BIHAREAN BIOLOGIST 15 (1): 48-52
Article No.: e201307
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http://biozoojournals.ro/bihbiol/index.html

Data on the reproductive biology of two Anguids in Turkey

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Received: 27. December 2019 / Accepted: 09. September 2020 / Available online: 10. September 2020 / Printed: June 2021

Abstract. Information on the reproduction biology of *Anguis fragilis* complex and *Pseudopus apodus* living in Turkey was very limited or rare. Here we dissected a total of 80 female Anguid (61 *A. fragilis* complex and 19 *P. apodus*) lizards to examine the ovaries for eggs and embryos. According to the frequencies concerning ripe eggs and embryos (for *A. fragilis* complex only) it can be suggested that the potential reproduction period of *A. fragilis* complex from April to July, whereas the potential reproduction period of *P. apodus* is between April and June. We also observed *A. fragilis* complex individuals gave birth in the laboratory and, the SVL values of newborn individuals varies between 44 mm and 56 mm whereas the SVL values of stillborns varies between 42 mm and 49 mm.

Key words: *Anguis fragilis* complex, *Pseudopus apodus*, Anguidae, reproduction biology, Turkey.

Introduction

Generally, three main reproduction model is reported in reptiles; viviparity, ovoviviparity, and oviparity (Başoğlu & Baran 1977, Vitt & Caldwell 2009). Most of the reptiles lay eggs on spring or early summer seasons and the hatchlings hatch at the end of summer or fall seasons. Oviparous reptiles generally make nests several places such as digged holes, hollows, etc., in order to protect the eggs and to stabilize the temperature and humidity. Any conditions could affect the nest in terms of incubation time, development rate, hatching success or newborns length (Vitt & Caldwell 2009).

Anguis fragilis Linnaeus, 1758 lives in Western and some part of Central Europe while *A. colchica* (Nordmann, 1840) inhabits Northern Eurasia, some part of Central and Eastern Europe (Gvoždík et al. 2010, Gvoždík et al. 2013, Szabó & Vörös 2014, Jablonski et al. 2016, Strzała et al. 2016). According to some researchers, *A. fragilis* distributed in Turkey (Bird 1936, Bodenheimer 1944, Baran et al. 2012, Keskin et al. 2013a) whereas some other researchers state that *A. colchica* lives in Turkey (Baran 1977, Başoğlu & Baran 1977, Baran et al. 1992, Kumlutaş et al. 1998, Çevik 1999, Gvoždík et al. 2010, Gvoždík et al. 2013, Jablonski et al. 2016, Yaşar, pers. comm. 2018). Because of the taxonomically contentious situation, here we used the name of the species as *A. fragilis* complex. The other Anguid species, *Pseudopus apodus* (Pallas, 1775) which is widespread species ranges eastwards from the Balkan region of Europe to Turkey, the Caucasus region, Central Asia and the Levant (Böettger 1892, Baran et al. 1988, Keskin et al. 2013b, Nasrabadi et al. 2018, Lavin & Girman 2019).

It is mentioned that there are few information on reproduction of the Anguid lizards except some species (Greer 1967, Trauth 1984, Stumpel 1985, Smith, pers. comm. 1990, Capula & Luiselli 1993, Galán & Ferreiro 2004, Rifai et al. 2006, Barros & Teixeira 2007, Kukushkin et al. 2013). There are only few studies on reproduction of the some populations of ovoviviparous *A. fragilis* complex and oviparous *P. apodus* (Stumpel 1985, Smith, pers. comm. 1990, Capula & Luiselli 1993, Galán & Ferreiro 2004, Rifai et al. 2006, Kukushkin et al. 2013). We aimed to determine the potential breeding season of Anguid species live in Turkey by counting the number of the ovas and embryos of the dissected samples in both species.

Material and Methods

Both species were collected on the fieldworks (52 fieldworks one of which takes 5 or 6 days, almost all months that the species were active) between 2008-2012 in scope of a TÜBİTAK project from the north of the 40° latitude of Turkey.

A total of 80 female specimens (sixty-one *A. fragilis* complex, nineteen *P. apodus*) belonging to the two Anguid species used in the study were collected between 2008 and 2012. The localities where the samples were captured are given in Tables 1 and 2. The specimens were caught by hand and transferred in the bags to the laboratory. The specimens examined via palpation due to a possible gravidity. The gravid specimens were kept in 55x35x35cm sized terrariums which covered with wood chips, leaves and moss on the ground. The other females were dissected and the ovaries were preserved in 70% alcohol for further analysis. Ovaries were examined under a stereomicroscope, Olympus SZ51 and the number of the ova with miscellaneous dimensions were recorded (Table 1, 2). Some of the gravid specimens gave birth in terrariums and the number of the hatchlings and stillborns were recorded. All specimens were deposited at the Collection of the Molecular Zootaxonomy Laboratory of Çanakkale Onsekiz Mart University (ÇOMU-ZDEU). Snout-vent length (SVL) of all specimens including the newborns and stillborns were measured using a digital caliper with an accuracy of 0.01 mm (Table 1, 2).

Results

A total of sixty-one *A. fragilis* complex specimens were dissected and both number of the ovas and embryos were determined. While the highest number of the ova which were not completed the vitellogenesis belongs to a specimen (SVL: 188 mm) caught at the end of May in 2010 from Tekirdağ, the lowest number of the ova belongs to a specimen (SVL: 183 mm) caught on June in 2010 from Asian part of İstanbul (Table 1).

The specimen of *A. fragilis* complex (from İstanbul) which was transferred to the laboratory in 2010 gave birth to five juveniles in the terrarium (Figure 1). The SVL of the juveniles was measured as 44 mm, 52 mm, 50 mm, 51 mm and 48 mm respectively. Another specimen of *A. fragilis* complex

Table 1. Capture Dates, Number of the ova, Number of the Embryo and SVL of *A. fragilis* complex individuals.

COMU-Zoology Research Lab- NO	Capture Dates	Locality	Number of the ova	Number of the Embryo	SVL (mm)
84/2010-1	26.5.10	Şarköy/Tekirdağ	112	1	188
84/2010-4	26.5.10	Şarköy/Tekirdağ	40	-	136
97/2010-3	12.6.10	Şarköy/Tekirdağ	56	-	147
97/2010-2	12.6.10	Şarköy/Tekirdağ	51	-	168
131/2009	20.10.09	Silivri/İstanbul	95	-	148
98/2010-3	21.5.10	İğneada/Kırklareli	19	-	110
98/2010-1	21.5.10	İğneada/kırklareli	55	-	166
134/2009-2	25.5.09	Sapanca/Adapazarı	34	-	153
134/2009-3	25.5.09	Sapanca/Adapazarı	53	-	148
Bursa-2-2		Bursa	41	-	160
Bursa-2-3		Bursa	52	-	152
Bursa-1-3		Bursa	48	-	175
9/2007-1	5.5.07	Beykoz/İstanbul	47	-	166
86/2010	1.6.10	Dereeski/İstanbul	7	2	193
85/2010	27.6.10	İstanbul	3	12	183
101/2010-2	11.6.10	Akçakoca/Düzce	56	-	177
136/2009-2	24.5.09	Çaycuma/Zonguldak	46	-	147
136/2009-1	24.5.09	Çaycuma/Zonguldak	48	-	168
141/2009	16-18.5.09	Kastamonu	34	-	139
90/2010-1	17.5.10	Pınarbaşı/Kastamonu	48	-	170
90/2010-2	17.5.10	Pınarbaşı/Kastamonu	63	-	159
106/2010-2	8.6.10	Vala köyü/Kastamonu	44	8	157
106/2010-3	8.6.10	Vala köyü/Kastamonu	61	-	137
106/2010-4	8.6.10	Vala köyü/Kastamonu	61	9	157
106/2010-5	8.6.10	Vala köyü/kastamonu	31	-	165
106/2010-6?	8.6.10	Vala köyü/Kastamonu	71	-	150
106/2010-7	8.6.10	Vala köyü/Kastamonu	52	6	165
106/2010-8	8.6.10	Vala köyü/Kastamonu	70	-	155
106/2010-9	8.6.10	Vala köyü/Kastamonu	65	-	143
143/2008-3	16.6.08	Sinop	36	-	153
89/2010	27.7.10	Sinop	46	-	127
185/2009-2	11.4.09	Karakum/Sinop	40	-	129
113/2009	19.6.09	Karakum/Sinop	45	-	157
143/2008-1	16.6.08	Sinop	85	-	245
145/2008	1.5.08	Çarşamba/Samsun	97	-	229
140/2009	22.5.09	İkizce/Ordu	-	16	214
144/2009	21.5.09	Gölköy/Ordu	56	-	162
146/2009-5	21.5.09	Perşembe/Ordu	36	-	161
146/2009-6	21.5.09	Perşembe/Ordu	34	-	142
180/2009-1	19-22.5.09	Yörükler/Samsun	52	-	187
180/2009-3	19-22.5.09	Yörükler/Samsun	65	-	163
91/2010-1	27.6.10	Dumlusu/Trabzon			189
91/2010	27.6.10	Dumlusu/Trabzon			142
91/2010-4	27.6.10	Dumlusu/Trabzon			162
Rize-1		Rize	87	14	189
Rize-2		Rize	44	-	172
Rize-5		Rize	69	11	162
96/2010-1	26.6.10	Ardanuç/Artvin	61	-	159
96/2010-2	26.6.10	Ardanuç/Artvin	59	-	169
96/2010-3	26.6.10	Ardanuç/Artvin	59	-	151
96/2010-4	26.6.10	Ardanuç/Artvin	17	-	113
95/2010-2	27.6.10	Esenkıyı/Artvin	86	-	208
184/2009-2	13.7.09	Yomra/Trabzon	-	1	177
Borçka-3		Borçka	88	9	167
93/2010-1	27.6.10	Fındıklı/Rize	3	4	179
93/2010-2	27.6.10	Fındıklı/rize	48	9	181
91/2010-2	27.6.10	Dumlusu/Trabzon	64	9	186
92/2010-1	27.6.10	Araklı/Trabzon	48	-	141
92/2010-2	27.6.10	Araklı/Trabzon	49	-	165
51/2011	25.6.11	Posof-Ardahan	38	-	202
1/2012	4.7.12	Şenkaya/Erzurum	48	-	132

Figure 1. *Anguis fragilis* complex individual with a newborn.

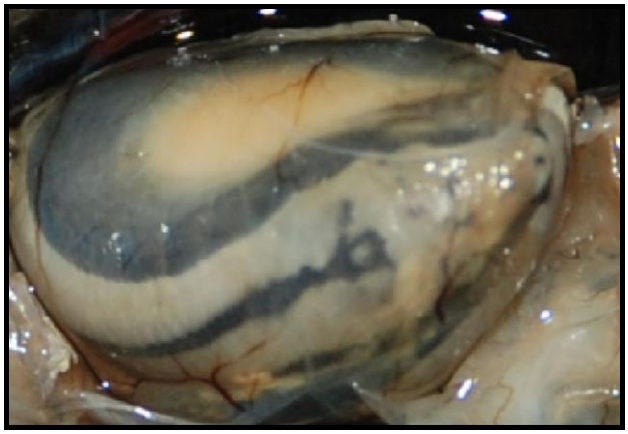

Figure 2. *Anguis fragilis* complex stillborns in sack.

(from Ordu) gave birth to five juveniles and four stillborns in sacks. The SVL of the juveniles was determined as 52 mm, 55 mm, 53 mm, 56 mm and 49 mm respectively. The SVL of the stillborns was measured as 49 mm, 48 mm, 45 mm and 42 mm respectively (Figure 2).

Figure 3. Eggs with miscellaneous dimensions in a dissected *P. apodus*.

A total of nineteen *P. apodus* specimens were dissected and the highest number of the ova which were not completed the vitellogenesis belongs to an individual (SVL: 372 mm) caught on May in 2009 from Gökçeada (Imbros), Çanakkale while the lowest number of the ova belongs to a road-killed individual from Gülpınar, Çanakkale. For the individuals caught from the Marmara and Black Sea regions, number of the ovas which were not completed the vitellogenesis were found to be quite high between April and June, while the number of the ovas was determined 30 for an individual collected from Eastern Anatolia (Digor, Kars) (Table 2) (Figure 3).

Discussion

There are not only few studies but also lack of information on reproductive biology of Anguids (Greer 1967, Trauth

Table 2. Capture Dates, Number of the ova and SVL of *P. apodus* individuals.

COMU-Zoology Research Lab- NO	Capture dates	Locality	Number of the ova	SVL (mm)
36/2010	29.4.2010	Çanakkale	37	375
122/2009	25.6.2009	Bigadiç/Balıkesir	45	360
14/2009	3.4.2009	Behramlı/Çanakkale	38	360
49/2009-3	25.4.2009	Gökçeada/Çanakkale	65	361
81/2010	17.6.2010	Beykoz/İstanbul	26	328
5/2012	3.6.2012	Gülpınar/Çanakkale	8	damaged
75/2009	5.5.2009	Gökçeada/Çanakkale	54	379
80/2010	8.5.2010	Bayramiç/Çanakkale	65	390
125/2010-2	27.5.2010	Hoşköy/Şarköy-Tekirdağ (1)	36	320
103/2009	13.6.2009	Kavak/Çanakkale	36	390
125/2010-1	27.5.2010	Hoşköy/Şarköy-Tekirdağ (2)	64	-
61/2004	24.4.2004	Ezine/Çanakkale	53	370
85/2009	17.5.2009	Gökçeada/Çanakkale	75	372
82/2010-2	7.5.2010	Bafra/Samsun	57	355
82/2010-7	7.5.2010	Bafra/Samsun	45	330
82/2010-3	7.5.2010	Bafra/Samsun	52	315
82/2010-6	7.5.2010	Bafra/Samsun	63	345
64/2011	25.6.2011	Digor/Kars	48	360
83/2010	6.10.2010	Digor/Kars	30	330

1984, Stumpel 1985, Smith, pers. comm. 1990, Capula & Luiselli 1993, Galán & Ferreiro 2004, Rifai et al. 2006, Barros & Teixeira 2007, Kukushkin et al. 2013, Ortiz et al. 2017, Kukushkin & Dovgal 2018).

The ovoviviparous Anguids living in North America [*Elgaria coerulea* (Wiegmann, 1828)] and Western Europe (*Anguis fragilis*) are among the northernmost anguid lizards. In order to withstand cold climates in the north, viviparity has a great role to survive and maintain their generations in the high altitudes (Greer 1967). *Anguis fragilis* complex that distributes generally the colder regions of Turkey is also ovoviviparous, and the mature ovas were found in the period between April and July which could be the breeding period for the Turkish population of this species.

The ovaries of *Celestus sepsoides*, *Celestus haetianus*, *Celestus crusculus crusculus*, *C. c. moleswhorthi*, *C. curtissi aporus*, *Diploglossus pleii*, *C. costatus*, *D. delasagra*, *Ophiodes vertebralis*, and *O. intermedius* were examined and on some species (*C. haetianus*, *C. cruculus cruculus*, *C. curtissi aporus*, *D. pleii*, *C. costatus*, *O. vertebralis*, and *O. intermedius*), both totally developed juveniles and oviductal eggs were detected (Greer 1967). On the other hand, the totally developed juveniles and oviductal eggs were detected on the same period (Greer 1967). Thus, it is possible to see different staged eggs at the same time which is similar to our results.

Stumpel (1985) were examined *A. fragilis* specimens and it is considered as the sex of the individuals those smaller SVL than 120 mm could not be determined. But our results showed, two of the individuals has 113 mm (Ardanuç/Artvin) and 110 mm (İğneada/Kırklareli) SVL respectively, has developed eggs. It could be suggested that sex of the individuals could not be determined by external morphological characteristics, thus we dissected the specimens to determine the sex of the individuals. Also, it could be thought that the ecological conditions of the species could cause an early sexual maturity.

An Italian Alps population of *A. fragilis* was studied for reproductive biology and it is found that the specimens of Alpin population have a biennial reproduction like the sympatrically living viperid *Vipera berus* and the clutch size of the *A. fragilis* reported between six and 13. It also was mentioned that the biennial reproduction could occur because of the cold climate (Capula & Luiselli 1993). There is no such information for the Turkish population. It is reported on Coruña population of the *A. fragilis* breed annually unlike others, and it is also mentioned that there could be a possible relationship between annual reproduction of females, the latitude and the environmental temperature (Galán & Ferreiro 2004, Ortiz et al. 2017). Thus, for Turkish populations, an annual breeding could be possible because most of the regions where the *A. fragilis* complex live have similar environmental conditions as Coruña.

Also in Coruña, the first newborns appeared in the countryside from the middle of August and the reproductive period of the species continues from mid-March to mid-September (Galán & Ferreiro 2004). Some other studies show that the reproductive period was shorter than three months (85 days in England) (Smith, pers. comm. 1990). In Turkish populations, the reproductive period starts on early April which similar to Coruña populations, and possibly continues to end of July.

On the other hand, the result showed that the potential breeding season of the Marmara and Black sea populations of *P. apodus* were between April and June. The full developed eggs were found in the Eastern Anatolian specimens of *P. apodus*. Thus the active period of this population could be extended till November or there could be an estivation for eastern population which is mentioned on Kukushkin et al. (2013).

Vitellogenic ovarian follicles were determined in the specimens of *Ophisaurus attenuatus attenuatus* Baird, 1880 that collected mid-April and early June (Trauth 1984). Barros and Teixeira (2007) investigated the female individual of *Ophiodes striatus* (Spix, 1824) and reported that the number of the oviductal ova/embryo varies between three and eleven. They also found a significant relationship between SVL and fecundity. In our study except an individual from Eastern Anatolia, all specimens were caught between April and June and all females have eggs with miscellaneous dimensions and no embryos were examined.

Although very few juveniles were detected during the fieldworks in the current study, no nest or newborn was found. Kukushkin et al. (2013) reported that pregnant females of *P. apodus* have a low occurrence in nature which could be as a result of the protection of clutches. They also stated that newborns of *P. apodus* are hard to see because of having a cryptic life, low number and high mortality rates. A natural nest belonging to the *P. apodus*, where the eggs are protected by the female, was discovered in July (Milto 2010).

We gave information on the number of eggs with miscellaneous dimensions in two Anguid lizards; *A. fragilis* complex and *P. apodus*. The reproductive ecology of these species still lack of information and cannot state exact truth as it mentioned Kukushkin et al. (2013). Thus more comprehensive both in vivo and in vitro studies are needed.

Acknowledgement. This study was a part of a PhD thesis of Batuhan Y. YAKIN and was supported by the Çanakkale Onsekiz Mart University Scientific Research Projects Commission (FDK-2018-1137). The specimens used in this study were collected for a TÜBİTAK project-TBAG-108T559.

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