Reproductive cycle of the Snake-eyed Lizard Ophisops elegans MÉNÉTRIÉS, 1832 in western Iran (Squamata: Sauria: Lacertidae)

Fortpflanzungszyklus des Schlangenauges Ophisops elegans MÉNÉTRIÉS, 1832 im Iran (Squamata: Sauria: Lacertidae)

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KURZFASSUNG

Die vorliegende Arbeit untersucht die Fortpflanzung des Schlangenauges *Ophisops elegans* MÉNÉTRIÉS, 1832 aus dem Zagros-Gebirge im Westiran. Im Jahreslauf gewonnene männliche und weibliche Fortpflanzungsorgane wurden hinsichtlich ihrer Größe vermessen und histologisch befundet. Die Ergebnisse zeigen einen testikulären Aktivitätszyklus, an dem drei Phasen unterscheidbar sind: aktive Phase, Übergangs- und Ruhephase. Auch am weiblichen Reproduktionszyklus lassen sich drei Phasen unterscheiden: prä-aktive, aktive und inaktive Phase. Während der aktiven Phasen findet im männlichen Geschlecht die Spermatogenese statt, Spermatozoen finden sich in den Gängen von Testis und Epididymis; bei Weibchen sind die Beschalungsdrüsen im Ovidukt aktiv. Wie für gemäßigte Kimazonen mit ausgeprägten Jahreszeiten zu erwarten, ist der Fortpflanzungszyklus von *O. elegans* saisonal, in seinem Ablauf an die Jahreszeiten gekoppelt.

ABSTRACT

Reproduction was studied in male and female Snake-eyed Lizards *Ophisops elegans* MÉNÉTRIÉS, 1832 from the Zagros Mountains in western Iran. For this purpose testis, epididymis and oviduct were subject to metric and histological analyses. The results show that the testicular cycle can be subdivided into three phases: active, transitional and resting phase. The female reproduction cycle allows for distinction between three phases as well: preactive, active and inactive phase. In the active phases, spermatogenesis occurred and spermatozoa were found in both testis and epididymis, while shell glands were active in the oviduct Typical for temperate climate zones with their pronounced seasons, the reproductive cycle of *O. elegans* is of the seasonal type, and strongly correlates with the time of the year.

KEY WORDS

Squamata: Sauria: Lacertidae: *Ophisops elegans*, reproduction, ecology, reproductive biology, reproductive cycle, testicular cycle, oviduct epithelial cycle, histology, Iran, Lorestan, Zagros Mountains

INTRODUCTION

Lizards present two general types of reproductive cycle: aseasonal (reproduction and gametogenesis occur all over the year) and seasonal (reproduction and gametogenesis are restricted to certain periods of the year). Aseasonal or continuous reproductive cycles are typical to (aseasonal) tropical habitats where mating and spermatogenesis occur almost all over the year (FITCH 1970, 1982; SHERBROOKE 1975; SOMMA & BROOKS 1976; JENSSEN & NUNEZ 1994; VIAL & STEW-ART 1985; VIEIRA et al. 2001; HERNANDEZ-GALLEGOS et al. 2002). Aseasonal reproduction is limited to the Inter Tropical Convergence Zone (ITCZ) while seasonal reproduction occurs outside the ITCZ (CHURCH

1962; GORMAN & LICHT 1975; VITT & GOLD-BERG 1983; TORKI 2006). In regions close to the ITCZ aseasonality is weakly expressed (VENCES et al. 2004). In seasonal tropical regions as well as in both warm and cool temperate regions the reproductive cycle is seasonal (DANIEL 1960; HEIDEMAN 1995; AL-JOHANY et al. 1996), and the reproductive activity in both males and females is related with the seasons.

Seasonal reproductive cycles may be subdivided into two well defined types: dissociated, and associated. In the dissociated reproductive type spermatogenesis and mating are synchronized but fertilization occurs later at a suitable time independently from





Fig. 1: Monthly average of maximum (Tmax) and minimum (Tmin) temperature (°C) and rainfall (mm) across the year in the study area (Zagros Mountains, northern Lorestan, Iran). From late November to February Tmin is below zero and snowfall is considerable. Rainfall during spring is higher than in autumn, rainfall in December is high, in contrast to January when precipitation is mainly present in the form of snow.

Abb. 1: Monatsmittel der maximalen (Tmax) und minimalen Temperaturen (Tmin) sowie der Regenmenge (mm) im Jahreslauf des Untersuchungsgebietes (Zagros Gebirge, nördliches Lorestan, Iran). Von Ende November bis Februar ist Tmin < 0 °C und der Schneefall beträchtlich. Die Regenfälle sind im Frühjahr ausgiebiger als im Herbst, im Dezember fällt viel Regen, anders als im Jänner, wo der Niederschlag hauptsächlich als Schnee fällt.

Figs. 3a (testis), 3b (epididymis) and 5a, 5b (oviduct) of *Ophisops elegans* Ménétriés, 1832 from the Zagros Mountains, northern Lorestan, Iran (opposite page).

Fig. 3a: Cross section of testis. Active reproductive phase with spermatozoa in the lumen of the seminiferous tubules. Sg - spermatogonia, PS - primary spermatocytes, SS - secondary spermatocytes, St - spermatids, Sz - spermatozoa; LS - diameter of tubulus seminiferus; GS - thickness of germinative layer of the wall of the tubulus seminiferus; IT - Interstitial tissue.

Abb. 3a: Testis-Querschnitt. Fortpflanzungsaktive Phase mit Spermatozoen im Lumen der Tubuli seminiferi.
Sg - Spermatogonien, PS - Primäre Spermatozyten, SS - Sekundäre Spermatocyten, St - Spermatiden,
Sz - Spermatozoa; LS - Durchmesser des Tubulus seminiferus; GS - Dicke des Stratum germinativum der Wand des Tubulus seminiferus; IT - Interstitielles Gewebe.

Fig. 3b: Cross section of epididymis. Active reproductive phase with spermatozoa in the lumen of the epididymis duct (LE). Sz - spermatozoa; EL - epithelium layer; AM - amorphous materials; CT - connective tissue.
Abb. 3b: Epididymis-Querschnitt. Fortpflanzungsaktive Phase mit Spermatozoen im Lumen des Ductus epididymidis (LE). Sz - Spermatozoen; EL - Epithelschicht; AM - amorphes Material; CT - Bindegewebe.

Fig. 5a: Cross section of oviduct. Active reproductive phase (April) after mating and before oviposition. G1 - G4 - shell glands; OT - oviduct wall thickness; CT - connective tissue; OS - oviduct septum.

Abb. 5a: Ovidukt-Querschnitt. Fortpflanzungsaktive Phase (April) nach der Paarung und vor der Eiablage. G1 - G4 - Beschalungsdrüsen; OT - Dicke der Oviduktwand; CT - Bindegewebe; OS - Ovidukt-Septum.

Fig. 5b: Cross section of the oviduct during its inactive phase (August) after oviposition with oviduct histological structures degraded. OT – oviduct wall thickness; CT - connective tissue.

Fig. 5b: Ovidukt-Querschnitt während seiner inaktiven Phase (August) nach der Eiablage. Die Gewebestrukturen des Oviduktes sind desintegriert. OT – Dicke der Oviduktwand; CT - Bindegewebe.

Abb. 3a (Testis, Hoden), 3b (Epididymis, Nebenhoden) und 5a, 5b (Ovidukt, Eileiter) von *Ophisops elegans* MÉNÉTRIÉS, 1832 aus dem Zagros Gebirge, nördliches Lorestan, Iran (gegenüberliegende Seite).

Reproductive cycle of Ophisops elegans MÉNÉTRIÉS, 1832 in western Iran



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Table 1: Descriptive statistics of histological and macroscopic measurements of male and female specimens of *Ophisops elegans* MÉNÉTRIÉS, 1832 (Zagros Mountains, northern Lorestan, Iran) by month from March to October. Number of Eggs – Number of mature eggs per oviduct. SEM – Standard Error of the Mean.

Tab 1: Beschreibende Statistiken histologischer und makroskopischer Messungen an männlichen und weiblichen *Ophisops elegans* MÉNÉTRIÉS, 1832 (Zagros Gebirge, nördliches Lorestan, Iran) für die Monate März bis Oktober. Eizahl – Anzahl reifer Eier in jedem Ovidukt. N / Mean / SEM – Stichprobengröße / Mittelwert / Standardfehler des Mittelwertes.

Month Monat	N Mean SEM	SVL (mm)	TV (mm ³)	GS (µm)	LS (µm)	LE (µm)	OT (µm)	Number of Eggs Eizahl
Mar	N	18	12	9	9	9	4	4
	Mean	45.09	21.15	75.55	79.88	328.66	86.25	0
	SEM	1.07	0.01	1.27	1.61	2.43	5.54	0
Apr	N -	18	15	10	10	10	3	3
	Mean	41.68	15.81	78.50	63.60	329.60	102.67	1.33
	SEM	0.69	0.07	1.50	4.59	3.27	9.33	1.33
May	N	15	13	10	10	10	2	2
	Mean	44.23	13.36	68.00	45.60	135.80	105.00	5
	SEM	0.92	0.07	2.54	2.47	8.14	5	1
Jun	N	19	16	9	9	9	3	3
	Mean	40.23	7.14	24.22	39.22	84.44	106.67	5.33
	SEM	0.73	0.09	0.98	1.30	2.47	8.81	0.66
Jul	N	18	14	10	10	10	3	3
	Mean	42.36	4.64	23.20	32.00	75.20	115.67	5.33
	SEM	0.75	0.10	1.21	0.97	3.56	2.33	0.66
Aug	N	17	13	8	8	8	4	4
	Mean	45.69	4.27	12.50	7.50	18.50	67.50	1.50
	SEM	1.13	0.10	2.06	0.90	1.05	5.95	1.50
Sep	N	15	12	10	10	10	3	3
	Mean	43.22	4.08	6.20	1.10	19.20	38.33	0
	SEM	0.90	0.09	0.24	0.48	0.99	4.41	0
Oct	N	18	13	9	9	9	3	3
	Mean	46.64	3.81	6.33	0.00	19.11	20.00	0
	SEM	1.02	0.04	0.33	0.00	0.88	2.88	0

mating. In this type where oogenesis is not synchronized with spermatogenesis, sperm is stored in the female reproductive system and several months or years may pass between mating and fertilization/oviposition/ hatching (CONNER & CREWS 1980; POUGH et al. 1998; Sever & HAMLETT 2002; YAMA-MOTO & OTA 2006). Dissociated reproductive cycles are common in temperate zones; the mating period is brief and sperm storage takes place in the female reproductive ducts until fertilization (MÉNDEZ DE LA CRUZ et al. 1988; VAN WYK 1995; SEVER & HAMLETT, 2002; SEVER & HOPKINS 2004). In the associated reproductive type spermatogenesis, and oogenesis as well as mating and fertilization are synchronic or closely time-related (WAPSTRA & SWAIN 2001; TAYLOR 2004), and oviposition and hatching occur soon after mating. This latter type is common in species inhabiting predictable environments

in the temperate zone (DIAZ et al. 1994; HUANG 1997; WAPSTRA & SWAIN 2001; TAYLOR 2004; TORKI 2005) but is also known from seasonal tropical regions (e.g., CENSKY 1995). Spermatogenesis of the agamas *Trapelus lessonae* (DE FILIPPI, 1865), *Laudakia nupta* (DE FILIPPI, 1843), and the gecko *Tropiocolotes helenae* NIKOLSKY, 1907, are being described (TORKI 2006, in press A, B, C) but the reproductive cycle of other lizards in the Iranian plateau has not been detailed so far.

The purpose of this study is to describe the reproductive cycle and the timing of the reproduction activity in *Ophisops elegans* MÉNÉTRIÉS, 1832 from the western Iranian plateau. *Ophisops elegans* was chosen because this species is present all over the Mid-Zagros area and its population density is high (e.g., ANDERSON 1999).

MATERIALS AND METHODS

The author collected 138 specimens (108 males and 30 females) of O. elegans by hand from August 2002 to July 2005. The study site is in the north of Lorestan province at 34° 05'N, 47°55'E, up to 2000 m a.s.l. This location is rocky, the vegetation is dominated by Astragalus sp. Average annual rainfall is 395 mm, including some snowfall in winter. Average annual maximum temperature is 19.4°C, average annual minimum is 5.51°C, the climate is cold temperate (Fig. 1). Due to the cold climate conditions all herpetofauna is hibernating from late October to late February and no specimens were collected during this time (TORKI 2005). The size of O. elegans (males and females) was 38.9 < SVL < 47.2 mm. Individuals with SVL longer or equal to 40 mm were considered adult as this was the SVL of the smallest lizard presenting spermatozoa in the testis. The left testis or ovary of all individuals was removed by dissection. In each lizard maximum length and width of the left testis was measured by vernier callipers to the nearest 0.1 mm, and the Testis Volume (TV, mm³) was estimated using the ellipsoid formula $v = 4abc\pi/3$, where v is the volume, and a, b and c represent half testicular height, width and length (e.g., TORKI 2006). For histological analysis, testes and oviducts were fixed in 3.7% formalin, dehydrated in a graded series of ethanol, cleared in xylol, and embedded in paraffin. Histological sections $(5-7 \mu m)$ were stained in hematoxylin with eosin counterstain. Observations and photographs were made with an Axiophoto[®] Zeiss[®] microscope. Sections of testes and oviducts were examined. For this study the following parameter states were measured: Diameter of the lumen of the seminiferous tubules (LS), thickness of the germinative layer of the seminiferous tubules (GS), diameter of the lumen of the epididymis duct (LE), oviduct wall thickness (OT) and shell gland density (number of shell glands arranged along an imaginary line through the oviduct wall). Tukey HSD test (post-ANOVA pairwise comparisons) to find significant differences, and Canonical Discriminant Function Analysis (DFA) to determine different phases of the gonadal cycle as defined in the present paper and other studies of the author (TORKI 2006, in press A, B, C) were applied. For determination of the juvenile status in the field and the hatching time, a series of 500 specimens collected during three years was measured for their SVL, FL (Femur length), and HL (Head Length); all of these animals were released after measurement.

Since 33 male and 5 female specimens were destroyed during the histological procedures, only 75 male and 25 female specimens were used for histological purposes. Testis volume (TV) measurements were available from all 108 male specimens.

RESULTS

Male reproduction

Descriptive statistics of four testicular parameters are shown in Table 1. From March to October testis volume (TV) decreased by 82% and the thickness of the germinative layer of the seminiferous tubules (GS) by 88%. Based on DFA and Tukey HSD tests (α = 0.01), in *O. elegans* the testicular cycle outside the hibernation period can be subdivided into three phases (Figs. 2 and 6): (I) active phase, in late winter and spring – from March to May, (II) transitional phase, during June-July, and (III) resting phase, from August to October. During the active phase spermatozoa were present in the lumen of both seminiferous tubules and epididymis (Fig. 3), but not so during the resting phase. During the transitional phase spermatozoa were found in the lumen of the seminiferous tubules of many specimens while spermatocytes were never found in the epididymis.

Female reproduction

Descriptive statistics of oviduct histology, diameter and number of mature eggs outside the hibernation period are shown in 62

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Variablen 'Testis-Volumen' (TV), 'Lumendurchmesser des Ductus epididymidis'(LE), 'Lumendurchmesser der Tubuli seminiferi' (LS), 'Dicke des Stratum germinativum der Tubuli seminiferi' (GS) (gruppierende Variable = Fangmonat) zur Darstellung des testikulären Zyklus in der Phase der biologischen Aktivität bei reifen Ophisops elegans MENETRIÉS, 1832 (Zagros Gebirge, nördliches Lorestan, Iran). Die Männchen aus den Fangmonaten (3+4+5), (6+7) und (8+9+10) bildeten drei Cluster.

Table 1. Oviduct wall thickness increased from March to July and decreased towards October; maximum thickness was found in July. Based on Tukey HSD test ($\alpha = 0.01$) and DFA (Fig. 4), oviduct histological studies revealed the sequence of three phases (Fig. 6): (I) pre-active phase – from March to April, (II) active phase - from May to July, and (III) resting phase – from August to October. In phases I and III the thickness of the oviduct wall tissue is smaller than in phase II (Fig. 5). In phases I and II shell glands are found which are bigger in phase II than in phase I; in phase III oviduct tissue lacks shell glands. An oviduct septum is found in phases I and II (Fig. 5a), but not in phase III (Fig. 5b). From April to August, especially from May to July, mature eggs are present in the oviducts (Table 1), but not in



Fig. 4: Canonical Discriminant Functions Analysis of the variables 'oviduct wall thickness' and 'shell gland density' (grouping variable = month of capture) describing the oviductal cycle during biological activity in mature *Ophisops elegans* MÉNÉTRIÉS, 1832 (Zagros Mountains, northern Lorestan, Iran). Three clusters were formed by the females caught in the months (3+4) (5+6+7) and (8+9+10).

Abb. 4: Kanonische Diskriminanzanalyse der Variablen 'Dicke der Oviduktwand' und 'Dichte der Schalendrüsen' (gruppierende Variable = Fangmonat) zur Darstellung der zyklischen Veränderungen des Oviduktes in der Phase der biologischen Aktivität bei reifen *Ophisops elegans* MÉNETRIÉs, 1832 (Zagros Gebirge, nördliches Lorestan, Iran). Die Weibchen aus den Fangmonaten (3+4), (5+6+7) und (8+9+10) bildeten drei Cluster.

March and from September to October and not always in April. Many female specimens had twelve (6+6) or ten (5+5) eggs in their oviducts, others had eight (4+4). In March, follicles were found in the ovaries and from April to May (especially in late May) mature eggs were present in all female specimens. In summer, the ovaries were devoid of mature eggs but showed follicles. From June to August mature eggs were found again in many but not all female specimens (Table 1).

Hatching time

For determination of the hatching time and the maturation-status (juvenile of the current year or older), the measurements of SVL, FL, and HL taken from more than 500 Reproductive cycle of Ophisops elegans MÉNÉTRIÉS, 1832 in western Iran

specimens in the field were analyzed. Tukey HSD test ($\alpha = 0.05$) revealed the absence of significantly different size classes in individuals caught from March to July and the presence of two significantly different size classes among those caught from July to November, viz juveniles of the current year and older specimens. Juveniles of the current year are less than 27 mm long in SVL and can be found from July to November; in older specimens SVL is more than 37 mm and they are found during the whole activity period from March to October. In June four eggs of *O. elegans* were found under less than 5 cm of soil and six eggs in a nest, approximately 10 cm deep. As observed from several female specimens kept in the lab during oviposition, the egg shell of *O. elegans* is soft, not calcareous.

DISCUSSION

Male reproduction

Spermatogenesis of lizards from temperate regions passes through two periods: degeneration (during biological activity), and regeneration (during hibernation) (TORKI 2006, in press A, B, C). In male *Ophisops elegans* the degeneration period is subdivided into three phases: active, transitional and resting phase, as in another species of the Zagros Mountains, *Trapelus lessonae* (TORKI 2006). In *Laudakia nupta*



Fig. 6: Male and female reproductive cycles in *Ophisops elegans* MÉNÉTRIÉS, 1832 from the Zagros Mountains, northern Lorestan, Iran. The external ring shows the two periods (Re- and Degeneration) of the cycle. On the right side, the intermediate ring shows three phases (Active, Transitional and Resting) of male reproduction during biological activity, the internal circle shows three phases (Pre-Active, Active and Inactive) of female reproduction during biological activity. Hibernation which parallels the regeneration period and is synchronic between the sexes (November to February) is shown in the left of the figure.

Abb. 6: Männliche und weibliche Fortpflanzungszyklen bei Ophisops elegans MÉNÉTRIÉS, 1832 aus dem Zagros Gebirge, nördliches Lorestan, Iran. Der äußere Ring zeigt die beiden Perioden - Regeneration und Degeneration. Der mittlere Ring der rechten Seite beinhaltet die drei Phasen (aktive, Übergangs- und Ruhephase) des männlichen, der innere Ring die drei Phasen (prä-aktive, aktive und inaktive Phase) des weiblichen Fortpflanzungszyklus in der biologisch aktiven Zeit. Die Überwinterungsperiode auf der Inken Abbildungsseite fällt mit der Regenerationsperiode zusammen und dauert bei beiden Geschlechtern von November bis Februar.

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which occurs in the same area, spermatogenesis during biological activity consists of three phases; pre-active (spermatogenesis is observed, spermiogenesis is not), active and resting phase (TORKI in press B). In Tropiocolotes helenae the annual cycle of spermatogenesis passes through five phases, resting, pre-active, active, transitional and inactive (from its histology but not from its timing equalling the resting phase) phase. After hibernation, sperm production started in the seminiferous tubules of the West Iranian O. elegans just as in Trapelus lessonae and Tropiocolotes helenae (TORKI 2006) and other lizards of the temperate region (e.g. WAPSTRA & SWAIN 2001; TAYLOR 2004). In contrast, sperm production in Laudakia nupta occurred later in the post-hibernation phase during late spring and early in the summer (TORKI in press B). In L. nupta this phase is what I call the preactive phase during which spermatogenesis is active but spermiogenesis does not occur (TORKI in press C). Further studies show that in Trapelus lessonae a similar phase which I call activation phase occurs during the late hibernation period (TORKI in press C). The same may apply to O. elegans, because spermatogenesis timing in these two species is similar as are the climate conditions and other ecological factors (see TORKI 2006, in press C). The duration of the spermatogenesis activity is remarkably different among the lizard species of the Zagros Mountains: in L. nupta less than two months (TORKI in press B), in T. lessonae and O. elegans approximately five months, and finally in T. helenae nearly six months (TORKI unpubl.).

Body size is biggest in *L. nupta*, *T. helenae* is smallest, while *T. lessonae* and *O. elegans* are of intermediate size. Duration of spermatogenesis activity may be related with body size (TORKI & RASTEGAR-POUYANI 2006), or phylogenetic relationship (DUNHAM & MILES 1985) rather than climate conditions and other ecological factors that are similar for all species. In summary spermatogenesis was observed in the posthibernation phase (when lizards left their hibernacula) and did not occur in the prehibernation phase (when lizards went to their hibernacula) (TORKI 2006, in press A, B, C).

Female reproduction

Based on the histological status, the oviduct epithelial cycle of *O. elegans* underwent three phases: (I) pre-active phase (pre-oviposition phase, post-hibernation phase – oviductal eggs and shell glands smaller than in phase II), (II) active phase (oviposition phase – shell glands active and at their maximum size), and (III) inactive phase (post-oviposition phase, pre-hibernation phase). In the active phase (II), mating and fertilization occurred followed by egg growth, and oviposition.

Hatching time

Based on SVL data, hatching started in July. This holds true also for *Trapelus lessonae* (TORKI 2006). In contrast, some geckos inhabiting the costal Persian Gulf hatch more or less all over the year (TORKI 2007). The biological activity of juvenile specimens lasted until November, of mature specimens only until October. Juveniles may utilize this extra time of surface activity for growth and storage of fat reserves. This is also assumed for other lizards of the Zagros Mountains (TORKI 2007). In summary, the hibernation period in juvenile specimens is shorter than in adults.

Reproduction timing

The reproductive cycle of O. elegans is seasonal and associated (Fig. 6), like in Trapelus lessonae, Laudakia nupta, and Tropiocolotes helenae on the western Iranian plateau (TORKI 2006, in press B), and most other lizards of the temperate region (e.g., TAYLOR 2004), as spermatogenesis, oogenesis, mating and fertilization are synchronized and oviposition and hatching occur soon thereafter. In many lizards especially geckos of southern Iran at the costal Persian Gulf, reproduction is more or less aseasonal (TORKI 2007). In this region spermatogenesis activity as well as eggs are found all over the year. Lizard reproductive timing in southern Iran is different from that in western Iran but resembles that in arid south-western Madagascar (VENCES et al 2004). Climate conditions in arid, southwest Madagascar and along the costal

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Persian Gulf are fairly similar. Differences and similarites in reproductive timing can be explained by climate conditions (e.g., VENCES et al. 2004; TORKI 2006). Reproductive activity in tropical Madagascar is related to the rainy season (GLAW & VENCES 1996), in contrast, reproductive activity in the temperate region of western Iran is related to the temperature of the environment (e.g., TORKI 2006, in press A, C). For instance, male reproductive activity in lizards started earlier in southern than central Zagros (TORKI in press A).

During the hibernation period, the reproductive system in male and female

lizards is regenerated (hibernation period = regeneration period in Fig. 6). That fat body resources play an important role in this process (WAPSTRA & SWAIN 2001; TORKI 2005, 2006) applies to all studied lizards of the Zagros Mountains, in the western Iranian plateau (TORKI 2005, 2006, 2007, in press A, B, C).

Based on my observations during long-term studies of the Zagros Mountains herpetfauna from 2000 (TORKI & GHARZI 2001) up to the present, mating of *O. elegans* occurred exclusively during the active phase from March through May.

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