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REPRODUCTION OF THE ROMAN SNAIL (*HELIX POMATIA* L.) FROM A LOCAL NATURAL POPULATION IN FARM CONDITIONS AND IN A NATURAL HABITAT

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Abstract

The aim of this study was to compare the quality of clutches and reproduction results of two groups of Roman snails (*Helix pomatia*) from the same local population, laying eggs simultaneously in semi-natural farm conditions and in a natural habitat. The study material were Roman snails aged 2 or more years which had entered the third phenological season of their life and thus the first season of sexual maturity. Observations were conducted at an earthen enclosure in a greenhouse belonging to the experimental farm for edible snails at the National Research Institute of Animal Reproduction in Balice near Kraków (Poland) as well as at a site where a local population naturally occurs in the uncultivated park surrounding the Radziwiłł Palace. In the June-July season, differences among such parameters as weight of clutch, number of eggs in clutch, mean egg weight, and hatchling percentage when compared to the total number of eggs in the clutch were compared. It was determined that clutches of eggs from the natural population laid in the greenhouse were of lesser weight ($P<0.01$), contained fewer eggs ($P<0.05$), and the mean weight of individual eggs was less ($P<0.05$) than in clutches laid simultaneously in a natural habitat. Both in the greenhouse and in the natural habitat, in the first phase of laying eggs (June) the weight of the clutch and number of eggs it contained were greater than in the second phase (July). However, only for snails laying eggs in the greenhouse were these differences significant ($P<0.05$) and highly significant ($P<0.01$), respectively. Significant differences were not observed in hatchling percentage between eggs laid in the greenhouse and the natural habitat. The lower number of eggs laid in the farmed conditions of the greenhouse was successfully compensated for by the absence of mass destruction by rodents which occurred in the natural habitat.

Key words: *Helix pomatia*, farmed reproduction of natural population, natural reproduction

According to information included in the IUCN Red List of Threatened Species (Neubert, 2011), the Roman snail is considered to be in the Least Concern category. However, the need for greater protection of individual species as well as of whole natural complexes of malacofauna is becoming increasingly apparent due to the ongoing warming of the climate (Nicolai and Ansart, 2017), the impact of industriali-

sation (Barga-Więcławska and Świercz, 2015), and the expansion of intensive agriculture (Wall et al., 2017). These phenomena are responsible for the fragmentation or destruction of natural habitats of individual species of land snails, particularly of those with large body sizes such as the Roman snail. The fragmentation of habitats often causes destruction of the natural breeding areas of the snails. The Roman snail, as an edible snail, is considered difficult to breed on a large-scale in the artificial conditions of a farm, which is why it has not become the object of intensive commercial production, as has been the case with the common garden snail, *Cornu aspersum*. In Poland, experimental studies have been conducted in laboratory conditions and at experimental farms on increasing the effectiveness of reproduction of Roman snails originating from natural local populations (Dziabaszewski, 1975; Łysak et al., 2001; Gołąb and Lipińska 2009; Ligaszewski et al., 2005). These were successfully continued in conjunction with multi-year studies aimed at developing principles for active protection of a selected local natural population of Roman snails. The basis for this protection was the introduction into the natural habitat of partially reared hatchlings from this same population which had been obtained on a mass scale in farmed conditions (Ligaszewski et al., 2005; Ligaszewski et al., 2007; Ligaszewski et al., 2014 a, b; Ligaszewski et al., 2016 a, b). These studies allowed for the determination of the precise values and fluctuations of such values as clutch weight, number of eggs in clutch, mean weight of a single egg, ratio of clutch weight to breeder weight, and seasonal variability in the intensity of reproduction. The numbers of eggs in a clutch reported in the literature was quite varied, depending on the results of laboratory studies by various authors. According to Dziabaszewski (1975), the mean number of eggs in a clutch was 50, while Nica et al. (2012) reported 39.4, Hatziioannou et al. (1989) – 48.3, and Kramarenko (2013) – 33.8. Until now, however, no studies have been conducted to compare the quality of clutches and the reproduction results of two groups of Roman snails from the same natural population, laying eggs simultaneously, in the semi-natural farmed conditions and in the natural habitat. Such a comparison is the aim of this study.

The research hypothesis of the work discussed below assumes that the reproduction efficiency of Roman snails from the same natural population under controlled farm conditions will be higher than that of snails laying eggs in natural conditions. Obtaining such an effect should be related to the production of a special, dry, plant-based, high-protein feed mixture for breeders placed in an earth greenhouse enclosure. The second reason should be regular sprinkling of breeding pens, which prevents stopping breeding with periods of snail aestivation during natural drought. A third reason would be the effective protection of the egg deposit against animal pests, i.e. predatory rodents and ants. Such a comparison is the aim of this study.

Material and methods

Selection methods for snails intended for the greenhouse study

Material for the two-year long study (2006 and 2007) consisted of adult Roman snails (*Helix pomatia* L.) aged two or more years, originating from the local natural

population inhabiting the vicinity of the manor house park at the Radziwiłł Palace in Balice near Kraków (Poland). The age of 2 or more years meant that after having undergone two seasons of hibernation, the snails were entering their third spring-summer season of life activity and their first reproductive season. The studies were conducted during the natural reproductive season of this hermaphroditic species of snail, which in the Polish climate falls in the period from mid-May to mid-July. At the beginning of May, a single collection was taken of snails which were adult in the somatic and reproductive sense, meaning that the lips of the shell were hard and curled. The collected snails were placed in an earthen experimental greenhouse enclosure with a density of about 50 individuals per 1 m^2 surface area of the enclosure.

Preparation of the experimental enclosure

In mid-April, common agrimony (*Agrimonia eupatoria*) was sown in an earthen and wooden enclosure located in an unheated greenhouse with tilt-window ventilation and a glazed roof along with turf containing white clover (*Trifolium repens* L.) The enclosure was fitted with a misting system and feeding boxes in the form of wooden pallets on which a specialised, dry vegetable snail feed mixture was placed. Between the stands of vegetation, open spaces were left to allow for convenient observation of the Roman snails laying eggs in uncovered areas.

2006 study

The study was conducted exclusively in the greenhouse enclosure. It was treated as a preliminary and exploratory study vis-à-vis the larger study planned for the following year. Four hundred and thirty individual Roman snails were observed, all of which had been collected on a single warm, damp day when the entire natural population was exhibiting high levels of activity and was easily accessible to the collectors. Before releasing the snails into the experimental enclosure, their shells were marked with individual numbers, using a fast-drying wood paint. During further observations, the body weight and shell diameter of the snails were measured, and the parameters of their first mating season in the earthen greenhouse enclosure were recorded. Individual snails laid clutches of eggs most often twice in the season. Each snail which buried itself in the earthen floor in order to lay a clutch of eggs was covered by a heavy, clay flowerpot. This allowed appropriate measurements of the snails' and clutches' biometric parameters to be taken after 24 hours. Eggs were removed from the soil using a plastic spoon. At the end of July, after completion of the study, the breeding Roman snails were released back into the natural habitat from which they were collected. The mean body weight of the snails after laying the first clutch was 21.2 g (SD 3.6) and after the second 21.1 G (SD 3.9) The mean shell diameter in both cases was 33.9 mm (SD 1.8) and 33.9 mm (SD 1.7) respectively. The following parameters of the first and second clutches were measured: total weight of the clutch (g), number of eggs in the clutch, ratio of clutch weight to breeder weight (%), and mean weight of eggs in the clutch (mg). Next, the studied clutches were stored one next to another in specially prepared incubation trays on a layer of soil with a pH of 6.5. The hatchlings obtained from these, not being objects of study, were released into the natural environment.

For comparison of the significance of the differences in mean values of the studied parameters of the eggs laid in the first and second clutches by the same snails, Tukey's HSD test for unequal groups was applied. The remaining results of statistical analysis (median, quartile, minimum and maximum) are included in this work in the form of whisker plots.

2007 study

Taking into consideration the reproductive effectiveness of the Roman snail determined in the previous year's study, in the following year twice as many individuals were included in the reproduction programme in the earthen enclosure in the greenhouse, that is 920 individuals. At the same time, the process of laying eggs was observed in the natural habitat, and on the day when the largest number of snails were seen to be digging themselves into the ground in order to lay eggs, these locations were marked with flags which were small but still visible from afar. This procedure was carried out twice, on a defined day in June and July. On these same days, the snails laying eggs in the earthen enclosure were covered with clay flowerpots. From those snails transferred from the natural habitat to the earthen enclosure in mid-May, 50 to 58 clutches of eggs were selected randomly on the 18th of June and 12th of July. This number was adjusted to match the number of clutches collected from snails laying eggs in the natural habitat. Only those clutches from the natural habitat were taken into consideration which had not suffered any damage from rodents. This time the snails themselves were not weighed and measured due to the difficulty of managing the egg-laying snails of the natural population in the uneven terrain of the natural habitat, which made it difficult to cover the snails with a clay flowerpot. For this reason also, the ratio of clutch weight to breeder weight was not calculated. Instead, a comparison was made of the percentage of hatchlings from each clutch for those laid in the greenhouse and in the natural biotope (in June). The eggs were placed separately in special round plastic boxes covered with perforated lids on a thin layer of soil with a pH of 6.5. The temperature in these incubators was maintained at between 22°C and 24°C. For comparison of significant differences between mean values for the parameters of clutches of eggs obtained from the same population in greenhouse conditions and in the natural habitat, Tukey's HSD test for unequal groups was applied. The remaining results of statistical analysis (median, quartile, minimum and maximum) are included in this work in the form of whisker plots.

Results

Studies conducted in 2006

Of the 430 Roman snails exhibiting the features of adult breeders transferred from the natural population to the earthen enclosure in mid-May, 126 individuals, that is 29.35%, laid clutches of eggs twice from June to mid-August.

The mean body weight and shell diameter did not change in any statistically significant way nor did they increase in number in this period (Table 1). However, snails

weighed after laying eggs the second time in the season exhibited greater variability of body weight than in the period of laying the first clutch of eggs (Figure 1), both in terms of weight gain among some of the individuals and weight loss in others, while still maintaining similar mean values for this parameter (Figure 1). Such differences were not observed in the case of shell diameter (Figure 2). The mean weight of the first clutch, the number of eggs which it contained, and the ratio of clutch weight to snail body weight were significantly ($P<0.01$) higher than the values of the same parameters in the case of the second clutch. Mean egg weight in the first clutch was higher than in the second in a significant manner ($P<0.05$). These differences for the values of the four parameters mentioned above were 37.8%, 42.3%, 45.4%, and 3.8% respectively. The same dynamic of differences can be seen in Figures 3–6, broken down into results for the first and second clutches in median and quartile.

Table 1. Characteristics of Roman snails and clutches of eggs laid twice in the 2006 season

No. of clutch	Body weight (g)		Shell diameter (mm)		Weight of clutch (g)		Number of eggs in clutch (N)		Ratio of egg weight to body weight (%)		Egg weight (mg)	
	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
First	21.2	3.6	33.9	1.8	6.2 A	2.1	49.8 A	16.1	32.0 A	0.1	136 a	0.02
Second	21.1	3.9	33.9	1.7	4.5 B	1.9	35.0 B	14.6	22.0 B	0.1	131 b	0.02
All	21.2	3.7	33.9	1.7	5.6	2.3	42.4	17.0	0.3	0.1	130	0.02

A, B – highly significant differences ($P<0.01$).
x, y – significant differences ($P<0.05$).



Figure 1. Body weight of Roman snails after laying eggs in the greenhouse, 2006

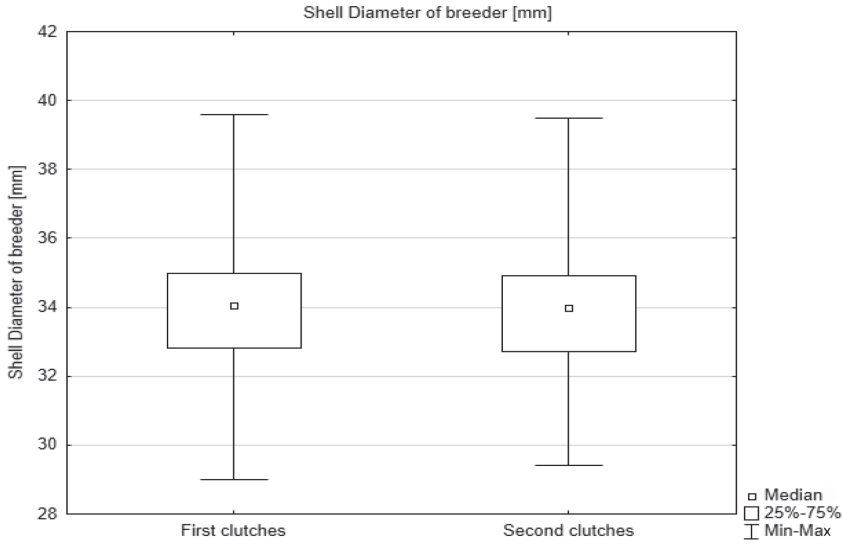


Figure 2. Shell diameter after laying eggs in the greenhouse, 2006

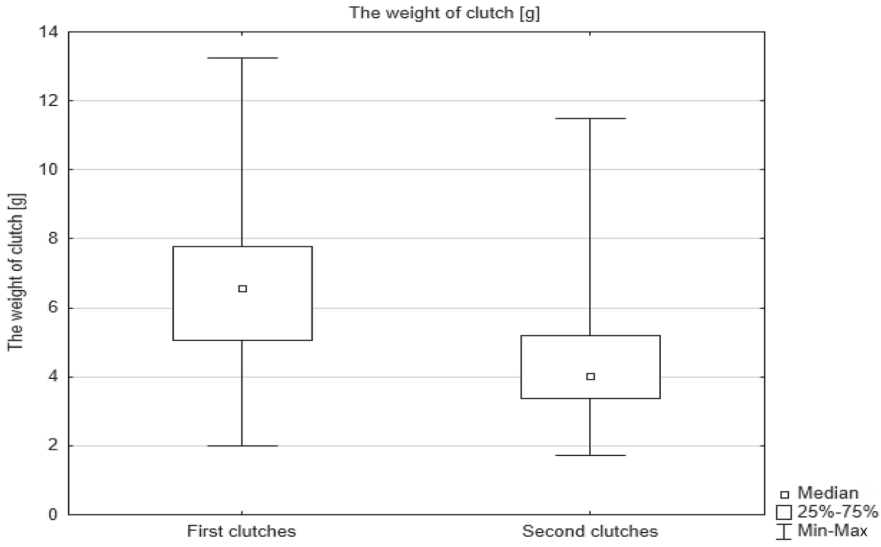


Figure 3. Weight of first and second clutches, 2006

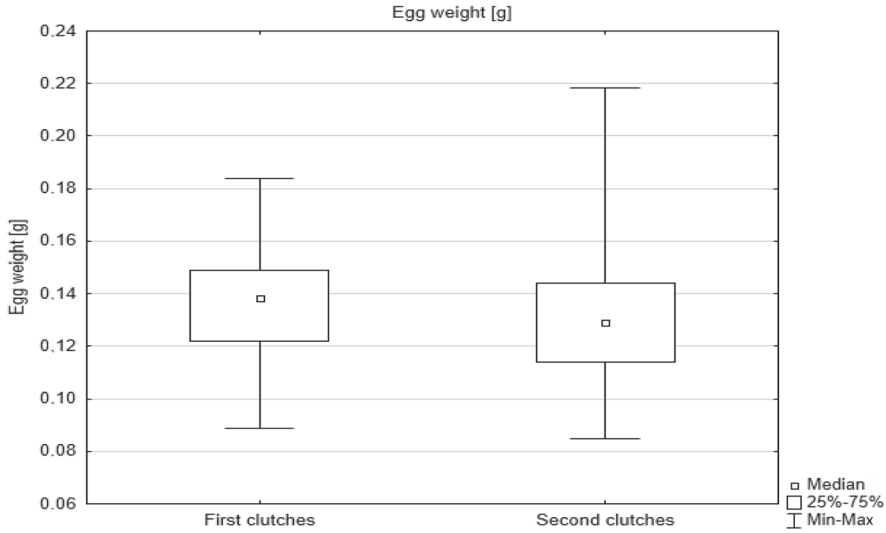


Figure 4. Egg weight in the first and second clutches

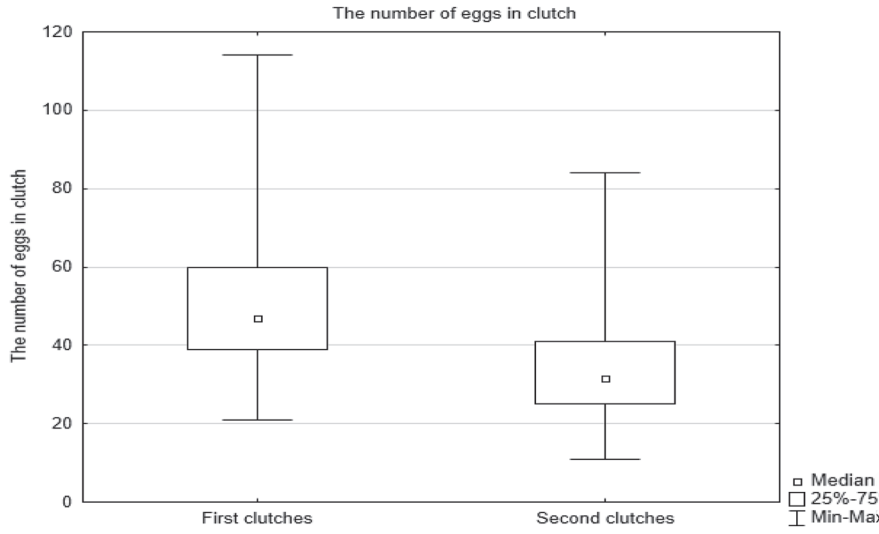


Figure 5. Number of eggs in the first and second clutches, 2006

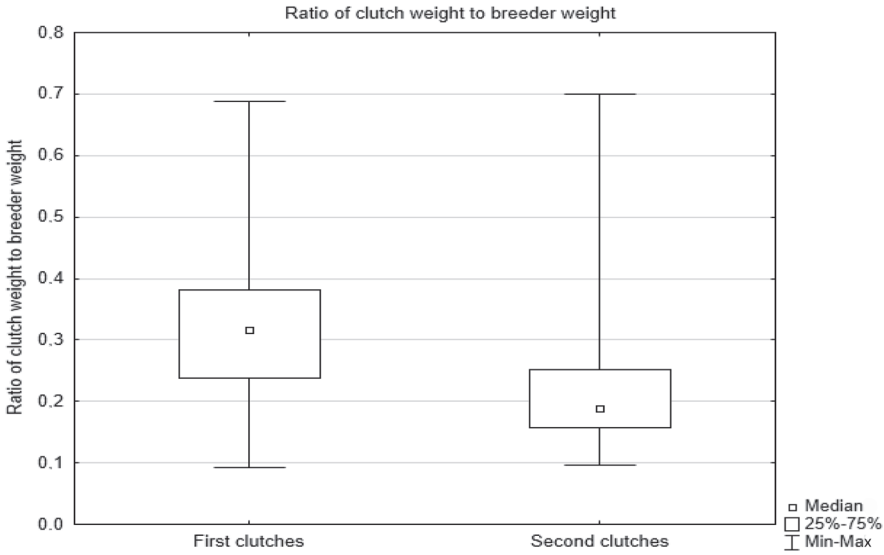


Figure 6. Ratio of first and second clutch weight to body weight of breeder, 2006

Studies conducted in 2007

From the 920 snails transferred from the natural habitat to the earthen enclosure in mid-May, roughly 50 clutches of eggs were selected randomly on the 18th of June and 12th of July. This number was adjusted to match the number of clutches collected from snails laying eggs on the same days in the natural habitat. It was determined that of the 242 egg deposit sites marked in June and the 120 sites marked in July, respectively 64.2% and 40.8% of the clutches were at the time of collection destroyed by rodents which had dug up to them from underneath or in another way damaged the eggs.

Table 2. Characteristics of Roman snail egg clutches laid in greenhouse and natural habitats, 2007

Place and date of sample collection	Weight of clutch (g)		Number of eggs in clutch (N)		Egg weight (mg)		Hatchling percentage (%)	
	mean	SD	mean	SD	mean	SD	mean	SD
Natural 18 June	6.4 A	2.1	50.3 a	17.7	130 A	0.03	31.6	13.0
Greenhouse 18 June	4.35 B,x	1.3	42.4 b,X	11.4	100 B	0.02	29.7	12,5
Natural 12 July	5.7 A	1.8	44.3 A	14.3	130	0.02	–	–
Greenhouse 12 July	3.5 B,y	1.2	32.3 B,Y	11.3	110	0.02	–	–
All	5.9	2.0	42.4	15.2	120	0.02	30.6	12.7

A, B – highly significant differences (P<0.01) depending on the place where the samples were collected for a given collection date.

x, y – significant differences (P<0.05).

X, Y – highly significant differences (P<0.01) between the two sample collection dates for eachtype of habitat.

x, y – significant differences (P<0.05).

Both in the samples from June and from May, the mean weight of the clutches laid by snails transferred from the natural population to the earthen enclosure in the greenhouse was lower in a highly significant manner (P>0.01) than that obtained among snails left in their natural habitat (Table 2). These differences were 47.1% and 62.9%, respectively. The number of eggs in the clutch in the natural habitat in June was higher in a significant manner (P<0.05), while in July this difference was higher in a highly significant manner than in the case of reproduction in the greenhouse, by 18.6% ad 37.1% respectively. The differences identified above between the clutches from the natural habitat and the greenhouse conditions thus widened as the reproductive season passed. The mean weight of eggs laid in clutches by snails in their natural habitat in June was higher by 30%, a significant difference (P>0.01) over those laid in the greenhouse, while in July this difference amounted to 18.2%, though it was not statistically significant. Statistically significant differences were not observed in hatchling percentage between eggs laid in the greenhouse and the natural habitat. Therefore, just as in 2006, by means of analogy to the first and second clutches, the mean clutch weight in the earlier June period was higher than one month later, but only for those clutches laid in the greenhouse were these differences highly significant (P>0.01), while such differences did not occur among the snails reproducing in the natural habitat. These differences amounted to 24.3% in greenhouse conditions and 12.3% in the natural habitat. The same dynamic of differences in the mean weight of an individual egg was observed, where the statistically insignificant difference in the natural habitat was 13.5%, but for greenhouse conditions this same difference was statistically highly significant, amounting to 31.3%. These dynamics of differences have been illustrated with division into data for medians and the remaining two for quartiles in Figures 7 to 10.

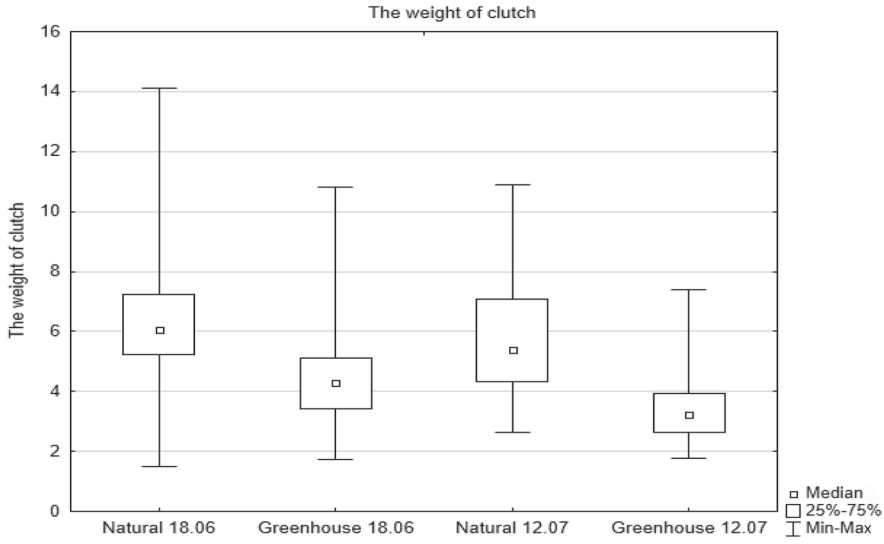


Figure 7. Clutch weight of clutches laid in the greenhouse and natural habitat, 2007

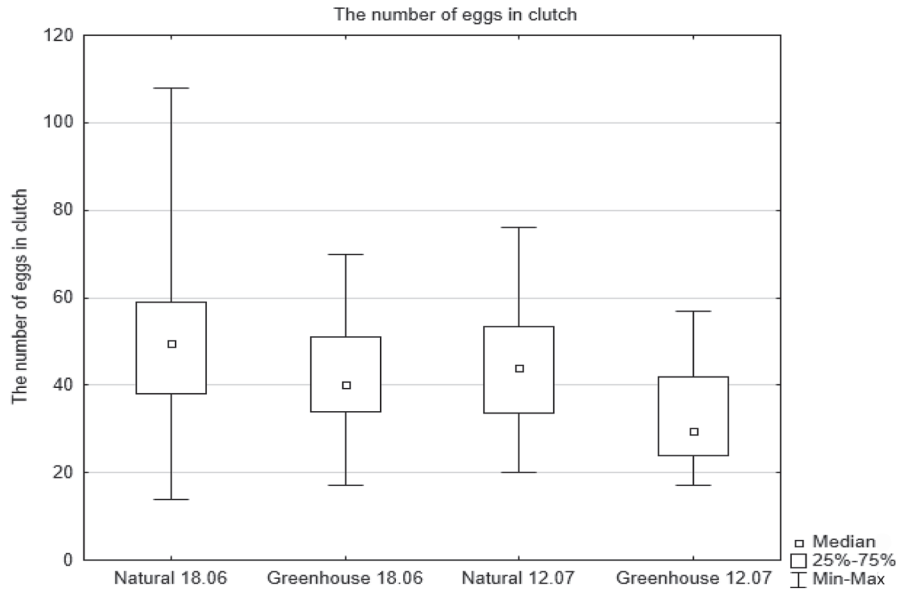


Figure 8. Number of eggs in clutches laid in the greenhouse and natural habitat in June and July, 2007

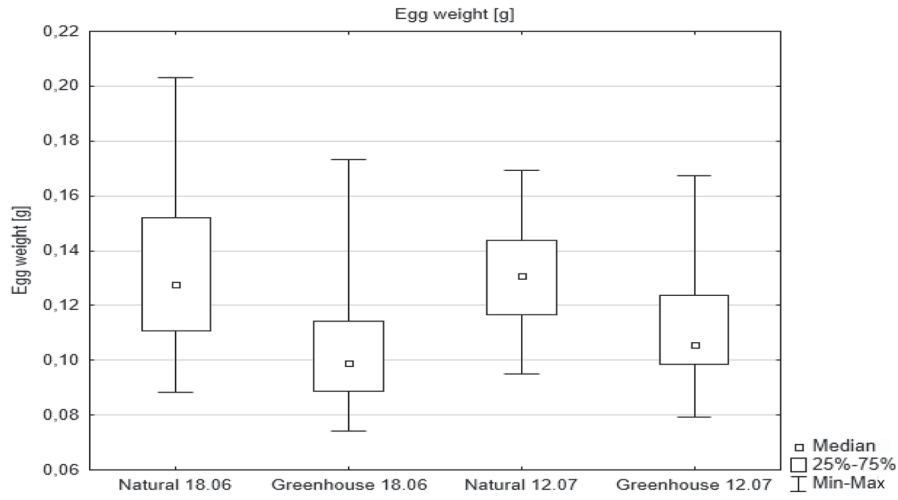


Figure 9. Egg weight in clutches laid in the greenhouse and natural habitat, 2007

Therefore, the results obtained in two reproductive seasons, 2006 and 2007 can be summarized in the following points:

in 2007 it was determined that in two successive egg-laying periods (June and July), significant ($P<0.01$) and highly significant ($P<0.05$) differences were observed

between the fundamental features of clutches laid in the farmed enclosure and in the natural habitat:

– lower clutch weight: by 47.1% in June and 62.9% in July;

– lower number of eggs in the clutch: by 18.6% and 37.1% respectively;

– 30% lower mean egg weight for clutches laid in June;

– complete or partial destruction of the egg clutches by rodents was observed in 64.2% and 40.8% respectively among the eggs collected from the natural habitat, while in the greenhouse conditions such losses were not noted;

– statistically significant differences were not observed between clutches of eggs collected from the natural habitat in 2007 based on the date of laying;

– statistically significant differences were not observed in hatchling percentage between eggs laid in the greenhouse and the natural habitat.

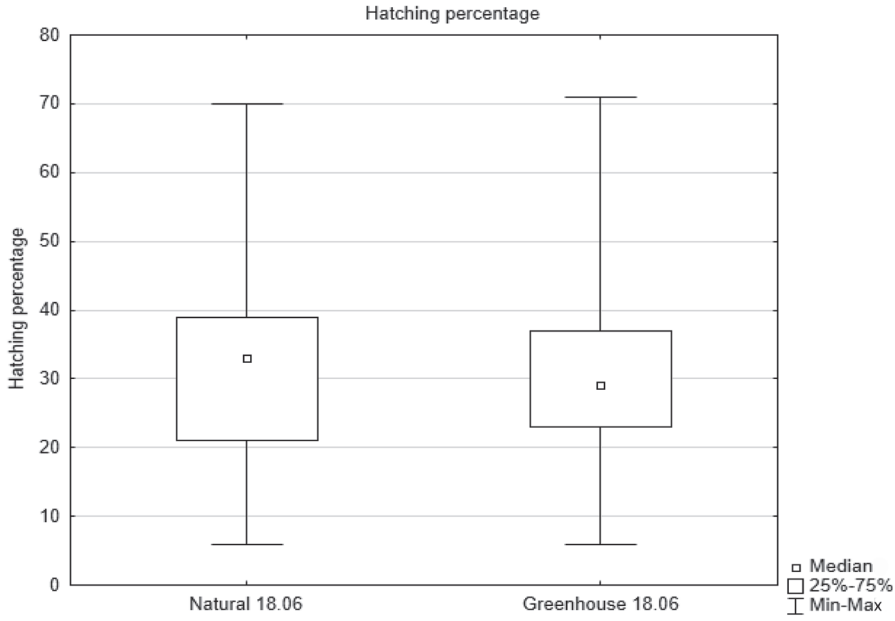


Figure 10. Percentage of hatchlings from clutches laid in the greenhouse and natural habitat, June 2007

Additionally, in 2006 and 2007, the following highly significant ($P<0.01$) and significant ($P<0.05$) differences between clutches of eggs laid in the greenhouse in June (the first clutch of the season) and July (the second clutch) were observed:

– higher weight of the first clutch (June), respectively by 37.8% in 2006 and 24.3% in 2007;

– higher number of eggs in the first clutch, respectively by 42.3% and 10.1%.

Statistically significant differences were not observed between clutches of eggs collected from the natural habitat in 2007 based on the date of laying.

Statistically significant differences were not observed in hatchling percentage between eggs laid in the greenhouse and the natural habitat.

Discussion

The aim of the study was to compare the quality of clutches of eggs and the reproductive results of a natural population transferred to a semi-natural farm environment with those left in their natural habitat.

No citations were found in the malacological literature which directly addressed the issues discussed in this study. There are, however, separate publications addressing the reproduction results of snails from the genus *Helicidae*, which are cited below, including Roman snails studied in laboratory and farm conditions, as well as a smaller number of citations addressing this issue in the natural habitat. The latter, however, do not cover the reproductive results of the Roman snail. Therefore, the discussion of the causes of the occurrence of statistically significant differences between features of egg clutches from the same natural population laid in farm conditions and in the natural habitat was based on these sources.

Table 3. Comparison of parameters of clutches of Roman snail eggs from a natural population in Balice at various times during the study

Date of study	Type of sample	Weight of clutch (g)	Number of eggs in clutch (N)	Egg weight (mg)	Ratio of eggs to body weight (%)
Clutches of eggs from Roman snails which laid eggs twice in the reproductive season					
2003	Greenhouse:				
(Ligaszewski et al., 2007)	First clutch	6.8 A	49.6	139	32.5 A
	Second clutch	4.5 B	35.2	131	22.2 B
2006	Greenhouse:				
	First clutch	6.2 A	49.8 A	136 a	32.0 A
	Second clutch	4.5 B	35.0 B	131 b	22.0 B
Clutches of eggs laid at different stages of the reproductive season.					
2003	Greenhouse:				
(Ligaszewski et al., 2007)	June	6.6 A	48.1 A	139 A	30.4 A
	July	4.7 B	35.9 B	133 B	23.3 B
2007	Greenhouse:				
	June	4.4 A	42.4 A	100	–
	July	3.5 B	32.3 B	110	–
2007	Natural clutch:				
	June	6.4	50.3	130	–
	July	5.7	44.3	130	–

Gołąb and Lipińska (2009) stated that the number of eggs in clutches laid by Roman snails in laboratory conditions was dependent on the shell diameter. In contrast, in this study no statistically significant changes over time in the shell diameter and body weight of the snails marked with identification numbers laying eggs in farm conditions were observed. Therefore, the confirmed significant differences between the first and second clutches in the 2006 reproductive season, and also between clutches laid by snails collected at an earlier or later date, did not result from differ-

ences in the shell diameter of the breeders. According to Baur (1992), the association of Roman snails for breeding purposes in natural populations is random, that is these snails do not select a mating partner based on shell diameter. The Roman snails at the age of two or more years from the population discussed here were collected in a random manner. This collection took place on a single occasion at the beginning of May, among those snails which exhibited the most mature characteristics. In this situation, slower-growing snails which matured in May and June and may thus have achieved a greater shell diameter, and consequently greater variability in this parameter, were not included in the sample. Therefore, the lower number of eggs observed with on average a lower clutch weight from Roman snails in farm conditions when compared to snails laying eggs in their natural habitat must have been caused by this smaller shell diameter and by the relatively low variability in values of this parameter in the first experimental group. In studies conducted earlier, in 2003, among the population from Balice discussed here it was determined that the shell diameter was correlated in ascending order in a highly significant manner with such features of the clutch as clutch weight and mean weight of an individual egg. The Roman snails gathered during that study had a mean shell diameter of 24.1 mm (Ligaszewski et al., 2007), while the snails used in the study currently under discussion from 2006 had a smaller diameter amounting only to 33.9 mm on average. Therefore, such features of snail egg clutches in greenhouse conditions as clutch weight, number of eggs in a clutch, mean weight of an individual egg, and ratio of clutch weight to breeder weight had generally lower values in the snails discussed in this study than in the study conducted three years earlier (Table 3). In 2007, these values decreased further when compared to 2003 and 2006, with the caveat that in 2007 the snails were not marked and their shell diameters were not measured. In 2007, the clutches of Roman snail eggs from the greenhouse had in general slightly lower values in terms of the parameters studied here than those collected from the natural habitat. This concerned in particular the later July phase of reproduction. While the numerical values of the parameters of the clutches of eggs laid in the greenhouse sharply decreased along with the passing of the reproductive season, in the clutches laid in the natural habitat this phenomenon occurred only to a slight degree which was statistically insignificant. As Lazaridou-Dimitriadou and Bailey (1991) observed, in clutches of eggs of various other species of snails from the genus *Helix* laid in the later phase of the reproductive season, there were also fewer eggs than in clutches from the early part of the season. The same conclusion was reached by Madec et al. (2000) when studying a population of *Cornu aspersum aspersum* (synonym *Helix aspersa aspersa*), in which the second clutch was also of a lower weight than the first clutch. The authors also reached this conclusion in this study, both regarding clutches laid in farm conditions and in the natural habitat. Nicolai et al. (2010) stated that the weight of the clutch and the size of the eggs of *Cornu aspersum aspersum* from a natural population were both closely correlated with the life strategies of the snails in relation to the nutritional resources of their environments. Eggs laid by snails which matured at an earlier age in an environment rich in nutritional resources were small, while the eggs of snails which matured later in a less nutritionally rich environment were larger and heavier. A confirmation of this dependency was also

provided by the results of this study, as the life environment of the snails laying eggs in the earthen enclosure in the greenhouse, in which the snails were fed with a specially prepared high-protein feed mixture, was more rich in nutrients than the natural habitat. Additionally, for reproduction in these conditions, snails aged two years or more which had matured in spring as the first of their group were se-lected for this study. Due to this early-onset maturity, these snails were selected from the natural population for farmed reproduction in May, before the beginning of the two-month long (June-July) phenological reproductive season. As the cited researcher observed (Nicolai et al., 2010), as in the case of *Cornu aspersum aspersum*, Roman snails from the earthen enclosure in the greenhouse laid eggs of lower weight than those which reproduced in the less energy- and protein-rich environment of the natural habitat, feeding on less nutritious vegetation (Ligaszewski et al., 2016 a).

The previously mentioned study by Gołąb and Lipińska (2009) achieved a percentage of hatchlings in a clutch in laboratory conditions of on average 48%, while Turcek (1970) achieved as much as 80% of all the eggs laid in the studied clutches. This result was higher than in the currently discussed study, in which in both clutches laid in the greenhouse and in the natural habitat, this percentage was 30.0% and 32.0% respectively. However, in the latter study, the hatchling percentage was only calculated one week after the hatching of the first individuals, that is after the final cessation of the natural phenomenon of infant cannibalism among *Helicidae* which occurs in the first days of the life of these snails (Baur 1988, 1990; Desbuquois et al., 2000).

The losses caused by the intense pressure of rodents on the clutches in the natural habitat were more than compensated for in the relatively safe conditions of the earthen enclosure in the greenhouse.

As it was mentioned previously, breeders in phenological reproduction comprised snails aged two years or more which had achieved somatic maturity at the beginning of May, that is as the earliest from among their age group in the natural population. Therefore, the genetic variability of the hatchlings obtained in farm conditions was certainly lower than in the case of the hatchlings in the natural habitat obtained from all snails aged two years or more and maturing gradually throughout May and June.

Conclusions

The statistical significance of the differences between the values of the basic parameters of reproduction of Roman snail (*Helix pomatia*) from the same natural population laying eggs in the conditions of the ground farm and in the natural environment was influenced by the following differences between both environments:

• trophic, resulting from differences in the nutritional value of the feed mixture and natural vegetation

• genetic, associated with harvesting for reproduction in greenhouse conditions of a relatively small part of the natural population of the Roman snail

• physiological, related to the young age (2+) of snails collected for reproduction in greenhouse conditions, laying eggs for the first time in their lives

The deposits of eggs laid in the greenhouse pen were not exposed to destruction by predatory rodents, which made it possible to obtain more broods per one stud under these conditions.

Reproduction in greenhouse conditions allowed for environmental compensation of the endangered population of the vine, although it could affect the genetic balance of the natural population, depending on the selection and abundance of breeders.

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