

# REPRODUCTION AND GROWTH OF *XEROLENTA OBVIA* (MENKE, 1828) (GASTROPODA: EUPULMONATA: GEOMITRIDAE) IN LABORATORY CONDITIONS

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**ABSTRACT:** Laboratory observations made it possible to ascertain some life cycle parameters of *Xerolenta obvia*. The eggs are laid in clutches of 15–65 (mean 32) or rarely singly; they are partly calcified, milky white, oval or almost spherical, with a mean size of 1.37 × 1.46 mm; time to hatching is 11 to 28 days (mean 18); hatchlings have shells of 1.5–2.0 whorls (mean 1.7); hatching success is ca. 57%. Sexual maturity (first egg) is reached by snails at 4.25–5.2 whorls (mean 4.85) within 290–660 days. The average life span is two years. Neither uniparental reproduction nor egg or sibling cannibalism was observed. The life-history traits of *X. obvia* are compared with some xerophilous species of similar size.

**KEY WORDS:** Geomitridae; land snails; life history; growth rate; xerophilous snails

## INTRODUCTION

*Xerolenta obvia* (syn. *Helicella obvia*) originates from south-eastern Europe (WIKTOR 2004, WELTER-SCHULTES 2012), from where it was introduced into Central and Western Europe. It has since extended its range further (URBAŃSKI 1957, WOJTAŚ & WOJTAŚ-STOKŁOSA 2001). The snail dispersed mainly along railway embankments and rivers (RIEDEL 1988). Now its distribution extends from Asia Minor to the eastern and central Balkans, N. Italy, Carpathian countries (in the east to Moldova and N. Ukraine) and to the Baltic Sea (WELTER-SCHULTES 2012). In Central Europe it inhabits Poland, the Czech Republic, Slovakia, Hungary, Switzerland, Austria and Germany, reaching SE. France (KERNEY et al. 1983). In Poland *X. obvia* occurs in almost the whole country, occupying mainly lowlands (WIKTOR 2004), while in Bulgaria and the Alps it reaches altitudes up to 2,000 m a.s.l. (WELTER-SCHULTES 2012). Outside Europe it was introduced to Canada (in 1969 and

1972 to Bethany, Ontario) and the United States (by 2002 to Wayne County, Michigan and by 2012 to Cascade County, Montana) (GRIMM & WIGGINS 1975, FORSYTH et al. 2015, BIRDSALL et al. 2019).

The systematic position of *X. obvia* has changed. It was included in the Helicidae Rafinesque, 1815 (KERNEY et al. 1983) or the Hygromiidae Tryon, 1866 (BOUCHET & ROCROI 2005, SCHILEYKO 2006). Now it is classified in the Geomitridae C. Boettger, 1909 (RAZKIN et al. 2015), whose members mostly occur in xeric habitats. The information on geomitrid life cycles and/or seasonal dynamics has been reported in several studies (e.g. LAZARIDOU-DIMITRIADOU 1981, STAIKOU & LAZARIDOU-DIMITRIADOU 1991, HÄNSEL et al. 1999, KISS et al. 2005, BAKER 2012). The information on the biology of *X. obvia* includes field observations on climate-dependent variations in life histories in the Mediterranean and Central Europe (LAZARIDOU-DIMITRIADOU 1995,

LAZARIDOU & CHATZIOANNOU 2005, MARZEC et al. 2020); there is also a study further east in Belarus (ZEMOGLYADCHUK 2019). Some fragmentary data are also provided by FRÖMMING (1954) and BIRDSALL et al. (2019).

## MATERIAL AND METHODS

The source material for the laboratory culture of *X. obvia* (131 individuals of different ages, i.e. 1.5–3.5 whorls) was collected on two occasions: 55 snails kept in groups and 15 snails kept singly, collected as 128 eggs on the 17th of October 2015; and 50 snails kept in groups and 11 snails kept singly, collected as juveniles (mean number of whorls: 2.96; range 2.25–3.5; SD = 0.25; n = 61) on the 18th of June 2016. Additionally, a group of 50 snails (2.0–4.0 whorls) were kept in a glass vivarium since October 4th, 2017. All the snails and eggs originated from the village of Piotrkowiczki (SW. Poland, 51°16.12'N, 17°02.12'E; 190 m a.s.l.).

To check the possibility of uniparental reproduction, 26 individuals were kept singly from their earliest juvenile stages until death. The remaining 105 snails were kept in pairs (10 pairs) and groups: 2 groups of 3 individuals, 5 groups of 5 individuals, 3 groups of 8 individuals, 3 groups of 10 individuals.

The snails were kept in plastic containers of different sizes depending on the number of individuals (6×7.5×5 cm for singly kept snails and pairs; 11.5×7×7 cm for groups of 3 individuals; 15.5×12.5×5 cm for groups of 5 individuals; 15.5×12.5×7 cm for groups of 8 and 10 individuals). Eggs and hatchlings were kept in separate Petri dishes (5–10 cm diameter) lined with damp tissue paper and moist soil. The substratum of the containers consisted of a layer of damp tissue paper with a thin layer of gardening soil (natural, with no supplements) on top, which was replaced with sandy gravel after October 4th, 2017. The gravel was brought from the natural habitat and sterilized at 70 °C for 72 h. The containers were aired and the substratum was changed at least once a week. Water and food (iceberg lettuce) were supplied as needed. Dolomite tablets constituted the source of calcium. The dishes

This paper presents the results of laboratory observations on the life cycle of the species and compares the results with those obtained by other authors and/or from other regions for some European xerophilous species of similar size.

es and containers with snails were kept in a climate chamber in regular conditions of temperature (day 22 °C, night 18 °C) and humidity (80%), with a 12:12 h lighting regime (fluorescent lighting). The glass vivarium (30×30×45 cm) was placed in the laboratory room, where the temperature ranged from 17 °C in winter to 28 °C in summer, and a humidity of ca. 80% was maintained by regularly wetting the tissue paper. In these conditions one fragmentary copulation and six egg clutches were recorded.

The observations in a climate chamber were to investigate the capability/incapability of uniparental reproduction (n = 26), egg laying, clutch size, time to hatching, hatching success, growth rate (105 snails kept in groups vs 26 snails kept singly), maturation (i.e. until the first egg laying was observed, n = 5), life span (period from hatching to death of snails with at least 4.8 whorls, 884 days, n = 12) and possible cannibalism (n = 45). Survivorship was calculated based on Kaplan-Meier estimate.

The growth rate of individuals was assessed through counting whorls at 30-day intervals using EHRMANN's method (1933). To ascertain the clutch size and hatching success, likely egg-laying places were searched at least once a week and during periods of intense observations (e.g. reproductive period) every day or two, the eggs and hatchlings were counted, and the time to hatching recorded. A sample of eggs (n = 100) were measured with a calibrated eyepiece to the nearest 0.025 mm. The measurements included 60 eggs laid by snails during their collection in the field and transport to the laboratory as well as two clutches of 20 eggs each laid by snails in the laboratory on December 18th, 2017 and March 9th, 2018. To test cannibalism, hatchlings (test 1) and adults (test 2) were offered conspecific eggs at different stages of development.

## RESULTS

### COPULATION, EGG LAYING, TIME TO HATCHING AND HATCHLINGS

The fragmentary copulation (its last stage: retracting the copulatory organs) was observed in the laboratory on September 16th, 2017 and February 23rd, 2018, and in the field on October 4th, 2017

(Fig. 1). During copulation the snails were in a horizontal position, attached to each other side-to-side. Copulations and sperm transfer were reciprocal. After the copulations, each partner was placed in a separate container in the climatic chamber. Only after the mating in September, one of the snails laid 30 eggs in a single clutch 16 days later. Of these, seven



Fig. 1. Mating of *X. obvia* in the field (4 Oct 2017, photo E. KUŹNIK-KOWALSKA)

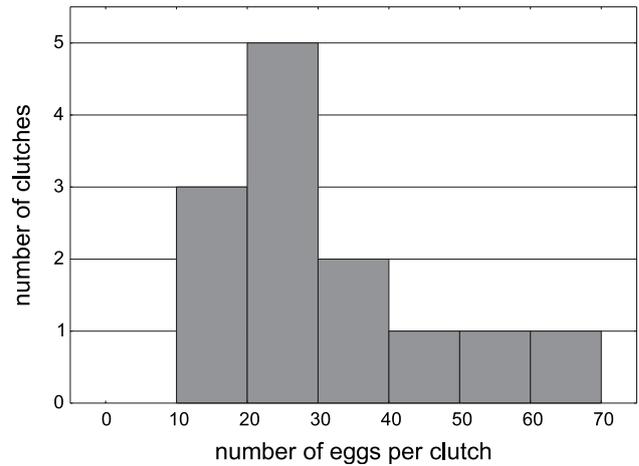
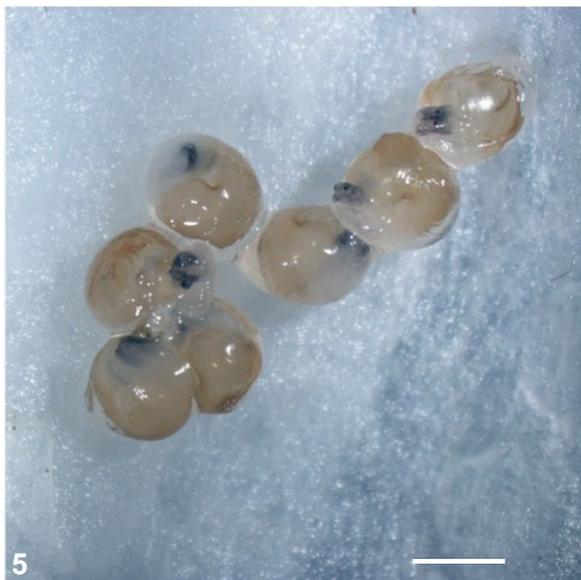
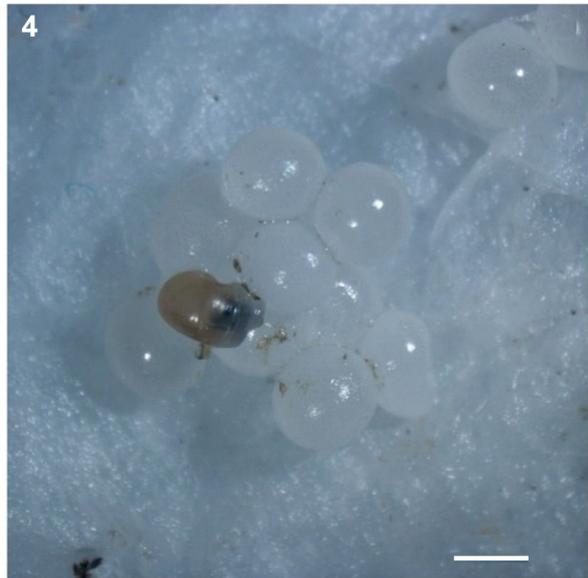
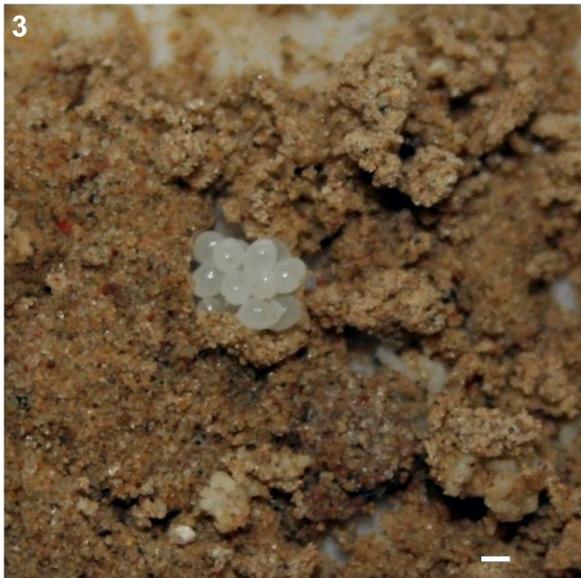


Fig. 2. Frequency distribution of number of eggs per clutch in *X. obvia*



Figs 3–6. *X. obvia*: freshly laid eggs (3), juvenile during asynchronous hatching (4), nearly hatched juveniles in semi-transparent envelope (5), and five-day hatchlings (6) (photos E. KUŹNIK-KOWALSKA). Scale bars 1 mm

eggs hatched and five hatchlings survived until the end of the study. The other snails observed copulating did not lay eggs.

None of the 26 snails kept singly laid eggs whereas 13 out of the 155 snails kept in groups did lay eggs. The difference in proportion was not statistically significant (Fisher's exact test  $p = 0.2$ ). Eggs were laid mainly in autumn (October 2016,  $n = 28$ ; October 2017,  $n = 290$ ), then in winter (December–January 2017,  $n = 60$ ) and spring (March–April 2017,  $n = 45$ ). Of the total of 423 eggs, 6 were laid singly and the rest in 13 clutches. The mean clutch size was 32 eggs (range 15–65;  $SD = 14.8$ ;  $n = 13$ ) (Fig. 2). The eggs were laid mainly in holes dug in the sandy gravel, but also directly on the tissue paper. Newly laid eggs were partly calcified, milky white, and oval or almost spherical. Initially they were shiny as a result of the covering mucus layer; then they became cream-coloured and matt, and finally light brown, semi-translucent, so that the young snail could be seen (Figs 3–6). The mean egg width was 1.37 mm (range 1.2–1.6 mm;  $SD = 0.11$  mm;  $n = 100$ ), while the mean egg length was 1.46 (range 1.2–1.8 mm;  $SD = 0.12$ ;  $n = 100$ ).

The first egg-laying (sexual maturity) was directly observed in snails with 4.25–5.2 whorls (mean 4.91;  $SD = 0.38$ ;  $n = 5$ ). These sizes were reached 290–660 days after hatching (mean 448.6;  $SD = 158.35$ ;  $n = 5$ ). The smallest egg-laying snail (4.25 whorls) laid 28 eggs, which did not hatch. This snail kept growing for the next five months to reach 4.5 whorls. The juveniles hatched from eggs laid by one snail with 5.0 whorls, which grew to 5.1 whorls within five months, and by three other snails (with 5.0, 5.1 and 5.2 whorls), which did not grow further.

The time to hatching ranged from 11 to 28 days (mean 18;  $SD = 8.04$ ;  $n = 100$ ). The hatching was asynchronous; juveniles of the same clutch hatched over 1–3 days. The newly hatched juveniles had translucent shells and bodies; their shells had 1.5–2.0 whorls (mean 1.7;  $SD = 0.11$ ;  $n = 100$ ). The hatching success was 57% (variation between clutches 24–100%;  $SD = 22.6\%$ ;  $n = 423$  eggs;  $n = 13$  clutches). Out of six single eggs, five (83%) hatched.

No egg cannibalism was observed among juveniles or adults, even in the absence of other food; likewise, no juvenile-juvenile and adult-juvenile cannibalism occurred.

## GROWTH, SURVIVORSHIP AND LIFE SPAN

The growth rate of individuals of *X. obvia* kept in isolation and in groups was similar (Fig. 7), suggesting that it did not depend on the density. The proportional growth rate was more or less consistent throughout whole growth unless there occurred an unexpected acceleration in snails between ca. 3.5 and 4.5 whorls. The juvenile snails suffered very high mortality in the laboratory culture. Among 131 young snails observed, 13 survived 884 days, i.e. to the end of the laboratory culture (10%). 25% of all juveniles died during 130.5 days, 50% snails survived 300 days and 25% survived longer than 582 days (Fig. 8). The life span of *X. obvia* ranged from 655 days (ca. 22 months) to 884 days (ca. 30 months) (mean = 772;  $SD = 69$ ;  $n = 12$ ).

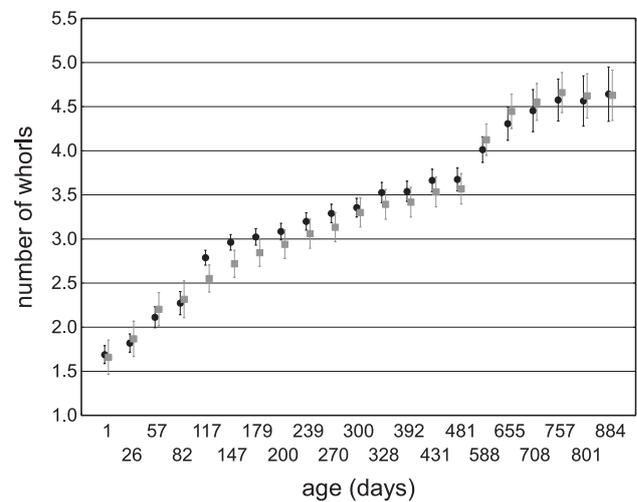


Fig. 7. Mean growth of *X. obvia* individuals kept in groups (circle,  $n = 105$ ) and singly (square,  $n = 26$ ). The error bars represent 95% confidence intervals

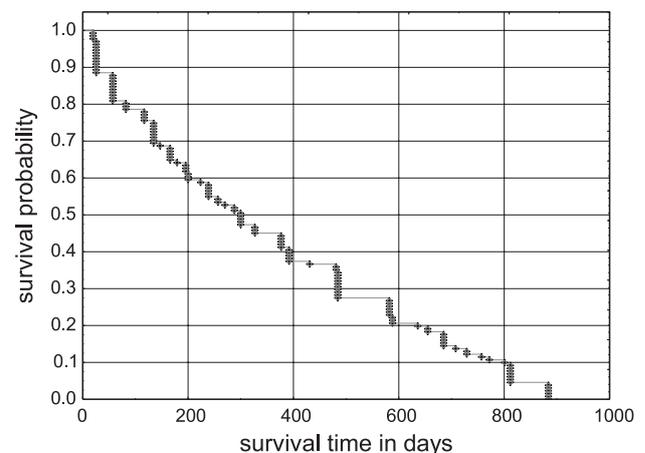


Fig. 8. Survivorship function of 131 *X. obvia* individuals



## DISCUSSION

The knowledge of pulmonate life cycles is still very scanty, and varies much among the various families (HELLER 2001). Members of the Geomitridae sensu RAZKIN et al. (2015), which thrive in predominantly xeric habitats, are no exceptions. We managed to observe egg and clutch size, time to hatching, the appearance of hatchlings and the growth of *X. obvia* in the laboratory, and – in an earlier study – the differences in growth pattern, population dynamics and shell morphology between two natural populations (MARZEC et al. 2020). These field observations pertained to two distant populations from areas which varied in terms of climatic regime. Here we attempt to place the information in the context of a few arbitrarily chosen European xerophilous species of similar size.

In many xerophilous gastropods from the Mediterranean region reproductive and egg-laying periods are similar regardless of the location. In this area the reproduction takes place in autumn (MOQUIN-TANDON 1855, BERNER 1941, STAIKOU & LAZARIDOU-DIMITRIADOU 1991, LAZARIDOU & CHATZIOANNOU 2005). In Central and Eastern Europe *X. obvia* also reproduces in autumn (ZEMOGLYADCHUK 2019, MARZEC et al. 2020), but snails introduced to Montana in the USA can lay eggs both in autumn and spring (BIRDSALL et al. 2019). In French populations of *Xeropicta derbentina* (Krynicky, 1836) (syn. *X. arenosa*) prolonged egg-laying was observed from September to January, with some eggs also recorded in March (KISS et al. 2005). In a xerophilous helicid *Theba pisana* (Müller, 1774) the breeding period included summer and autumn in Britain and northern France, but the reproduction occurred in autumn and winter in the Mediterranean (COWIE 1984).

In SW. England a smaller snail *Candidula intersec-ta* (Poiret, 1801) laid eggs in November–December and early summer, the timing apparently varying according to rainfall and moisture balance (BAKER 1968). We observed egg-laying by the snails collected in October. Field studies revealed that the snails from this population in SW. Poland were more plastic compared to the population from the distant geographic region in NE. Poland. The SW. population reproduced mainly in autumn, but juveniles of the smallest recorded age class were present from April to August. Few adults could survive for more than one season (MARZEC et al. 2020).

The field studies indicate a predominantly annual life cycle of *X. obvia* and show that its growth rate depends on climatic conditions and is negatively correlated with the age of snails (MARZEC et al. 2020). Since the juveniles of *X. obvia* survived in the labora-

tory very poorly, doubts may arise whether the mean life span, estimated for two years, was real. However, our data did not differ from those published by other authors, who reported longevity of *X. obvia* for 1–3 years (LAZARIDOU & CHATZIOANNOU 2005, WELTER-SCHULTES 2012). As determined by width, weight and number of whorls, *X. obvia* exhibits high variation in growth rate but develops fastest at 20 °C. Individuals at both 12 °C and 28 °C were noticeably smaller than snails reared at 20 °C. Moreover, snails reared at 28 °C experienced high mortality (BIRDSALL et al. 2019). Variations in growth rate and life span are suspected to be a response of *X. derbentina* to an attenuation of summer drought due to occasional rain (KISS et al. 2005).

*Xerolenta obvia* reached maturity in the year following hatching, similarly to that observed in the French populations of *Ceriuella virgata* (Da Costa, 1778) and *Helicella itala* (Linnaeus, 1758) – two species of size, shell structure and habitat requirements similar to those of *X. obvia* (MOQUIN-TANDON 1855). There is, however, substantial variation in the life cycles, depending on the habitat type or climate (KISS et al. 2005, LAZARIDOU & CHATZIOANNOU 2005, BAKER 2012, MARZEC et al. 2020). *C. virgata* normally had a single generation annually with massive egg hatching in autumn and fast juvenile growth until the next spring in Australia (POMEROY 1969). Its life cycle may also vary between habitats: annual in pasture–cereal rotations or biennial in permanent pastures (BAKER 1996, 2008). *T. pisana*, *X. derbentina* and *X. obvia* are able to switch from an annual to a biennial cycle in response to climatic conditions or population density (COWIE 1984, KISS et al. 2005, LAZARIDOU & CHATZIOANNOU 2005). Given such plasticity as well as fast growth and reproduction of these snails, and the fact that they form very abundant populations, it is likely that, with a climate change, the species will be able to invade new regions of the world. *T. pisana* and *C. virgata* invaded North America and southern Australia, becoming serious pests of crops and pastures and difficult to control (COWIE et al. 2009, BAKER 2012). There are no published observations in Europe of *X. obvia* being an agricultural pest. Nevertheless, its recent introduction in Montana, USA, is seen as a potential risk for grain and hay production and for export markets. This snail is of particular concern since it is known to feed on fodder crops (BIRDSALL et al. 2019). *X. derbentina* was successfully introduced to south-eastern France from Eastern Mediterranean Europe (KISS et al. 2005). In the near future, we may expect a further increase in the number of alien gastropods to expand across Europe and other continents (HATTELAND et

Table 1. Life history traits of *X. obvia* and some other xerophilous species of similar size

	<i>Xerolenta obvia</i>	<i>Helicella itala</i>	<i>Cermeuella virgata</i>	<i>Xeropicta derbentina</i>
Adult shell diameter (WELTER-SCHULTES 2012)	13–20 mm	9–25 mm	12–23 mm	12–20 mm
Egg appearance	partly calcified, milky white, oval or almost spherical (this study) milky white (FRÖMMING 1954)	in matt whitish envelopes with small, rhomboidal calcareous crystals (MOQUIN-TANDON 1855) oval (FRÖMMING 1954)	globular, in slightly opaque, white envelope (MOQUIN-TANDON 1855)	
Egg diameter (mm)	1.2–1.6 × 1.2–1.8 (mean 1.37 × 1.46) (this study) 1.2–1.5 (FRÖMMING 1954) 1.00–1.46 (LAZARIDOU & CHATZIOANNOU 2005)	1.5 (MOQUIN-TANDON 1855) 1.2–1.6 (FRÖMMING 1954)	1.5 (MOQUIN-TANDON 1855)	1.84 (POPOV & DRAGOMASCHENKO 1997) 1.45–1.78 (mean 1.61) (KRAMARENKO 2002) 1.1–1.2 (KISS et al. 2005)
Clutch size	15–65 (mean 32) (this study) 25–40 (FRÖMMING 1954) 7–30 (mean 18.3) and 17–95 (mean 57) depending on location (LAZARIDOU & CHATZIOANNOU 2005) 42–85 (mean 69), decreasing in older snails 18–28 (mean 22) (LAZARIDOU-DIMITRIADOU 1995)	30–60 (MOQUIN-TANDON 1855) 18–94 (mostly 26–68) (FRÖMMING 1954)	30–60 (France, MOQUIN-TANDON 1855) 1–257 (mean 62.8) (Australia, BAKER 1991) up to 60 in 40 clutches (USA, WHITE-MCLEAN 2011)	mean 31.3 ± 12.9 or 64 ± 11.4 depending on location (Greek, LAZARIDOU-DIMITRIADOU 1981, STAIKOU & LAZARIDOU-DIMITRIADOU 1991) 39–128 (mean 87.2) (Crimea, POPOV & DRAGOMASCHENKO 1997) 6–111 (mean 41.1) (S. Ukraine, KRAMARENKO 2002)
Time to hatching (days)	11–28 (mean 18) (this study) 25–30 (LAZARIDOU-DIMITRIADOU 1995)	18–20 (MOQUIN-TANDON 1855) 25–52 (mostly 28–36) (FRÖMMING 1954)	15–20 (MOQUIN-TANDON 1855)	11–16 (lab, POPOV & DRAGOMASCHENKO 1997) 15–20 (lab, KISS et al. 2005) 20–25 (field, LAZARIDOU-DIMITRIADOU 1981, STAIKOU & LAZARIDOU-DIMITRIADOU 1991)
Reproductive period	Greece (E. Macedonia) – September–November (LAZARIDOU-DIMITRIADOU 1995) Belarus, Poland – mainly autumn (ZEMOGLYADCHUK 2019, MARZEC et al. 2020) USA – autumn and spring (BIRDSALL et al. 2019)	France – July–November (MOQUIN-TANDON 1855)	France – autumn (MOQUIN-TANDON 1855) Australia – autumn–winter (BAKER 1996)	Greece – October–November depending on location (LAZARIDOU-DIMITRIADOU 1981, STAIKOU & LAZARIDOU-DIMITRIADOU 1991) Crimea – September–November (POPOV & DRAGOMASCHENKO 1997) S. France – November–December (BONAVITA 1965) SE France – September–January (KISS et al. 2005)



al. 2013, SARMA et al. 2015, PROĆKÓW et al. 2019). Several species, e.g. *C. virgata*, *Arion vulgaris*, *Monacha cartusiana*, *Helix lucorum*, *Hygromia cinctella*, *Cornu aspersum* are well adapted to human-impacted environments (PELTANOVÁ et al. 2012, DEDOV et al. 2015, WALTON 2017, PÁLL-GERGELY et al. 2019).

The differences in life history of *X. obvia* reported by various authors indicate inter-population differences in life cycle traits. They are summarised in Table 1 and compared with some xerophilous species of similar size. The intraspecific differences in the egg size and the number of eggs could be due to population densities or to climatic differences among the sites studied. An adaptive evolutionary response to the climate may be manifested among others by the small number of eggs in more arid areas (LAZARIDOU-DIMITRIADOU 1981, STAIKOU & LAZARIDOU-DIMITRIADOU 1991, LAZARIDOU & CHATZIOANNOU 2005). The fecundity (i.e. total number of eggs produced, numbers of clutches and

clutch sizes) was positively correlated with shell size of *T. pisana* and *C. virgata* (BAKER 1991).

In our study the duration of egg stage in *X. obvia* varied widely and hatching was asynchronous. Such differences can be attributed to the temperature. A wide range of times to hatching is usually explained by possible egg retention (TOMPA 1984). However, *X. obvia* was kept in constant laboratory conditions with no signs indicating egg retention. Furthermore, variation in the egg size negatively changed rates of juvenile growth and the timing of maturation in a slug *Deroceras laeve* (O. F. Müller, 1774) (SHIBATA & ROLLO 1988).

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