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RESEARCH ARTICLE

The first observations of oocyte diameters for the pipefish from the southern Black Sea

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Abstract

Syngnathid specimens sample between July 2001-May 2002 was examined about measurements of oocyte diameter in the coastal waters of Sinop peninsula, southern Back Sea. The specimens of Syngnathid fishes (*Syngnathus acus*, *Syngnathus typhle, Syngnathus variegatus*, *Syngnathus tenuirostris*, *Nerophis ophidion*) catched with using hand dredge from the vegetated coastal area. According to results, the mean of hydrated oocyte diameter was calculated as, respectively: *Syngnathus acus*: 1.96 mm, *Syngnathus typhle*: 1.76 (♀) and 2.22 (♂) mm, *Syngnathus tenuirostris*: 1.81 mm, *Syngnathus variegatus*: 1.63 mm and *Nerophis ophidion*: 0.89 mm. Also with this study, oocytes diameter values of *Syngnathus tenuirostris, Syngnathus variegatus*, *Syngnathus typhle* and *Nerophis ophidion* determined for the first time from coast of Turkey.

Key words: Syngnathidae, oocyte diameter, southern Black Sea, Turkey.

Introduction

The pipefishes and seahorses have different reproductive and breeding strategies among the teleost fishes (Watanabe and Watanabe 2002). Polygamous mating system is reported in syngnathid fishes (Watanabe and Watanabe 2002; Wilson *et al*. 2003). In genus Syngnathus, one or several females transfer mature oocytes into the brood pouch of mature male (Kornienko 2001).

Parental care of eggs and developing embryos are exhibited in specialize pouch of mature males (Vincent *et al.* 1991; 1995; Kornienko 2001; Wilson *et al*. 2003; Ahnesjö 2008). Main functions of the brood pouch of mature male are: to protect from physical difficulties (predation etc.) and to provide osmoregulation and to provide nourishment for eggs and embryos (Watanabe and Watanabe 2002; Wilson *et al*. 2003; Silva *et al*. 2006).

In last ten years researches on the reproductive biology and breeding ecology of syngnathid fishes were investigated intensively (Kornienko 2001; Watanabe and Watanabe 2002; Wilson *et al.* 2003; Lyons and Dunne 2005; Ricatto *et al.* 2003; Kvarnemo and Simmons 2004; Jones *et al.* 2005; Silva *et al.* 2006; Rispoli and Wilson 2007; Ripley and Foran 2009). However, there is a single study about the reproductive biology of great pipefish, *Syngnathus acus* from the Turkish coasts (Gürkan *et al.* 2009).

The species of family Syngnathidae are found in shallow coastal areas with vegetation and they are important part of the ichthyofauna of estuaries (Howard and Koehn 1985). There are few data on the occurrence of Syngnathus in the coasts of Black Sea (Kence and Bilgin 1996; Cakić *et al.* 2002; Bilecenoğlu *et al.* 2002). According to Popov (1931), benthic fishes of the Black Sea include, Blennidae, Gobiidae, Pleuronectidae, Labridae, Syngnathidae, and also the genus *Syngnathus* is represented six species in the coast of Black Sea (Kence and Bilgin 1996).

The aim of the present study was to determine oocytes and eggs diameters of *Syngnathus acus, Syngnathus tenuirostris, Syngnathus variegatus*, *Syngnathus typhle* and *Nerophis ophidion* from the coast of Sinop Peninsula, the southern coast of the Black Sea, for the first time.

Material and Methods

A total of 114 syngnathid fishes were collected in different habitats (41˚54′N – 42˚00′N and 35˚10′E) a using hand dredge between July 2001 and May 2002, from shallow waters in the Sinop peninsula, Black Sea, Turkey.

The captured samples were kept in 10% formalin solution for laboratory analysis. The fish species were identified based on Washington (1984). Total length (TL, mm) and total weight (0.01 g precision) measurements were determined in the laboratory. In all samples, sex of individuals was identified by the pouch in males and ovary was observed in female. The samples with incomplete gonadal development were accepted as immature. The number of eggs found in brood pouch, and size of hydrated oocytes found in ovaries were counted in all mature individuals. And then the eggs and oocytes were fixed in a %10 formalin solution and their long diameters measured using a binocular microscope (having micrometric ocular with 10x3 magnification). (Development phases in oocytes (corticol alveoli, vitellogenetic, maturing, mature and oogonia) were determined according to Begovac and Wallace (1987). Oogonia (Phase I) and primer oocyte (Phase II) were considered as the eggs of the next year but were not evaluated.

Statistical tests were calculated by Statistica 6 software. For the statistical differences between sexes and oocyte diameters were determined by t-test (Sümbüloğlu and Sümbüloğlu 1993).

Results

Of 114 individuals, representing five species, collected in the study area, 93 samples were determined as mature and 21 samples immature. All descriptive results of examined five species are given in Table 1.

The minimal sexual maturity lengths of species were shown in Table 1. We consider that sexual length was 96 mm in females and 70 mm in males of *S. acus*. A significant difference was determined between sexes according to length ($P=0.018$, $P<0.05$). In *S. tenuirostris*, minimum sexual maturity length was 67 mm for females and 91 mm in males. It was showed that the total lengths of males longer than females ($P=0.006$, $P<0.05$). However, the minimal sexual maturity length of females and males in *S. typhle* were determined as 130 mm and 100 mm, respectively, but there was no significant difference ($P>0.05$). We found minimal sexually mature length of females in *S. variegatus* as 82 mm and 78 mm in males. In this species, females are bigger than the males, and there was no statistical difference by the length values ($P>0.05$). In *N. ophidion*, the minimal sexual maturity length was determined as 132 mm in females and 130 mm in males. There was no statistical differences of length between sexes ($P>0.05$).

In Table 2, range of oocyte diameter values and mean of diameters showed in ovaries and brood pouches.

According to number of size hydrated oocyte, there was no female carrying hydrated oocyte in *S. acus, S. variegatus* and *N. ophidion*. Whereas, number of hydrated oocyte size in ovaries of females *S. typhle* and *S. tenuirostris*, were examined 76 and 48 respectively. The number of eggs was counted in males 89 in *S. acus*, 104 in *S. typhle*, 49 in *S. variegatus* and 36 in *N. ophidian*.

By the results obtained, minimal oocyte diameter value was in *N. ophidion* (0.77 mm) (Figure 1). The highest oocyte diameter value was in *S. typhle* (1.75 mm) between five species. For Syngnathus genus, maximum oocyte diameter value was measured in *S. typhle* (2.49 mm) (Figure 2) and minimal oocyte diameter value in S. *variegatus* (1.23 mm) (Figure 3).

Between hydrated oocyte diameter values, statistical difference was determined in *S. variegatus* (Anova, $P< 0.001$). In *S. acus*, there was no differences in mature oocytes values, in contrast, significantly differences were calculated in maturing oocytes (Anova, $P<0.001$). In female gonads of *S. tenuirostris*, mature oocyte and maturing oocytes were observed in contrast, there was no egg in brood pouch of males (Figure 4).

Table 1. Sex ratio and values of total length and total weight of Syngnathid species (TL, mm; W, g)

Species	Female			Male			F/M	P
	N	TL Range	W Range	N	TL Range	W Range		
Syngnathus acus	20	70-100	0.11-0.62	7	96-233	0.11-6.46	1=0.6	P<0.05
Syngnathus typhle	8	130-292	0.48-13.35	4	100-167	0.30-1.98	1=0.5	P>0.05
Syngnathus variegatus	5	82-85	0.15-0.23	5	78-112	0.15-0.59	1=1	P>0.05
Syngnathus tenuirostris	16	67-100	0.03-0.29	3	91-167	0.37-1.98	1=0.2	P<0.05
Nerophis ophidion	15	132-170	0.24-0.53	8	130-177	0.22-0.55	1=0.5	P>0.05

*P<0.05: statistically differences between sexes.

Table 2. The range of oocyte diameter and mean of oocyte and egg diameters values (n=number of oocyte)

Species		N	n	Hydrated oocyte mean±SE	n	Mature oocyte mean±SE	n	Maturing oocyte mean±SE
Syngnathus acus	♀	5	-	-	8	1.89±0.13 (1.75-1.93)	104	0.74±0.27 (0.46-1.30)
	♂	5	89	1.96±0.25 (1.75±2.28)	41	1.25±0.19 (1.12-1.47)	-	-
Syngnathus typhle	♀	5	76	1.76±0.22 (1.75-2.49)	118	1.66±0.16 (0.88-2.21)	314	0.80±0.10 (0.40-1.30)
	♂	2	104	2.22±0.25 (1.75-2.28)	-	-	20	4.71±1.05 (0.50-0.60)
Syngnathus tenuirostris	♀	18	48	1.81±0.14 (1.54-2.21)	118	1.65±1.51 (1.05-2.03)	227	0.83±0.06 (0.25-1.58)
	♂	-	-	-	-	-	-	-
Syngnathus variegatus	♀	-	-	-	-	-	-	-
	♂	2	49	1.63±0.23 (1.23-1.93)	-	-	-	-
Nerophis ophidion	♀	1	-	-	-	-	135	0.55±0.04(049-0.63)
	♂	1	36	0.89±0.15 (0.77-1.09)	-	-	-	-
TOTAL		39	402		285		800	
P		P<0.001		P>0.001		P<0.001		

* P<0.001: statistically significant differences between eggs diameter

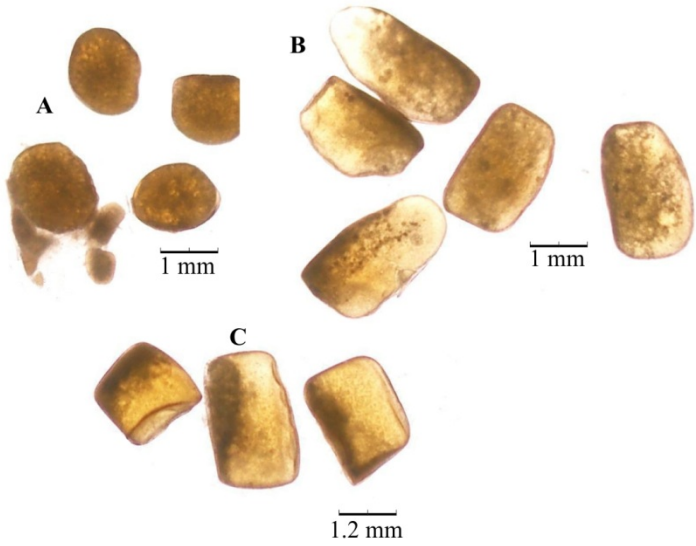

Figure 1. Maturing oocytes (A) and hydrated oocytes (B) in gonad, hydrated oocyte in brood pouch in male (C) for *N. ophidion*

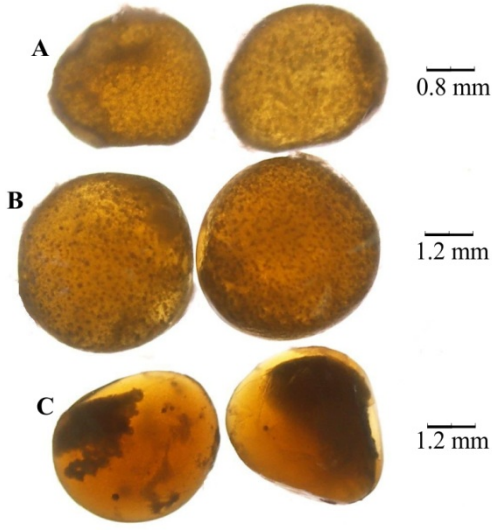

Figure 2. Mature oocytes (A) and hydrated oocyte (B) in gonad, fertilized egg in brood pouch in male (C) for *S. typhle*

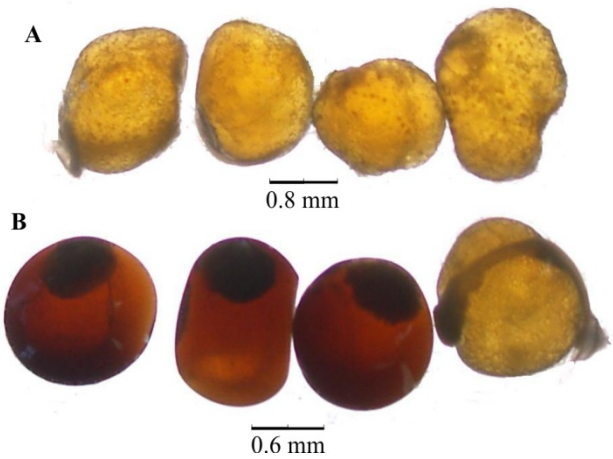

Figure 3. Mature oocyte (A) in gonad and fertilized egg and embryo in male pouch (B) for S. *variegatus*

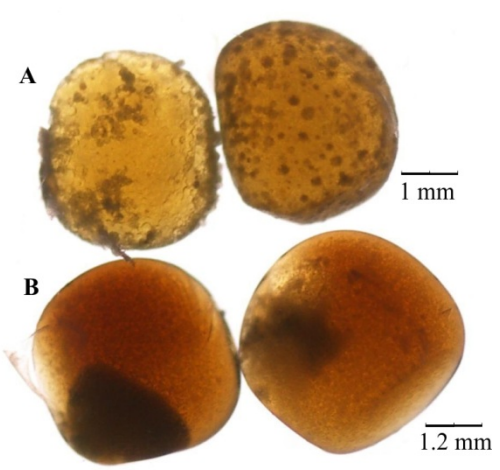

Figure 4. Mature oocyte (A) and hydrated oocyte in female gonad (B) in *S. tenuirostris*

Discussion

In this research, diameter values of oocyets for *S. acus, S. tenuirostris, S. variegatus, S. typhle* and *N. ophidion* were given for the first time at the Turkish coasts. Small sampling size, despite the hand dredge collection covering on vegetated habitats from the shallow coastal area, indicates the relatively small population density of the genus *Synganthus* and *Nerophis*.

The polygamous mating system has been reported for S. *typhle* (Jones *et al.* 1999), *S. acus* (Vincent *et al.* 1995) and *N. ophidion* (McCoy *et al.* 2001). Polygamy occurs in the genus *Syngnathus*, where both males and females mate multiple times during single breeding period (Wilson *et al.* 2003). The female transfers the eggs at mating by inserting her ovipositor into the brood pouch of males (Kornienko 2001; Watanabe and Watanabe 2001; Ahnesjö 2008). In contrast, males of genus *Nerophis* receive their entire brood from a female during a single mating (Wilson *et al.* 2003; Kvarnemo and Simmons 2004). This indicates that polygamous mating system differently occurs among two genera *Syngnathus* and *Nerophis*. Our results indicated that the oocytes have three different development stages (only hydrated egg, hydrated and mature oocyte, maturing oocyte were found in brood pouch of males and female of *S. acus*.

Female/male ratio reported by Vincent *et al.* (1995) is equal but our results agreed with the results given by Gürkan *et al.* (2009). It is indicated that *S. acus* was polygamous in the study area.

In this study, the egg diameteres ranged from 1.1 mm to 2.3 mm. Whereas, Gürkan *et al.* (2009) did not find larger eggs than 1.8 mm for the Aegean population. It seems that produced oocytes in northern population (Black Sea) of *S. acus* may have bigger diameter than those of the Aegean Sea population. Gasparini and Teixeira (1999) pointed out the number of eggs in male pouch were varied in different localities as fish length, brood pouch morphology, egg size and extreme abiotic conditions. Differences in egg diameter found in brood pouch at different localities suggest that growth rate of species could be different at different latitudes (Gasparini and Teixeira 1999) and also warmer sea temperatures can effect upon growth rates of species (Kirby *et al.* 2006).

In *S. typhle*, the oocytes found in brood pouch of males have two different development stages. The smaller than 0.50 mm diameter oocytes in brood pouch were considered as eggs given from the different female's gonad. The numbers of mature females more than males in their habitats during their reproductive season (Vincent *et al.* 1995). Ahnesjö (1996) claimed that oocytes have all development stage find in the gonad of female, whereas, males mates different females and so different development stages eggs were found in the brood pouch. Also, the same development stage in larvae and hydrated eggs had been observed in brood pouch of *S. typhle* male in population of Black Sea (Kornienko 2001). In our study, hydrated and fertilized eggs were recorded by same development stage in one male brood pouch. Moreover it was showed that one female also transferred oocytes into the brood pouch of several male due to reproductive success of females. Derived from Vincent (1990) and Kuiter (2000), highest oocyte diameter was found 2 mm in *S. typhle*. Our findings for diameter values were 2.3 mm for male and 2.5 mm for female and values bigger than the above researchers' results. The reasons of differences of egg diameters between studies may be differences growth rates at the different localities, differences of abiotic components (sea temperature *etc.*). These conditions may

affect the potential reproductive rates of the cold seas inhabitants (Kirby *et al*. 2006).

There was no knowledge about reproductive biology of *S. tenuirostis* in the literature, but hydrated and mature and also maturing oocytes in female ovary was found in this study. The results of observed oocytes in female ovary performed on fish of the same species proved that one female deposits offspring to several males (Berglund *et al.* 1988; Kornienko 2001).

According to results obtained, the eggs had the same developing stage were examined in the brood pouch of male *S. variegatus*. The eggs in the brood pouch were found as fertilized eggs or with embryos. Because there was no different developing stage eggs in male' pouch, we may accept that *S. variegatus* is a polygamous.

According to Vincent *et al*. (1995), *N. ophidion* male carries eggs from only one female per reproductive period and their offspring are attached to his ventral surface (Monterio *et al.* 2001; Ricatto *et al.* 2003; Lyons and Dune 2005). Whereas, males of Syngnathus species have potential for multiple mating and brood eggs in an enclosed and sealed brood pouch and carry several partial clutches from different females (Berglund *et al.* 1988). Dawson (1986) and Froese and Pauly (2005) reported that the highest oocyte diameter was 1.2 mm for *N. ophidion.* In our results, values of egg diameter were determined between 0.5 mm and 1.1 mm. Although our results are accordance with these researchers', the differences in egg diameters can be derived from specimens in different localities.

Consequently, oocyte values of *S. acus, S. tenuirostris, S. typhle, S. variegatus* and *N. ophidion* were represented for the first time from the Turkish Black Sea coasts.

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Güney Karadeniz'deki deniziğnelerinin yumurta çapları üzerine ilk gözlemler

Özet

Bu çalışmada, Temmuz 2001-Mayıs 2002 yılları arasında güney Karadeniz'de Sinop yarımadasının kıyısal sularındaki deniziğnelerinin yumurta çapları incelenmiştir. Deniziğnesi örnekleri (*Synganthus acus*, *Syngnathus typhle, Syngnathus variegatus*, *Syngnathus tenuirostris*, *Nerophis ophidion*) vejetasyonlu kıyısal bölgeden el dreji kullanılarak yakalanmıştır. Sonuçlara göre, ortalama hidrate yumurta çapları sırasıyla; *Syngnathus acus*: 1.96 mm, *Syngnathus typhle*: 1.76 (♀), 2.22 (♂) mm, *Syngnathus*

tenuirostris: 1.81 mm, *Syngnathus variegatus*: 1.63 mm ve *Nerophis ophidion*: 0,89 mm. olarak hesaplanmıştır.

Ayrıca bu çalışma ile *Syngnathus tenuirostris, Syngnathus variegatus*, *Syngnathus typhle* ve *Nerophis ophidion* için yumurta çapları Türkiye kıyılarından ilk defa tespit edilmiştir.

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