

# Reproductive biology of Caspian vimba, *Vimba vimba* (L.), in the coastal waters of the southwestern Caspian Sea

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**Abstract.** The reproduction biology of Caspian vimba, *Vimba vimba* (L.), was investigated on the southwestern coast of the Caspian Sea using 712 fish specimens collected at three fisheries catch stations located in Talesh, Anzali, and Kiashahr over a two-year period from April 2012 through June 2013. Seasonal changes in the gonadosomatic index, gonad histological status, size frequency distribution of oocytes, and the fecundity of the species were studied. The peaks of the gonadosomatic index (GSI) were observed both in females and males in June 2012 and May 2013 indicating the variable peak spawning time of this species. Histological examinations and oocyte frequency distribution patterns showed synchronous oocyte development in the Caspian vimba. Absolute fecundity ranged from 5873 to 35421 eggs. The size at first maturity for females was 120 mm FL. We concluded that the Caspian vimba is a total spawner with variable spawning dependent on environmental factors.

**Keywords:** Caspian Sea, *Vimba vimba*, gonadosomatic index, fecundity, oocyte development

## Introduction

Reproductive strategy is a fundamental topic in the study of population dynamics and the biology of fish species (Hunter et al. 1992, Murua and Sabrido-Ray 2003). Successful fisheries management must consider the reproductive ability of fish populations and assess accurately biological parameters including reproductive characteristics, such as spawning season duration, fish age and size at maturity, and fecundity along with growth and mortality estimates (Quinn and Deriso 1999). Moreover, determining spawning patterns is necessary to acquire an understanding of the population dynamics of different fish species (Hilborn and Walters 1992, Rinchard and Kestemont 2003). Reproduction studies provide basic quantitative data on reproductive capacity that are used to assess the effects of exploiting fish populations (Murua 2010). Finally, knowledge about fish reproductive processes at cellular levels is critical for the proper management of fish species rehabilitation (Jakobsen et al. 2009). Fish reproduction is a periodic process (Munro 1990) that is reflected in gonad weight, fecundity, and oocyte development (Narimatsu et al. 2005). Abiotic and biotic factors can cause variations in reproductive traits such as reproductive strategy, fecundity, and gonad weight (Fowler et al. 2000), and these factors are capable of

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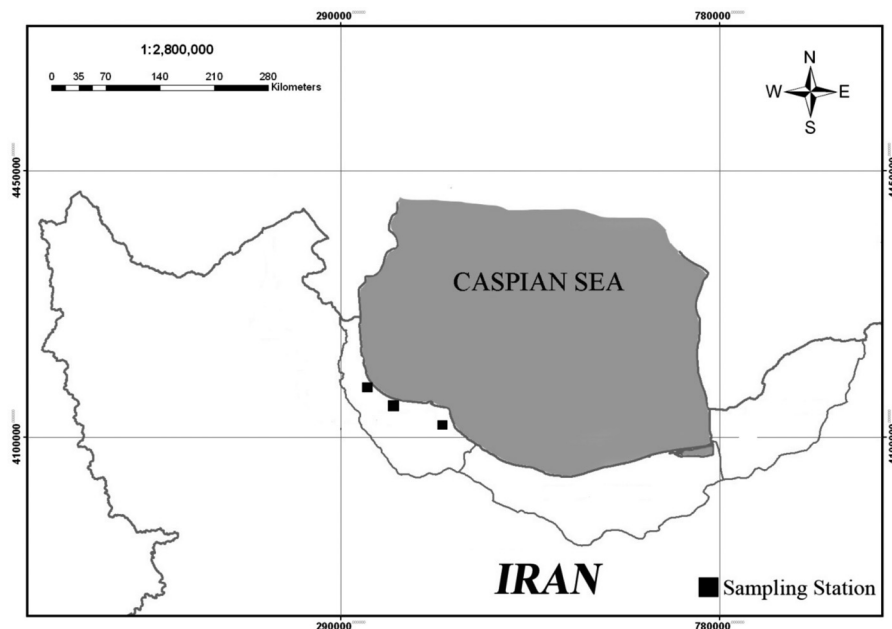


Figure 1. Map of the southern coast of the Caspian Sea. Rectangles represent sampling sites of *V. vimba*.

influencing reproductive ability (Marshall et al. 1998).

Several commercially important fishes inhabit the Caspian Sea (Chaichi et al. 2011) including the cyprinid species *Vimba vimba* (L.). *Vimba* is a migratory fish that mainly lives in fresh as well as brackish waters. It migrates into fresh water in spring to reproduce, after which the spawners return to brackish waters to feed and prepare for the subsequent reproduction cycle (Hänfling et al. 2009). Under natural conditions, *vimba* attains maturity at the age of 4-5 years (Łuszczek-Trojnar et al. 2008). Spawning occurs during May and July on gravel beds in fast flowing streams and rivers (Bontemps 1971).

Opinions about the reproductive strategy of *vimba* are varied. Kazanchev (1981) states that *vimba* is a total spawner, whereas Łuszczek-Trojnar et al. (2008) report that a total of 30000-120000 eggs can be deposited in two or three batches by *vimba* in Poland. A review of the literature on the reproduction biology of *vimba* reveals that several points require further study to gain a comprehensive understanding of the reproduction strategies of *vimba* on the southwestern coast of the Caspian Sea. To date, no histological studies have been performed on *vimba*

reproductive parameters in this region of the Caspian Sea. Therefore, the decision was taken to tackle the question of whether *vimba* from the southwestern regions of the Caspian Sea is a batch or total spawner. For this purpose, size at maturity, gonad histology, trends in gonadosomatic index, fecundity, and oocyte diameter frequencies were examined to shed light on the reproductive biology of the species. This information is vital since *vimba* constitutes an important segment of commercial catches of teleost fishes in the southern basins of the Caspian Sea. Finally, the results obtained are crucial for developing preliminary stock rehabilitation and management measures.

## Materials and Methods

### Sampling

The study was conducted in the southern basin of the Caspian Sea. Locations of the sampling sites are presented in Fig. 1. A total of 712 specimens (343 female, 369 male) was collected randomly from April 2012 through June 2013 – a period that covered two consecutive *vimba* spawning seasons. Sampling was performed monthly.

Gill nets with a mesh size range of 9–28 mm were used for sampling by commercial fishermen in three fishing zones including Kiashhr (37°25'10"N, 49°56'56"E), Bandar Anzali (37°28'0.12"N, 49°28'0.12"E), and Talesh (37° 46' 54.63" N, 48° 56'36.64" E) along the southern coast of the Caspian Sea.

### General biological data and determining spawning time

The fork lengths (FL, mm), total weights (TW, g) ( $\pm 1$  g), and gonad weights (GW) ( $\pm 0.001$  g) of all specimens were measured. Sex was determined by examining the gonads in the laboratory, and the macroscopic maturity stages were recorded based on the criteria outlined in Table 1.

The following formula was used to estimate the gonadosomatic index (GSI) of each fish with intact gonads (Brown-Peterson et al. 2001)

$$GSI = \frac{GW}{TW - GW} \times 100$$

where GW is gonad weight, and TW is total weight.

### Histological analysis and size-frequency distribution

Histological analysis was conducted to determine maturity stages at the microscopic level to assist in

determining the reproductive strategy of *V. vimba*. The ovaries were removed from a sub-sample of 150 females collected during the reproductive period. The gonads were weighed ( $\pm 0.001$  g), and the right lobes of the ovaries were preserved in 10% buffered formaldehyde. Tissue samples were taken transversely to avoid possible discrepancies in the developmental stage of oocytes because of their position in the ovary. Histological slides were prepared by dehydrating samples in 70% ethanol, and then, according to routine procedures, the samples were embedded in paraffin and 5  $\mu$ m sections were cut and then stained with hematoxylin and eosin. The histological classification of oocytes (Table 1) was done according to Brown-Peterson et al. (2011). Intact ovaries from ten females at each phase of maturity were used to determine the oocyte size-frequency distribution. Samples were placed in Petri dishes and separated using a needle, and the diameters of 100 to 300 oocytes were measured under a stereo-microscope (Olympus SZX12, USA) using transmitted light and bright-field illumination to determine each maturity stage. Oocyte developmental stages were classified as chromatin nucleolar, perinucleolar, cortical alveoli, vitellogenic yolk, and nucleolar migration (Davis and West 1993). Only those oocytes that were sectioned through the nucleus were included in size frequency analyses. Fecundity was estimated using 24 female fish sampled from March to June 2013.

**Table 1**

Macroscopic and histological staging criteria for the ovary of vimba, modified criteria by Brown-Peterson et al. (2011)

Phase	Macroscopic appearance	Histological features
I. Immature	Small ovary and blood vessels indistinct	Only oogonia and primary growth (PG) oocytes present, no atresia present
II. Developing: includes early developing sub-phase	Enlarging ovary	Early vitellogenesis: Vtg1 and Vtg2, PG, cortical alveoli (CA) present. In early developing sub-phase PG oocytes and CA only present
III. Spawning capable: includes actively spawning sub-phase	Large ovary, blood vessels prominent	Late vitellogenesis: Vtg3 oocytes present. In actively spawning sub-phase late oocyte with nuclear migration, hydration or ovulation present
IV. Regressing	Flaccid ovary	Post ovulatory follicles (POFs) and atresia present. Some CA or Vtg1 and Vtg2 present
V. Regenerating	Small ovary	Oogonia and PG present. Old atresia and POFs may be present

## Fecundity

To investigate the effect of ovary region on oocyte number, 0.15 g oocyte samples were taken from the anterior, middle, and posterior parts of both left and right ovaries of ten females (Dominguez-Petit 2006). Fish with intact ovaries in phase III, which is defined as spawning capable according to Brown-Peterson et al. (2011), and without signs of postovulatory follicles (POF) were used for fecundity estimations (Ganias et al 2004). No significant differences were noted in the numbers of oocytes among the three sections of each ovary ( $F = 0.057$ ,  $df = 2$ ,  $P > 0.05$ ) nor between the left and right ovaries ( $F = 0.004$ ,  $df = 1$ ,  $P > 0.05$ ). Therefore, 0.15 g oocyte samples collected from the middle part of the right ovary were used to estimate fecundity. Absolute fecundity was determined by multiplying the oocyte number per 0.15 g of ovary by the gonad weight. A power regression was fitted to determine the relationship between fish fork length and absolute fecundity. Relative fecundity was estimated using the following formula:  $RF = AF \times BW^{-1}$ , where  $AF$  is absolute fecundity, and  $BW$  is body weight (g).

## Size at maturity

A sample of 137 females 8-20 cm FL were used to estimate size at maturity in the 2013 spawning season (March to June 2013). Fish were considered mature when oocytes were in late vitellogenesis or in phases III-V based on macroscopic criteria (Table 1). Fish length was classified into 10 mm categories, and the logistic model fitted to the data was as follows:

$$P = 1 / (1 + e^{-r(L-L_m)})$$

where  $r$  is the slope of the curve, and  $L_m$  is the length at sexual maturity or the length corresponds of 0.5 (or 50%) in reproductive condition.

## Statistical analysis

Data are presented as means  $\pm$  standard error (SE). Two-way analysis of variance (ANOVA) was used to

understand the interaction of gender and month as independent factors on GSI, and the Duncan test was used to establish monthly differences in genders. One way ANOVA was used to compare the number of oocytes in the three sections of each ovary, and in the right and left ovaries. Linear regression was applied to observe the relationship between FL and fecundity. Statistical analyses were performed using SPSS (Version 16, Inc., Chicago, IL, USA). The level of significance for data analyses was 0.05.

## Results

### Biological Data and Spawning Time

The fork lengths of the females and males used in the study ranged from 8 to 21.4 cm and from 8 to 20.3 cm, respectively. Significant differences were observed in the length frequency distribution between females and males ( $\chi^2 = 58.25$ ,  $df = 12$ ,  $P < 0.05$ ). The chi square  $\chi^2$ -test indicated that the sex ratio was not significantly different from the theoretical 1:1 sex ratio ( $\chi^2 = 0.94$ ,  $df = 1$ ,  $P > 0.05$ ). Females constituted 48% ( $n = 343$ ) and males 52% of the samples. As shown in Figure 2, the frequency of males was notably higher than females in 2012 and 2013. The combination of gender and month affected changes in GSI significantly ( $F_{\text{Sex*Month}} = 13.43$ ,  $df = 13$ ,  $P < 0.05$ ). In both sexes, the GSI index increased gradually from April 2012 and peaked in June 2012, when the mean value was 13.59 for females and 5.05 for males; whereas in 2013, the pattern started in March and reproductive activity peaked in May when the mean GSI was 6.03 for males and 10.19 for females (Fig. 3).

### Histological analysis and size-frequency distribution

The oocyte development pattern of vimba from the coastal waters of the southwestern Caspian Sea was synchronous. Predominantly, oocyte development was synchronous at all phases of gonad development

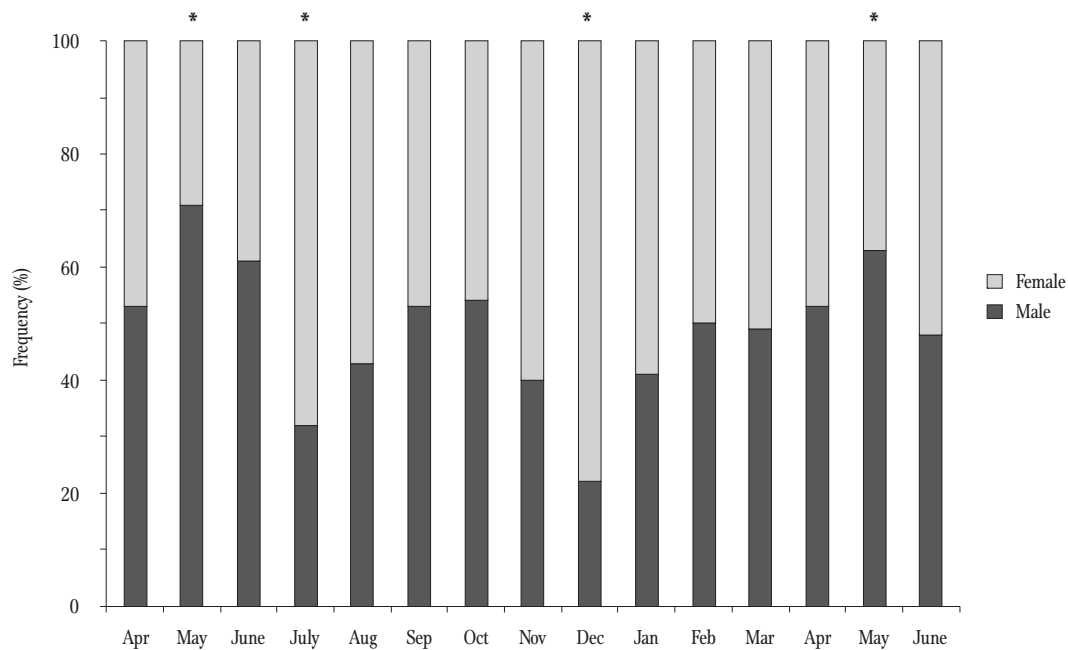


Figure 2. Monthly percent frequency of both sexes of *V. vimba* from April 2012 to June 2013 in the coastal waters of the southwestern Caspian Sea. \* indicates significant difference from the theoretical 1:1 sex ratio.

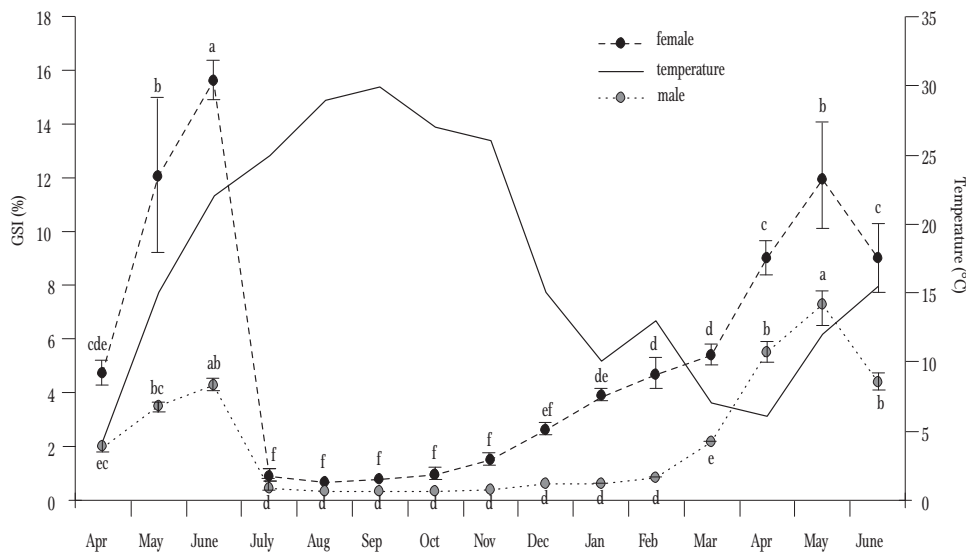


Figure 3. Monthly changes in the gonadosomatic index of *V. vimba* and the average water surface temperatures in the coastal waters of the southwestern Caspian Sea.

(Fig. 4). There was no evidence of post-ovulatory follicles (POFs) in ovaries in the active spawning sub-phase (Fig. 5), indicating that vimba is a total spawner in this region. The examination of oocyte diameter distributions indicated egg diameter below 240  $\mu\text{m}$  (Fig. 4) in the early developmental sub-phase, which was dominated by chromatin

nucleolar, perinucleolar, and cortical alveoli (Fig. 5). At the beginning of vitellogenesis, oocyte diameter increased (260–440  $\mu\text{m}$ ) (Fig. 4) because of the occurrence of early vitellogenesis (Vtg1 and Vtg2) (Fig. 5). The phase of late vitellogenesis (Vtg3) and large nucleus-migrated oocytes resulted in a significant increase in egg diameter (500–980  $\mu\text{m}$ ) (Fig. 4).

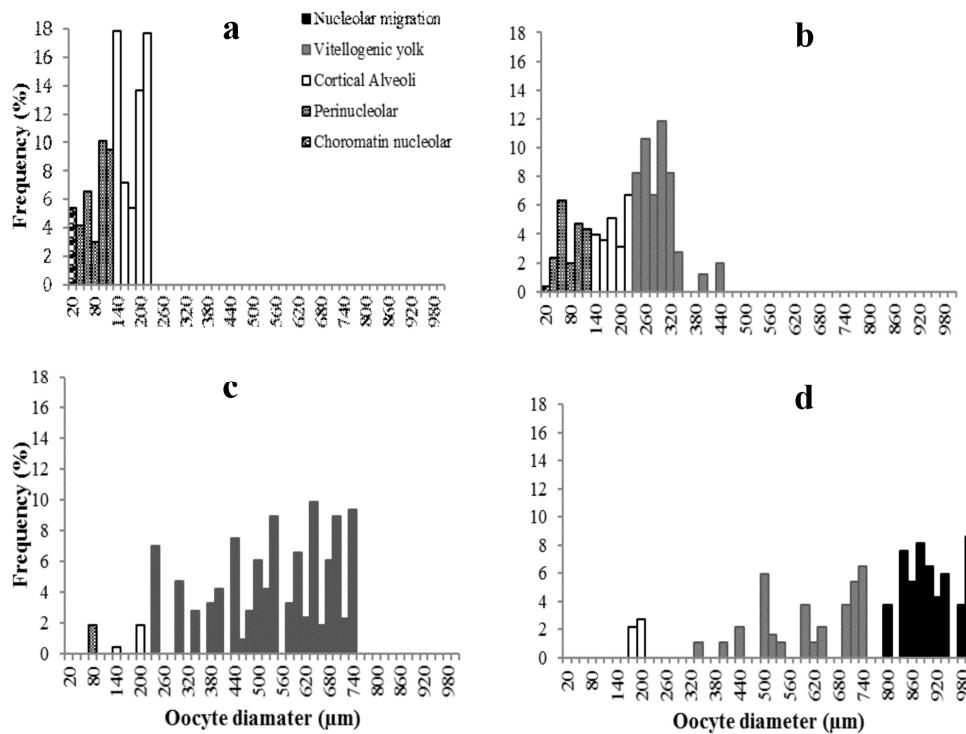


Figure 4. Size frequency distribution of oocyte diameter of *V. vimba*. A) phase II, early developing sub-phase, B) phase II, C) phase III, D) phase III, actively spawning sub-phase. n= 10 for each phase.

## Fecundity

No significant difference was noted between the weight of the right and left ovaries in the sub-sample of 20 females that were tested using the t-test presuming equal variance ( $t = -0.112$ ,  $df = 38$ ,  $P > 0.05$ ). Mean relative fecundity was  $934 \pm 68$  (SE), and ranged from 410 to 1771. Absolute fecundity (the number of mature oocytes per gram of fish body weight) was  $15822 \pm 157$  ranging from 5873 (for an individual with a fork length of 143 mm and a weight of 33.5 g) to 35421 (for an individual with a fork

length of 200 mm and a weight of 118.8 g). The relation between FL and fecundity was positive and significant ( $r^2 = 0.79$ ,  $df = 23$ ,  $P < 0.05$ ) (Fig. 6) and was defined by the following equation:  $Fec = 3180.2 \times FL - 36028$ .

## Size at maturity

The mean size at 50% maturity ( $L_{50}$ ) was 120 mm (Fig. 7). All females were mature at 180 mm FL, and the fork length of the smallest mature female was 114 mm (Table 2).

**Table 2**

Number of mature fish in each length class within the sampled population of *V. vimba* in the coastal waters of the southwestern Caspian Sea

	Length class (cm)												
	8-9	9-10	10-11	11-12	12-13	13-14	14-15	15-16	16-17	17-18	18-19	19-20	20-21
Number of mature fish	0	0	0	1	4	17	22	17	12	18	14	9	2



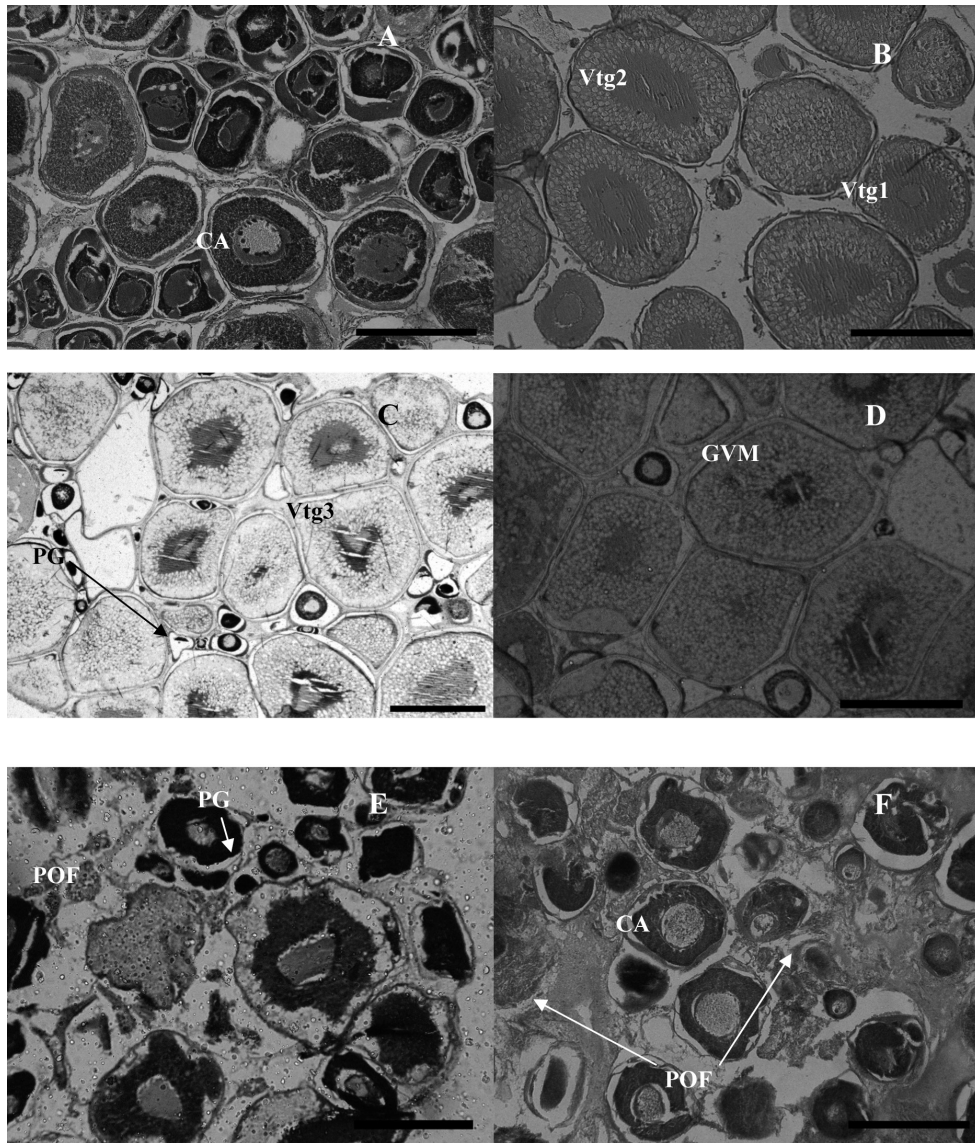


Figure 5. Histological sections of ovaries of *V. vimba*. A) early developing sub-phase, B) developing, C) spawning capable, D) late spawning capable sub-phase, E) regressing, F) regenerating. PG = Primary Growth, CA = Cortical Alveol, Vtg1 = Primar Vitellogenic Oocyte, Vtg2 = Secondary Vitellogenic Oocyte, Vtg3 = Tertiary Vitellogenic Oocyte, GVM = Germinal Vesicle Migration, POF = Postovulatory Follicle Complex. Scale bars 500  $\mu$ m.

## Discussion

A reliable method for estimating the duration of the reproductive season and the peak of spawning activity in many fish species is to combine the gonadosomatic index (GSI) with the proportion of various maturity phases (Fowler et al. 2000, Brown-Peterson et al. 2001, Lowerre-Barbieri et al. 2011). In this study, vimba exhibited inter-annual variation in the beginning and peak of spawning.

Previous studies on reproductive strategies of vimba also show a similar pattern. Schishabekov (1979) reported the onset of vimba spawning varies from one year to another under the influence of water temperature. Hosseini et al. (2010) reported the occurrence of adult vimba in Sefidroud River early in February indicating the early initiation of spawning. Different ecological and climatic conditions can affect the onset of vitellogenesis and the duration of spawning (Okgerman et al. 2011). The most significant

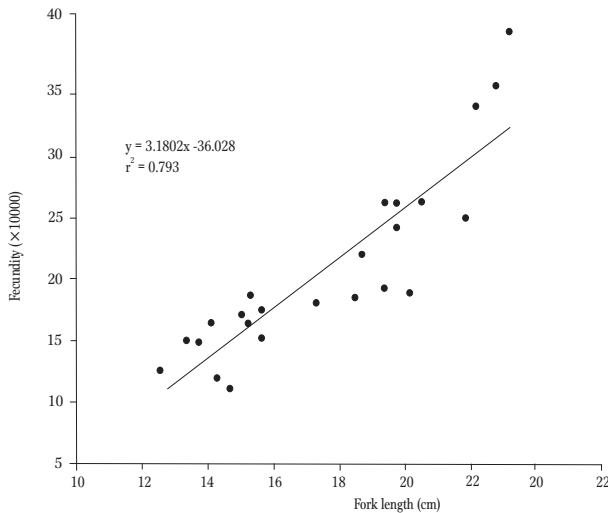


Figure 6. Relation between absolute fecundity and fork length (mm) of *V. vimba* (n= 24) in the coastal waters of the southwestern Caspian Sea.

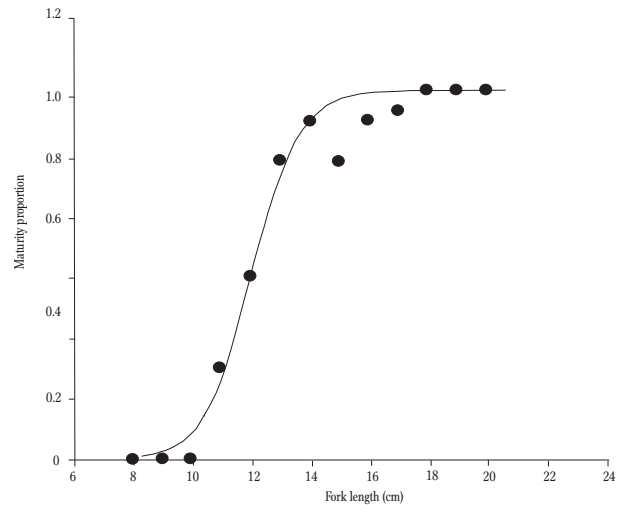


Figure 7. Proportion of mature female *V. vimba* at 10 mm length intervals. n = 137 (116 mature) in the coastal waters of the southwestern Caspian Sea.

environmental factor is water temperature, which can determine when the spawning period begins and also peak spawning (Wootton 1982, Lam 1983, Scott and Pankhurst 1992). Low water temperatures can influence vitellogenesis, and, consequently, delays in spawning can follow (Kjesbu 1994). In the present study, the mean water temperature ( $9.35 \pm 2.19^\circ\text{C}$ ) in February and March 2013 coincided with active vitellogenesis in vimba and also with the synchrony of the early onset of spawning as compared to 2012, when the mean water temperature in February and March 2012 was  $6.6 \pm 0.55^\circ\text{C}$ . Fish species use a variety of reproductive strategies to increase offspring survival (Balon 1985). The following was noted in this study and could confirm synchronous oocyte development and total spawning in vimba: (i) the absence of post-ovulatory follicles (POFs) in ovaries in different stages except for their dominant presence in ovaries in the spent regressing stage; (ii) lack of oocytes in different developmental stages at the whole ovary phases; (iii) invariability of the GSI trend during the spawning season and the occurrence of only one peak; (iv) the presence of only one clutch of growing oocytes in the size-frequency distribution. In species with synchronous oocyte development, oocytes develop and the fish ovulate

simultaneously, therefore, there is no replenishment from earlier stages (Wallace and Selman 1981, Murua and Saborido-Ray 2003). Species are known as total spawners when all clutches of yolked oocytes ovulate at one time and the eggs are shed over a short period or in a unique event (Tyler and Sumpter 1996). Obviously, vimba is a species with determinate fecundity, and during the spawning season increases in the mean diameter of advanced vitellogenic oocytes is expected. As the spawning season proceeds, the diameter of advanced yolked oocytes remains constant or decreases (Greer Walker et al. 1994). A previous study by Patimar and Safari (2010) on the biology of Caspian vimba reports a similar range and mean fecundity, with a range of absolute fecundity of 5436-36141 eggs for 3+ to 5+ fish (mean  $16672 \pm 6902.7$  eggs) for vimba caught in the Miankaleh Wildlife Refuge. In the present study, a positive relationship was observed between length and absolute fecundity. Length at 50% maturity was 120 mm smaller than the figure of 170 mm reported by Chaichi et al. (2011) from southeast Caspian Sea. A common response to environmental variations, which cause increased fish growth rates, is decreasing age and maturity size (Stearns and Crandall 1984). One of the main factors that could be



responsible for decreasing size at maturity is fishing mortality, which can lead to reduced population sizes (Tripple 1995). Therefore, lower age and size at maturity force individuals to participate in one or two spawning season before they are caught (Cardinale and Modin 1999) in order to survive their generation.

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**Author contributions.** All authors conceived the study, performed the research, wrote the paper, and read the final manuscript.

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