

Reproductive biology of the black-striped pipefish *Syngnathus abaster* Risso, 1827 (Pisces: Syngnathidae) in the Aegean Sea of Turkey

by

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Abstract

The paper presents the results of research on the reproductive biology of *Syngnathus abaster* – a species distributed in Çandarlı Bay. For this purpose, seine nets were collected from a depth of 0.5–2 m in 15 minute periods between April 2013 and March 2014, and a total of 185 individuals were sampled. It has been determined that 94 individuals (50.81%) were females, 79 were males (42.70%) and 12 were immature (6.49%). The sex ratio was 1:0.84. The average length values in female, male and immature individuals were 111.5 ± 7.35 , 109.9 ± 11.08 and 79.8 ± 5.30 mm, respectively. The maximum mean surface water temperature is in spring and summer seasons. When Gonadosomatic Index (GSI%) values were examined, the spawning period of the species was indicated as spring and summer. Three groups of species were identified as maturing (diameter: 0.61–1.20 mm), mature (diameter: 1.21–1.70 mm) and hydrated oocytes (diameter: 1.71–2.10 mm) in ovaries of female individuals. The number of eggs in the brood pouch of pregnant male fishes was on average 48 (mean \pm SD = 48 ± 14.09 eggs, range: 23–78 eggs). The hydrated oocyte/total length relationship was: $y = 0.8651x - 84.332$ ($n = 14$, $r^2 = 0.64$) and the number of eggs/total length relationship in the brood pouch of pregnant males was: $y = 1.0168x - 67.715$ ($n = 33$, $r^2 = 0.58$).

Key words: *Syngnathus abaster*, Syngnathidae, Reproduction, Fecundity, Aegean Sea

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Introduction

The *Syngnathus* genus belonging to the Syngnathidae family including pipefishes, seahorses and sea dragons is represented by 5 species in the Mediterranean and 6 species in the Black Sea (Bilecenoglu et al. 2014). The species *Syngnathus abaster* belonging to the *Syngnathus* genus occurs in the East Atlantic, from the south of Biscay Bay to Gibraltar, including the Mediterranean and the Black Sea (Dawson 1986). *S. abaster* is able to live in salt and fresh water. It is brown-green and has dark or pale spots on its tail. It occurs up to 5 m depth in sandy, muddy or *Zostera* regions. Males have a ventrally localized brood pouch (Whitehead et al. 1986), where they store fertilized eggs transferred by females. The eggs stay in the brood pouches through the incubation period (Luling 1983; Movčan 1988; Herald 1959).

When previous studies relating to *S. abaster* species were reviewed, particularly detailed studies on length-weight relationships, data on feeding ecologies and morphometric features were encountered (Howard, Koehn 1985; Campolmi et al. 1996; Diaz-Ruiz et al. 2000; Cakić et al. 2002; Kendrick, Hydnes 2003). Moreover, there are only a few studies on reproduction biology of the species. Tomasini et al. (1991) determined reproduction success of the *S. abaster* population from the Mediterranean lagoon. In the study by Franzoi et al. (1993) examining life cycles of *Syngnathus taenionotus* and *Syngnathus abaster* and feeding habitats in the Po River Delta (Adriatic Sea), reproduction features of the species were determined. Carcupino et al. (1997) studied ultrastructure organization of the brood pouch epithelium in males, while Silva et al. (2006) studied reproduction behaviors of embryonic development and behaviors of juveniles and mature individuals. Although there are some studies related to the distribution and abundance (Keskin et al. 2002), ecomorphological features (Gürkan, 2004) and length-weight relationships (Gurkan, Taskavak 2007) of the Syngnathidae family in our seas, there are no studies on reproduction biology of *Syngnathus abaster* in the Aegean Sea.

Although species belonging to the *Syngnathus* genus are not economically important, they attract the attention of scientists (Cakić et al. 2002) due to short life cycles (Franzoi et al. 1993; Campbell, Able 1998) and reproduction features (Berglund et al. 1986). Furthermore, it is profoundly important that further information on the reproduction of *S. abaster* is acquired as the species is in the Least Concern category on the Red List in terms of its sustainability

(IUCN 2015). The presented study has determined some reproduction features of the species such as the spawning period, the sex ratio, fecundity of female individuals and diameters of oocytes in their ovaries, and the number of eggs and larvae in brood pouches of pregnant *S. abaster* males occurring in the Aegean Sea of Turkey.

Material and methods

The study was conducted seasonally in Çandarlı Bay located between 38°57'37"N and 38°43'44"N latitudes and 26°44'58"E and 27°04'23"E longitudes of the northern Aegean Sea between April 2013 and March 2014. At four stations (Çaltidere, Eski Tuzla, Yenişakran, Kazıkbağları) selected in the bay, 15-minute samples were collected from a seine net at a depth of 0.5–2 m. Water temperature was measured (in situ) at the stations using a multiparameter device (WTW Multi 3420). Samples collected from a seine net were fixed in 10% formalin solution and transported to a laboratory. Afterward, species identification was performed using the study by Whitehead et al. (1986). To measure the total length of individuals (mm), we used a fish measuring board, while to determine the weight – a scale having 0.01 gram sensitivity. Sex determinations were conducted based on the brood pouch present in male individuals (Vincent et al. 1995), females and individuals without brood pouches were dissected. The maturity stages of gonads in female individuals were determined in accordance with Holden & Raitt (1974) (Table 1). The Chi-square test (χ^2) was performed to determine whether there were statistically significant differences between female and male ratios (Heithaus 2001). To determine the reproduction time, the Gonadosomatic Index (GSI) was calculated as follows:

$$GSI = \frac{\text{gonad weight}}{\text{body weight} - \text{gonad weight}} \times 100 \quad (\text{King 1995})$$

To determine batch fecundity, hydrated oocytes in ovaries of mature female individuals were calculated using the gravimetric method (Hunter et al. 1985). The number of eggs and embryos in brood pouches of male individuals were determined and oocyte diameters and prelarva and postlarva lengths were measured using an Olympus SZ60 stereo microscope. The regression analysis $y = a + bx$ was employed to determine the relationship between total length-batch fecundity relationship in females and total length-the number of eggs carried in males.

Table 1

Five-point maturity scale (Holden and Raitt, 1974)

Stage	State	Description
I	Immature	Ovary and testis about 1/3 of the body cavity's length. Ovaries pinkish, translucent; testis whitish. Ova not visible to naked eye.
II	Maturing virgin and recovering spent	Ovary and testis about 1/2 of the body cavity's length. Ovary pinkish, translucent; testis whitish, more or less symmetrical. Ova not visible to naked eye.
III	Ripening	Ovary and testis is about 2/3 of the body cavity's length. Ovary pinkish-yellow with granular appearance, testis whitish to creamy. No transparent or translucent ova visible.
IV	Ripe	Ovary and testis from 2/3 to full length of the body cavity. Ovary orange-pink with conspicuous superficial blood vessels. Large transparent, ripe ova visible. Testis whitish-creamy, soft.
V	Spent	Ovary and testis shrunken to about 1/2 of the body cavity's length. Walls loose. Ovary may contain remnants of disintegrating opaque and ripe ova, darkened or translucent. Testis bloodshot and flabby.

Results

A total of 185 individuals were examined, including 94 (50.81%) females and 79 (42.70%) males and 12 (6.49%) immature individuals. Length values in female, male and juvenile individuals were 91–135 mm (mean: 111.5 ± 7.35), 89–143 mm (mean: 109.9 ± 11.08), 68–85 mm (mean: 79.8 ± 5.30), respectively. According to t-test results performed for female and male individuals {LT [t-test, n = 173 (94 females

and 79 males), $p < 0.001$], weight values for female, male and juvenile individuals were 0.3–1.2 g (mean: 0.78 ± 0.18), 0.2–2.0 g (mean: 0.76 ± 0.36) and 0.1–0.2 g (mean: 0.11 ± 0.03), respectively (Table 2). The female to male ratio was 1:0.84, and *Chi-square* test (χ^2) was performed to determine whether the differences in the ratio were statistically significant ($\chi^2 = 0.65 < \chi^2_{t 0.05} = 3.84, p \leq 0.05$).

The water temperature, dissolved oxygen and salinity values depending on the seasons of the year

Table 2

Total length, total weight, Gonadosomatic Index values and sex ratio evaluation in the captured *Syngnathus abaster* female, male (with brood pouch, pregnant ones) and immature individuals. N: sample size; M: Mean; TL: total length in mm; W: weight in g; GSI: Gonadosomatic Index values; SD: Standard deviation) by seasons

FEMALE							
Seasons	N	MTL \pm SD (mm)	MW \pm SD (g)	N	GSI (%) \pm SD	♀:♂	
Autumn	7	114.00 \pm 10.03	0.63 \pm 0.24	7	0.97	0.35	
Winter	8	111.00 \pm 7.60	0.56 \pm 0.20	8	3.07	1	
Spring	23	110.00 \pm 8.40	0.83 \pm 0.20	23	6.86	2.09	
Summer	56	113.00 \pm 6.52	0.81 \pm 0.12	56	6.54	1.40	
Total (Range)	94	111.50 \pm 7.35	0.78 \pm 0.18	94	5.88	0.84	
MALES							
Seasons	Male (Total)			Pregnant male		Male with brood pouch	
	N	MTL \pm SD (mm)	MW \pm SD (g)	N	MTL \pm SD	N	MTL \pm SD
Autumn	20	105.00 \pm 10.20	0.42 \pm 0.12	3	107.7 \pm 13.3	17	103.9 \pm 9.92
Winter	8	109.00 \pm 11.90	0.53 \pm 0.20	-	-	8	79.18 \pm 11.9
Spring	11	104.00 \pm 8.70	0.78 \pm 0.20	11	109.9 \pm 8.22	-	-
Summer	40	113.00 \pm 10.49	0.95 \pm 0.32	35	114.1 \pm 11.5	5	110.4 \pm 6.73
Total (Range)	79	109.90 \pm 11.08	0.76 \pm 0.36	49	112.8 \pm 11.02	30	106.3 \pm 10.16
IMMATURE							
Seasons	N	MTL \pm SD (mm)	MW \pm SD (g)				
Autumn	8	79.25 \pm 6.56	0.11 \pm 0.04				
Winter	4	81	0.10				
Spring	0	-	-				
Summer	0	-	-				
Total (Range)	12	79.8 \pm 5.30	0.11 \pm 0.03				

Table 3

Physicochemical characteristics of sea water

	Station 1			Station 2			Station 3			Station 4		
	T	DO	S	T	DO	S	T	DO	S	T	DO	S
Spring	21.5	8.93	37.8	21.8	8.82	38.2	23.1	8.78	37.8	24.5	8.62	38.4
Summer	24.2	7.80	38.9	23.8	7.55	38.6	23.5	7.67	38.5	25.7	7.57	38.2
Autumn	18.6	9.13	38.1	18.6	9.40	37.7	18.2	9.36	38.4	18.5	9.10	38.8
Winter	15.5	10.31	37.5	16.1	10.12	34.3	15.3	10.11	34.2	17.2	10.9	38.2

T: Temperature (°C), DO: Dissolved oxygen (mg l⁻¹), S: Salinity (PSU)

are presented in Table 3. The surface water temperature varies between 15.3 and 25.7°C, dissolved oxygen varies between 7.55 and 10.9 mg l⁻¹ and salinity varies between 34.2 and 38.9 PSU during the year. Temperature is the most important parameter for the reproduction of marine fish species and mean surface water temperature values determined in our study were 22.7°C in spring, 24.3°C in summer, 18.5°C in autumn and 16°C in winter (Table 3). When GSI values determined for female individuals of *S. abaster* are examined, it appears that the highest value was recorded in spring (mean 6.86) and the spring and summer seasons are the spawning period of the species (Table 4 and Fig. 1). Reproduction occurred depending on the temperature increase.

When frequencies of mature male, female and immature individuals were examined, it was determined that mature males were mostly found in summer (21.62) and autumn seasons (10.81), mature females were found in spring (12.43) and summer seasons (30.27), immature individuals were only seen in autumn (4.33%) and winter (2.16%) seasons (Fig. 2).

When females carrying hydrated oocytes (OCF), pregnant males (PM) and males with empty brood pouches (MEP) were seasonally examined, it was determined that females carrying hydrated oocytes were mostly present in the spring season and their

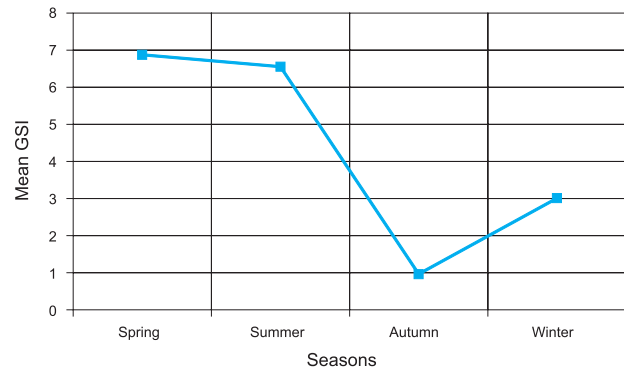


Figure 1

Mean GSI values of *Syngnathus abaster* over time

number decreased in the summer season, while inverse relationship was observed for pregnant males, i.e. their number increased from spring to summer (Fig. 1). When females carrying hydrated oocytes (OCF) and pregnant males (PM) were examined in relation to water temperature, similar results were obtained and served as a proof for the spawning period of the species (Fig. 3).

Fifty six ovaries from females were analyzed in spring and summer seasons determined as the

Table 4

Seasonal variation of GSI for female *Syngnathus abaster* during the period from April 2013 to March 2014, the Aegean Sea, Turkey

♀	No. of fish	Min.	Max	Mean ± SD	95% CI	
					Lower limit	Upper limit
Autumn	7	0.27	1.73	0.97 ± 0.19	0.50	1.43
Winter	8	1.95	4.70	3.01 ± 0.39	2.09	3.93
Spring	23	2.01	23.36	6.86 ± 1.13	4.51	9.21
Summer	56	1.74	14.99	6.54 ± 0.40	5.73	7.35

CI - Confidence Interval

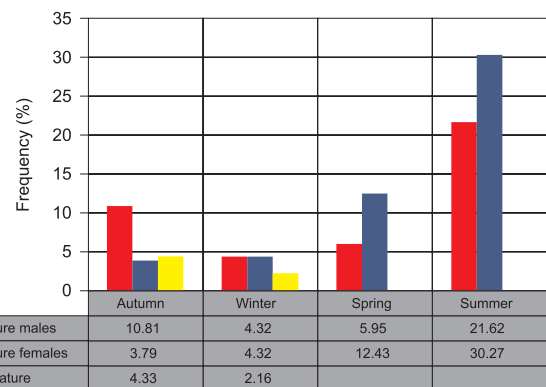


Figure 2

Frequency of mature males, mature females and juveniles in the samples in different seasons

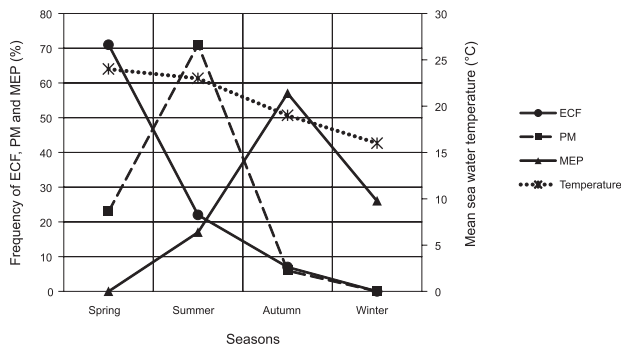


Figure 3

Frequency of hydrated-oocyte carrying females (OCF), pregnant males (PM), and males with empty brood pouches (MEP) in different seasons and relationships with water temperature

spawning period of the species. Three groups of oocytes were identified in ovaries of female individuals: 1) maturing oocytes (diameters: 0.61–1.20 mm), 2) mature oocytes (diameters: 1.21–1.70 mm) and 3) hydrated oocytes (diameters: 1.71–2.10 mm). When frequencies of these oocytes were examined, it was determined that oocytes having 1.51–1.60 mm diameter were particularly dominant (Fig. 4). The mean egg diameter was calculated as 1.84 ± 3.66 mm (min. 1.68, max 2.1 mm) in pregnant males.

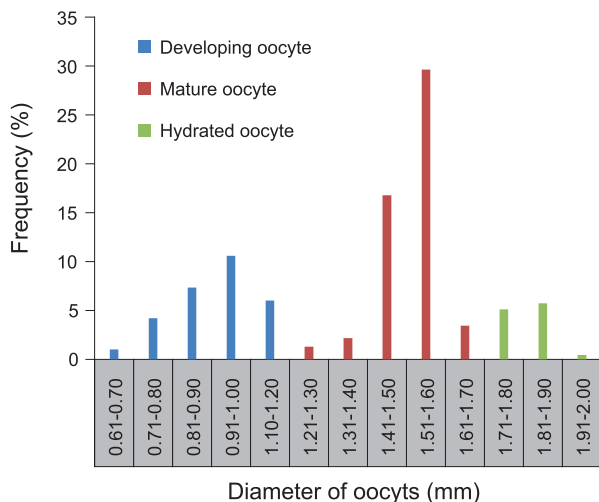


Figure 4

Frequency of oocyte diameters in the female ovary (n = 56)

A linear and positive relationship was found between hydrated oocytes in ovaries and the total length of individuals. The hydrated oocytes/total length relationship was $y = 0.8651x - 84.332$ (n = 14, $r^2 = 0.64$) (Fig. 5).

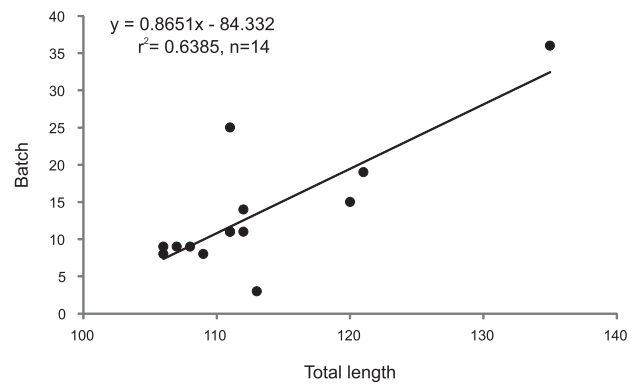


Figure 5

Total length-batch fecundity relationship in females

The number of eggs in male individuals carrying eggs in their brood pouches was on average 48 (mean \pm SD = 48 ± 14.09 eggs, range: 23–78 eggs), and the relationship between the total length of males and the number of eggs carried was examined. As a result of this examination, a positive relationship was found ($r^2 = 0.58$) (Fig. 6). The curve was fitted by $y = 1.0168x - 67.715$. The length of newborn larvae of the male individuals was 16.73 ± 3.01 mm (range: 12.6–22.0 mm).

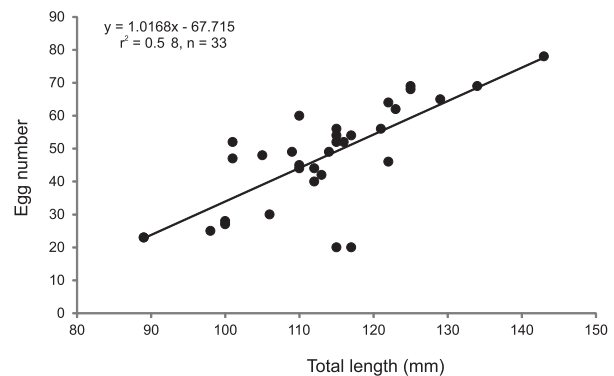


Figure 6

Relationship between the total length of males and the number of eggs carried

Discussion

As a result of the study performed seasonally in Çandarlı Bay between April 2013 and March 2014, the spawning period of the species was determined as spring and summer seasons. The studies by Franzoi et al. (1993) and Riccato et al. (2003) conducted on *S. abaster* indicated spring as a spawning period. In the studies conducted on some other pipefishes, the spawning period was spring for *Syngnathus typhle*, *Nerophis ophidion* (Riccato et al. 2003), March to

September for *S. acus* (Gurkan et al. 2009), end of April to June for *S. scovelli* (Bolland, Boeticher 2005), and this was similar to *S. abaster*.

The female to male ratio was determined as 1:0.84, and no significant differences between the sexes were found by the χ^2 test ($\chi^2 = 0.65 < \chi^2_{t 0.05} = 3.84$, $p \leq 0.05$). Franzoi et al. (1993) found that 47.5% of the population were females and 52.5% – males, and no apparent relationship was determined between females and males ($\chi^2 = 1.183$). For some species belonging to the *Syngnathus* genus, the female:male ratio was 1:0.58 in *S. acus* (Gurkan et al. 2009) and 1:0.4 in *S. scovelli* (Gaspini, Teixeira 1999).

In this study, the diameter of eggs in pregnant males was approximately equal to that in females. In the study conducted in Portugal (40°45'N; 8°40'W, Ria de Aveiro), Silva et al. (2006) determined the mean number of eggs in brood pouches of pregnant males as 37 (range: 10–64 eggs) and Franzoi et al. (1993) reported 109 ± 27 eggs on average. These values were much higher than in other studies. In this study, on average 48 eggs (range: 23–78 eggs) were found. This difference was ascribed to differences in the length of male individuals determined in these studies.

As evidenced by other studies, pregnant males of *S. abaster* have much fewer eggs than reported for other Syngnathidae (Monteiro et al. 2005). According to Monteiro et al. (2005), the maximum number of eggs in *Syngnathus abaster* is 100 and the maximum

number of eggs in other *Syngnathus* species varies between 110 and 500 according to the information they have found in studies carried out in various locations (Froese, Pauly 2005; Kuitert 2000; Dawson 1986; Vincent 1990). According to this study, the maximum egg diameter was 1.8 mm, while egg diameters determined by Silva et al. (2006) ranged from 1.09 to 2.06 mm. In addition, the diameter values in our study ranged from 1.21 to 2.10 mm, and these values seem quite similar to other studies.

A linear relationship between the length of male individuals and the number of eggs carried in the brood pouch was determined in the present study and in Silva et al. 2006, and the correlation coefficient according to Silva et al. 2006 was $r = 0.554$, and in our study – $r = 0.580$. Similarly to other Syngnathidae such as *S. typhle* and *S. schlegeli*, the relationship between the length of male individuals and the number of carried eggs proves that larger males can contain more eggs (Berglund, Rosenquist, 2003; Watanabe, Watanabe 2002). Length values of new larvae from brood pouches of pregnant males were 13.5 ± 1.0 mm as reported by Franzoi et al. (1993), 17.67 ± 0.22 mm by Silva et al. (2006) and 16.73 ± 3.01 mm as determined in the present study.

Embryos/postlarvae at different stages were found in some brood pouches of male individuals (Fig. 7). Although males of some species from the *Syngnathus* genus such as *S. taenionotus* takes eggs

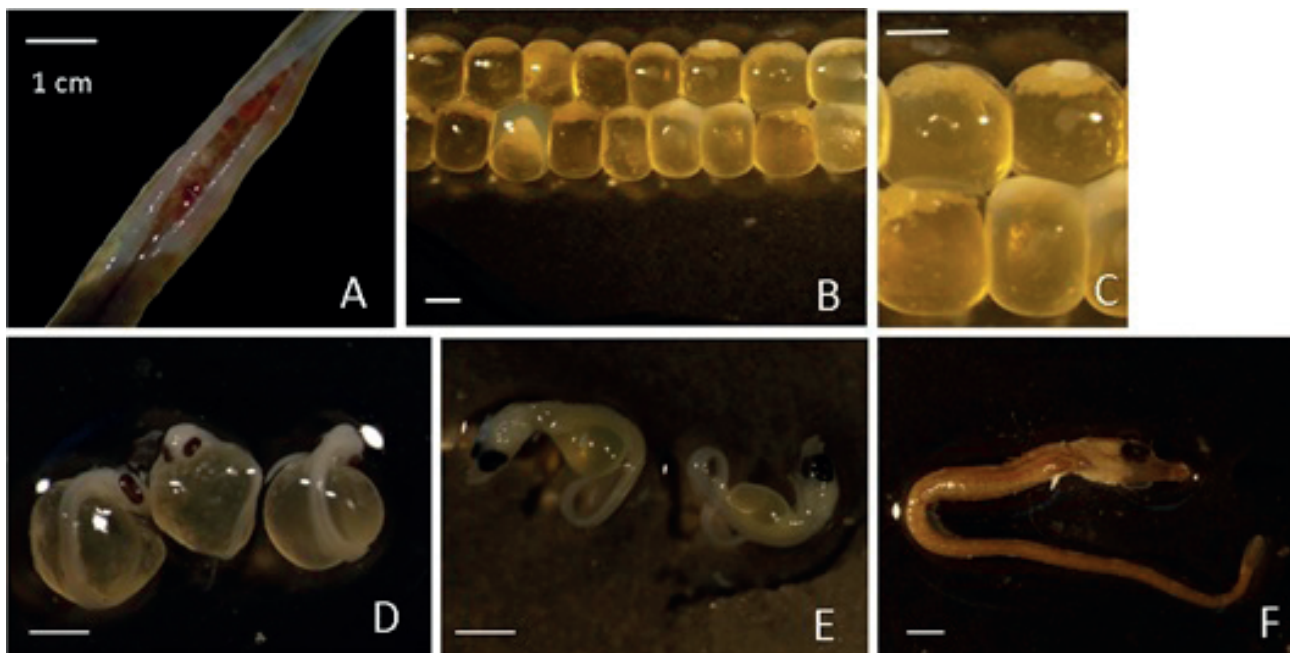


Figure 7

Different developmental stages in *Syngnathus abaster*; (A) brood pouch with eggs, (B–C) 7 days, (D) newborn head, (E) prelarvae; scale bars = 1 mm

from females simultaneously, males of *S. abaster* takes only eggs produced by one female in the spawning period. However, it was concluded that the presence of embryos/postlarvae of different stages in brood pouches of some male individuals was caused by the fact that females in some *Syngnathus* species can split egg groups among many males at the same time (Breder, Rosen 1966; Bayer 1980; Berglund et al. 1988).

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