, T: Theca layer, Vtg1: Early Vitellogenic Oocyte, Vtg2: Secondary Vitellogenic Oocyte, Vtg3: Tertiary Vitellogenic Oocytes, YGl: Yolk globules, Ygr: Yolk granules and ZR: Zona Radiate (H&E Staining).**

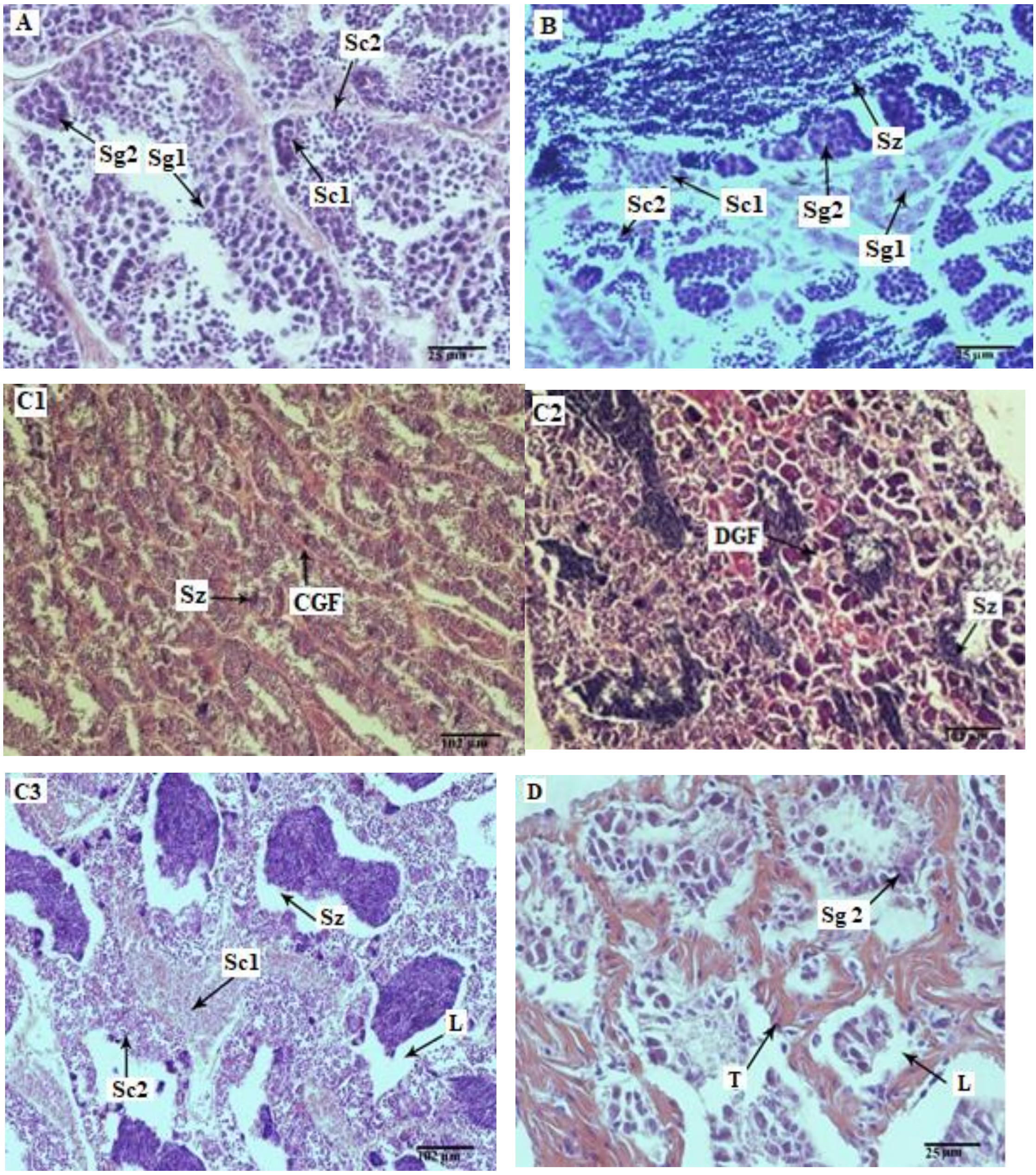



**Figure 7: Testis maturation stages in the Caspian goby (*Neogobius caspius*): A) Early developing stage, B) Developing stage, C1) First sub stage of the spawning capable, C2) Second sub stage of the spawning capable, C3) Third sub stage of spawning capable and D) Regression. CGE: Continuous Germinal Epithelium, DGE: Discontinuous Germinal Epithelium, L: Lumen of lobule, Sc1: Primary Spermatocyte, Sc2: Secondary Spermatocyte, Sg1: Primary Spermatogonia, Sg2: Secondary spermatogonia, Sz: Spermatozoa and T: Testicular tissue (H&E Staining).**

Proportions of mature ovaries were high from the first half of April to the second half of May with a peak in the second half of April (Fig. 8); the spent ovaries were observed from June to October. Mature testes were found from the first half of April to the second half of May, with a peak in the first half of May (Fig. 9).

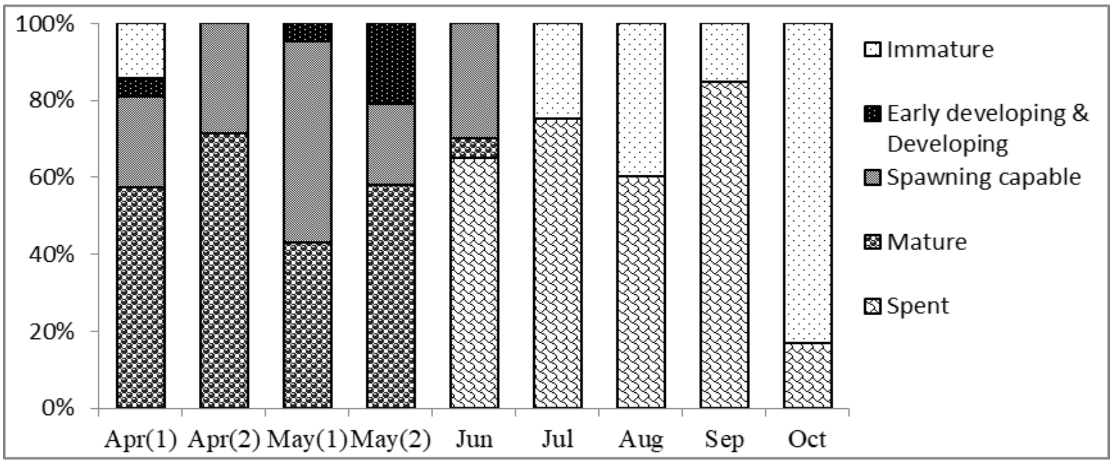



**Figure 8: Variation of the ovarian development stages for the Caspian goby (*Neogobius caspius*) in the southern Caspian Sea from April to October 2016.**

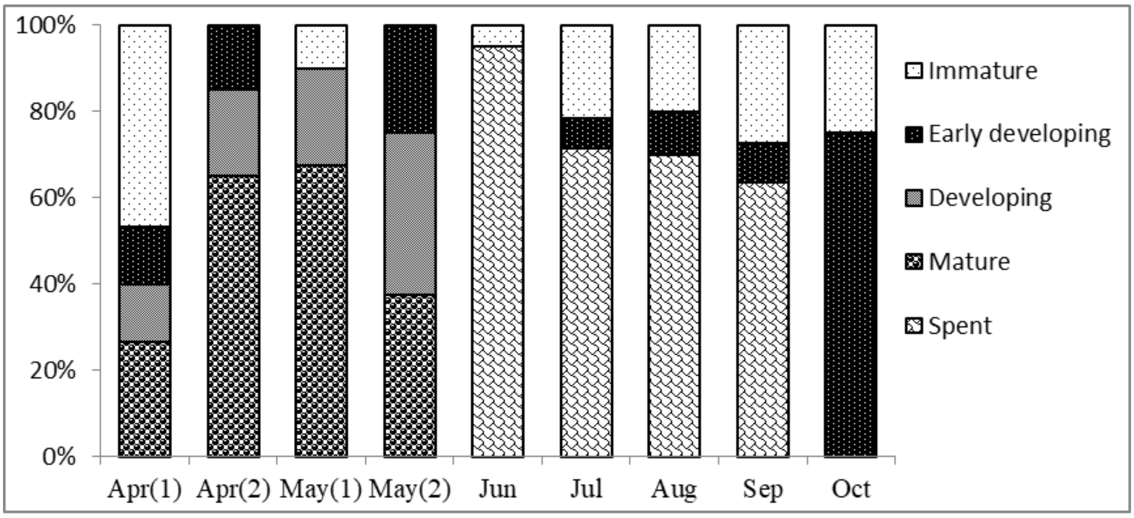



**Figure 9: Variation of the testis development stages for the Caspian goby (*Neogobius caspius*) in the southern Caspian Sea from April to October 2016.**

*Oocyte development*

The histological stages of oocyte development are described in Table 3. According to histological analyses, primary growth (oogonia, early perinucleolus and late perinucleolus in all seven months from April to October), previtellogenic (cortical alveolar in April and May), vitellogenic (Vtg1 and Vtg2 in May; Vtg3 in May and June) and mature (GVBD, GVM in April, May and June) oocytes were recognized.

**Table 3: Histological description of the oocyte developmental stages in the Caspian goby (*Neogobius caspius)* (Brown-Peterson et al., 2011 and Kagawa, 2013, was followed to classify stages).**

| Oocyte development Stage | Cell diameter (µm) Range | Average ± SD | Description |
|---|---|---|---|
| **Primary growth Oocytes:** | | | |
| Oogonia and chromatin nucleolus stage | 15.38-58.97 | 35.85±11.80 (n: 21) | Basophilic cytoplasm (purple), small irregular shape of oogonia that stained with hematoxylin. |
| Early perinucleolus stage | 50-111.22 | 80.12±17.26 (n: 23) | Small oocyte surround by single follicular layer, oolema formed and sparse nucleolus located mainly in inner surface of nucleus. |
| Late perinucleolus stage | 43.59-178.40 | 91.97±29.54 (n: 83) | Round oocyte, nucleus becomes greater and its membrane is clear. Nucleolus is located regularly at periphery of nucleus. |
| **Pre-vitellogenic oocyte:** | | | |
| **Cortical alveoli stage** | 125.64-492.97 | 293.64±94.02 (n: 50) | Oil droplets accumulate in cytoplasm of Oocyte, Yolk granules are absent, zona radiate, theca layer and nucleolus are visible but zona radiate have not developed. |
| **Vitellogenic oocyte** | | | |
| Vitellogenic stage | 275.67-940.54 | 659.68±173.14 (n: 27) | Presence of yolk granules, the size of oocyte increase, vitellogenic oocytes divide to three sub steps: Vtg1, Vtg2 and Vtg3, regarding oocytes diameter and number of yolk granules that fill cytoplasm. |
| **Mature pocyte** | | | |
| Maturation stage | 851.43-1482.16 | 1103.86±172.01 (n=11) | Germinal vesicle breakdown and germinal vesicle migration and mature oocyte are well-known in maturity stage. The nucleus migrates to animal pole in germinal vesicle Oocyte and disappears in germinal vesicle breakdown. |

## Discussion

Although males were outnumbered by females, our results revealed no significant differences in the sex ratio. This is contrary to the congener *N. melanostomus* with a male-biased sex ratio in its non-native range (M:F 2:1) and female-biased in its native range (MF 1:1.6-1.9) (summarized in Kornis *et al*., 2012). It is believed that stable native populations show an equal sex ratio as reported for another population of *N. caspius* from the southern Caspian Sea (Aghajanpour *et al*., 2016) or for the congeners *N. pallasi* (as *N. fluviatilis pallasi*) (Alavi-Yeganeh and Kalbassi, 2005; Patimar *et al*., 2008) and *N. fluviatilis* (Sasi and Berber, 2010) comparing with the male-biased in developing non-native populations (Corkum *et al*., 2004). On the other hand, Abdoli *et al*. (2002) reported a sex ratio of 1:3 (M:F) for a native riverine population of *N. fluviatilis* in the south Caspian drainage area. Nevertheless, sex ratios vary among

*Neogobius* species depending on sampling season (Konecna and Jurajda, 2012) and also because females may retreat from shallow spawning grounds after spawning but males remain protecting the nests (Kovtun, 1980).

Length frequency of specimens shows that males are larger than females which was also reported for the other species of the genus *Neogobious* such as *N. pallasi* (Alavi-Yeganeh and Kalbassi, 2005; Plachá *et al*., 2010) and *N. melanstomous* (Macun, 2018). Also, in mean size, males were larger than females, which resulted in a higher mean weight. Both sexes differed in the length distribution with the largest number of females in size class 90-100 mm and males in size class 100-110 mm. The maximum observed total length and weight of *N. caspius* were 120.6 mm/17.61 g for females and 175.0 mm/62.59 g for males, respectively. Although Berg (1965) reported a maximum length of 345 mm for a male from the northern Caspian Sea, while studies from the southern Caspian Sea reported much lower values, e.g. 157 mm TL/male (Aghajanpour *et al*., 2016) or 160 mm TL and 50.5 g weight for males and 15.0 cm TL and 45.5 g weight for females (SarpanahSarkohi *et al*., 2008). It is not known if these differences in the maximum size are based on environmental constraints differing between the northern and the southern Caspian Sea.

Both GSI and HSI indices as well as the percentage of mature gonad stages indicate that spawning season starts in spring and lasts till June. This was supported by data on monthly variation of GSI and HSI which indicated that Caspian goby migrate to shallow coastal areas in April when they are approximately ready for reproduction. GSI reached high values during April and May, matching the high proportions of mature ovaries and testis. Our results showed that the GSI values for males were lower than those of females, which is a common feature of most gobies (Miller, 1984).

Accumulation of energy in the liver during the feeding seasons and retrieving of this energy during periods of high demand and restricted feeding has been documented for several teleost fishes (Biswas, 1993). This storage is seemingly linked with the reproductive cycle (De Vlaming, 1971; Miller, 1979). Nevertheless, data on the relationship between reproduction and energy storage in the liver are rare for gobiid fishes (Miller, 1984; Fouda *et al*., 1993; Kovacic, 2007).

Data revealed a distinct difference in the HSI between males and females. While the decrease in HSI was relatively short in females, during the second half of April (it gradually increased again from May to October), the decrease of HSI started in males during the second half of April and continued till the end of May. This may be related to longer period of reproduction in males which guard the nests until hatching.

Raising temperature and salinity from April to June is synchronous with increase of GSI in both sexes of Caspian goby. The spawning season of many fish species is determined by water temperature (Munro *et al*., 1990; Lappalainen *et al*., 2003; Reichard *et al*., 2004), and there are many evidences regarding the optimum temperature range for yolk-conversion efficiency and development in teleost (Herzig and Winkler, 1986; Heming and Buddington, 1988; Tyler and Sumpter, 1996; Hardy and Litvak, 2004; Brown *et al*., 2011). Generally, with increasing temperature, rising in salinity could cause a decrease of the gonad weight but salinity changes alone cannot be considered as a prominent factor during reproduction (De Vlaming, 1971). From April to July pH is distinctly decreasing which could be related to decrease of local rivers influent at that time. But influence of pH on the reproduction of gobies in the Caspian Sea is not reported so far.

Fish condition, represent nutritional status and could be related to general health, prey or food availability, reproductive potential, environmental conditions like temperature and water level fluctuation (Gebremedhin and Mingist, 2014). As many previous reports indicated, males had a higher condition value than females which indicate a higher energy investment in females during the breeding season. On the other hands, males will have better energy (lipid content) for nesting and defending territories (Skolbekken and Utne-palm, 2001). A similar trend was observed for two condition indices ($K_f$ and $K_c$) indicating equal energy investment in body and viscera mass.

Estimation of the absolute fecundity will be helpful in assessment of potential productivity and reproductive capacity of a species which is more or less related to fish size, fish weight and ovary weight (Murua *et al*., 2003). It is clear that the gonad mass gives a better correlation with reproductive capacity than the body mass. The only previous report for fecundity of *N. caspius* is 212-1234 eggs as absolute fecundity (SarpanahSarkohi *et al*., 2008) which is relatively concordant with the result of the present study (400-1800; mean: 1000). In comparison with the other *Neogobius* species, lower range reported for *N. fluviatilis* (276-533) in Madarsoo stream from the Caspian Sea watershed basin (Abdoli *et al*., 2002) and wider range and higher number for *N. kessleri* (669-5644; mean: 2109) in middle of Danube River (Kovac *et al*., 2009) and *N. melanostomus* (1420-2009) in lake Karabogaz, Turkey (Macun, 2018). High fecundity and batch spawning strategy over a long period could increase survival of offspring in the Caspian goby (Murua *et al*., 2003).

Nearly all temperate and tropical gobies are iteroparous or potentially iteroparous (Miller, 1984). Batch spawning strategy is reported in the other *Neogobius* species like round goby *N. melanostomus* from May to

June in Detroit River of Canada (Macinnis and Corkum, 2000) and from its native distribution range in the Caspian Sea (Kulikova, 1985), Monkey goby *Neogobious fluviatilis* from May to August in Danube River in Bulgaria (Konecna and Jurajda, 2012) and Caspian sand goby *Neogobius pallasi* from April to June in the southern Caspian Sea (Abdoli *et al*., 2002; (as *N. fluviatilis*); Alavi-Yeganeh and Kalbassi, 2005) which all laying their eggs in the nest under the rocks.

Finding of the present study demonstrates that the Caspian goby is iteroparous and a batch spawner species, since the mature ovaries and testes contained different types of oocytes and spermatocytes, respectively.

There is limited study for *Neogobius* species in Ponto-Caspian area and these basic data could be used for potential reproduction assessment and conservation management of Caspian goby in the study region.

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