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OBSERVATIONS ON THE MATURATION AND DEVELOPMENT OF A ROMAN SNAIL (*HELIX POMATIA,* LINNAEUS, 1758) POPULATION OF FARMED ORIGIN IN NATURAL PLOTS*

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Abstract

Observations were made concerning active species protection of the Roman snail. Samples were collected from three natural plots in which 3,000 marked hatchlings of farmed origin, aged 1+ (three individuals per m^2), were placed in mid-May 2011. The hatchlings originated from breeding snails of the park, or 'source' plot. The other plots were a forest, or 'inhabited' plot, occupied by a foreign population and a cultivated 'empty' plot, which had been emptied of its natural population. By the end of June 2012, the introduced snails were aged 2+, when snails of this species reach maturity. During this period, as part of the analysis of collected samples, the snails in their final maturation period in this age group were divided into mature and immature groups. After thirteen months of observations, a greater density was observed for the farm-originated, naturalised population in the 'empty' plot than in the total populations for the other two plots. In the 'empty' plot the percentage of somatic and sexually mature farmed snails aged 2+ was significantly higher than in the same snail groups from the other two research plots. There were no statistically significant differences between the shell diameters of the mature farmed snails in all the research plots. The Roman snails of farmed origin considerably extended their territorial range, maintaining their high percentage share in the local natural populations.

Key words: naturalised population, snail maturation, farmed population, natural population

The present study is a follow-up to an experiment initiated in 2011 (Ligaszewski et al., 2014), which investigated the effectiveness of applying natural methods of active species protection for the Roman snail (*Helix pomatia*) in three experimental natural plots independently of the commonly used passive protection for the natural populations (European Community Directive on the Conservation of Natural Habitats and of Wild Fauna and Flora (92/43/EEC)). In the first year (2011), 3,000 marked

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specimens of farmed origin, aged 1+, were placed in each research plot with an average of three individuals per m^2. One scientific argument for undertaking this study was an observation on the absence of evidence that active protection methods of *Helix pomatia* could be as effective as passive protection methods. Apart from the important aspects of studies on passive protection of the species focused on monitoring natural populations in Poland (Zając, 2008), detailed ecological studies on the age structure and behaviour of the local population of the species are relatively rare (Łomnicki, 1971; Pollard, 1973; Woyciechowski and Łomnicki, 1977; Woyciechowski, 1980; Ledergerber, 1998; Andreev, 2006). Interesting research had already been undertaken on the influence on species behaviour of the experimental connection of adult specimens from two separate natural populations in the same area (Woyciechowski and Łomnicki, 1977). During the first year (2011), the initial effect of introducing juveniles to a native population or a habitat emptied of its natural population was studied. The study proved the hypothesis that 'the growth of farmed Roman snail hatchlings aged 1+, introduced to natural plots, will be in accordance with species biology, and this introduction will have an essential impact on the structure and numbers of local natural populations of the Roman snail' (Ligaszewski et al., 2014). In 2012, the objective of the study was to investigate late-spring quality structures of the observed populations, as influenced by the maturation of the first generation of snails of farmed origin, aged 2+.

Material and methods

The technique used for reproduction and rearing of the age 1+ Roman snail hatchlings from the population living near the 'source' plot was described previously (Ligaszewski et al., 2007). When identifying the age group of snails, the numbers 1, 2 and 3 were used to indicate the number of winter hibernation periods, while '+' designated a new phenological season of living activity. The present research was conducted on three experimental natural plots, each with an area of 1000 m^2, located about 20 km from Cracow, Poland (Ligaszewski et al., 2014). The three types of plot in the second year of the study, namely the 'source', 'inhabited', and 'empty' plots, are described below. The sampling method mandated collection of snails by one researcher for a period limited to 1 h to avoid damage to flora and snails on the experimental plots. The samples were collected on rainy and cloudy days, between 6 and 7 a.m., when the air temperature ranged from 16 to 17°C. The shells of farmed snails were marked with quick drying non-toxic wood varnish (Ligaszewski et al., 2014) which largely faded away 13 months after the introduction of the snails to the research plots; until then, traces of the coloured varnish on the shells were easy to see with a magnifying glass. After separation of the marked snails from the collected samples, a study of the age structure of the local populations was undertaken. The age of the Roman snails from the natural populations was determined using a mixed method of shell assessment: shell diameter, number of annual growth rings in the shell (Pollard, 1973), and incomplete shell growth as manifested in a soft and inflected shell lip. Roman snails from Polish populations continue their somatic development until autumn, mostly at age 2+, as reflected in the shell lip turning outwards and becoming hard. Farmed or natural snails begin to reach somatic and reproductive maturity aged 2+. Accordingly, the rate of their growth and maturation in different plots was studied after subdividing the specimens into mature and immature snails. Shell diameter measurements (Ligaszewski et al., 2009) were made on the collected samples.

Plant communities at surveyed stations were compared using a similarity factor as in the calculation formula of Sørensen (1948):

$$P = 2c \times 100 \times (a + b) - 1$$

where:

P is the similarity factor expressed as a percentage,
c is the number of species common to a given pair of communities (gathering),
a is the number of species in the first community of plants,
b is the number of species in the second.

Statistica software was used to perform t-test in order to identify any differences in natural snails' shell diameter between the source and the inhabited plot and between mature and immature snails (from both the natural and the farmed population) in each one of the three different plots. One way ANOVA, followed by the post hoc Bonferroni test (in cases of statistical significance), was implemented in order to detect any differences in farmed snails' shell diameter between the three plots (source; inhabited; empty).

Description of the natural conditions in the three experimental plots and structure of the Roman snail's natural populations in the second year of the study

a) The 'empty' plot in Będkowice, near Cracow

This Roman snail population was created in spring 2011 (Ligaszewski et al., 2014) after introducing *Helix pomatia* individuals in the second year of life (1+), raised under farm conditions, from the 'source' plot population into a habitat which had previously been free of any natural populations.

In the second year of the study (Ligaszewski et al., 2014), compared to the first, the floristic composition of wild species of herbaceous plants and those sown a year earlier had partly changed. This plot underwent a succession of natural plants in terms of species composition and of dominance relationships that were almost the same as prior to the two-year field study. However, from the viewpoint of trophic relationships, the fact that white clover (*Trifolium repens*), sown a year earlier, continued to be one of the dominant plants was beneficial for the Roman snail. In 2012 the plot also contained a mono-population of farmed snails aged 2+, which had been introduced to the plot a year earlier.

b) The 'inhabited' plot in Mydlniki-Wapiennik, near Cracow

In the spring of 2011, farmed Roman snails aged 1+, originating from breeding stock of a foreign 'source' plot population, were introduced to the local population.

Between 2010 and 2012, no changes were found in the floristic composition of different species of herbaceous plants and in their dominance relationships.

The structure of the natural population of Roman snails in 2012 was as follows: snails aged 1+: 12.7% (N=10); 2+: 57.0% (N=45); 3+ and above: 30.3% (N=24), in a sample of 79 individuals. There was also a mono-population of farmed snails aged 2+ which had been introduced to the plot a year earlier.

c) The 'source' plot in Balice, near Cracow

The area around this plot was inhabited by a natural population of Roman snails from which adult specimens were taken to enable reproduction to take place in greenhouse conditions. It was from this population that all the experimentally farmed snails were introduced to the experimental plots. No changes were found in the floristic composition of different species of herbaceous plants or in their dominance relationships between 2010 and 2012.

The structure of the natural population in 2012 was as follows: snails aged 1+: 15.3% (N=17); 2+: 50.4% (N=56); 3+ and above: 34.3% (N=38) in a sample of 111 individuals. There was also a mono-population of farmed snails aged 2+ which had been introduced to the plot a year earlier.

Results

Floristic comparison of the research plots

The obtained indicators of species comparison in the various research plots indicate their low level of floristic similarity; none of the variants of the comparative value of this indicator exceeds 50% (Table 1). By far the smallest similarities in the particular years of floristic research were found between the 'source' plot and the 'empty' plot. The flora of the 'source' plot was, moreover, the poorest in terms of species, particularly with respect to the number of legume and dicotyledonous species, compared with the other two plots (Table 2): the total plant species of this plot were on average 35.0% less than in the other two plots. In the 'empty' plot, where the flora had been modified by the partial cultivation of the soil and seeding in 2011 of several species of fodder and dicotyledonous legumes, the floristic similarity between successive years fell to about 50%, then began to grow, before stabilizing at around 70% (Figure 1). Of the fodder plants in this plot, only white clover maintained its position in 2012 as one of the dominant plants, occupying 10% of its surface.

Table 1. Indicators of similarity in species (Sørensen, 1948) of vegetation between plots

Plots	Study year		
	2010	2011	2012
Source – Inhabited	34%	35%	35%
Source – Empty	26%	19%	17%
Inhabitat – Empty	45%	37%	38%

Table 2. Number of plant species in different plots

Plant group	Plots								
	source plot			inhabited plot			empty plot		
	2010	2011	2012	2010	2011	2012	2010	2011	2012
Grasses	4	4	4	5	6	6	6	4	8
Legumes	1	1	1	4	4	4	5	4	7
Dicotyledonous plants	13	14	14	19	20	19	17	16	19
Total	18	19	19	28	30	29	28	24	34

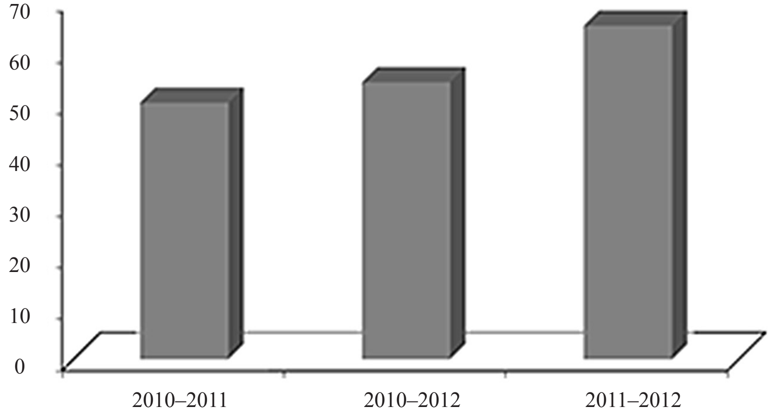

Figure 1. Changes in Sørensen's indicator of similarity in plant communities in the 'empty' plot, 2010–1012

Age structure of the populations

Sample sizes collected in late June using the same method from each research plot were highest for the 'empty' plot, followed by the 'source' plot and then the 'inhabited' plot (Table 3). The differences in the number of snails in the samples from the latter two plots in relation to the largest sample from the first plot, treated as relative differences in the number of the general populations analyzed during this period, were 21.4% and 41.9%, respectively. While a large population of exclusively farmed snails aged 2+ developed in the 'empty' plot, in the other two plots farmed snails accounted for 33–35% of the total populations and about 49% of all the snails aged 2+. In the latter two plots, natural and farmed snails aged 2+ constituted about 70% of the collected samples and dominated within the quantitative structure of the studied populations. In the 'empty' plot, the size of this population was the greatest of all plots and reached 0.21 snails per m^2. In the structure of all specimens aged 2+, the largest proportion of somatic mature specimens was found in the 'empty' plot (58.1%), which is 20.6% larger than in the 'inhabited' plot and 32.7% more than in the 'source' plot (Table 4). After collating out the results of the tests on natural and farmed specimens, it was found that in the 'source' plot the rate of maturation in the farmed and natural snails was similar, but in the 'inhabited' plot proportionally twice as many snails of farmed origin had already reached maturity as from the natural population (48.8% compared to 24.4%).

Table 3. Impact of the introduction of farmed snails on the structure and density of the population of Roman snails in different research plots

Plot	Age group							
	1+		2+		3+ or above		total	
	no	%	no	%	no	%	no	%
Sample from source plot								
Total sample including:	17	10.3	110	66.7	38	23.0	165	100.0
structure of natural population	17	15.3	56	50.4	38	34.3	111	100.0
natural snails in total sample	17	10.3	56	33.9	38	23.0	111	68.3
farmed snails in total sample*	-	-	54	32.7	-	-	54	32.7
farmed snails in 2+ group			54	49.1				
Total specimens per m^2 including:	0.02		0.11		0.04		0.17	
natural specimens per m^2	0.02		0.06		0.04		0.12	
farmed specimens per m^2			0.05				0.05	
Sample from inhabited plot								
Total sample including:	10	8.2	88	42.1	24	19.7	122	100.0
structure of natural population	10	12.7	45	57.0	24	30.3	79	100.0
natural snails in total sample	10	8.2	45	64.8	24	19.7	79	64.8
farmed snails in total sample*	-	-	43	35.2	-	-	43	35.2
farmed snails in 2+ group			43	48.9				
Total specimens per m^2 including	0.01		0.09		0.02		0.12	
natural specimens per m^2	0.01		0.05		0.02		0.08	
farmed specimens per m^2			0.04				0.04	
Sample from empty plot								
Absence of natural population	-	-	-	-	-	-	-	-
Total sample: farmed snails*	-	-	210	100.0	-	-	210	100.0
Farmed specimens per m^2	-	-	0.21				0.21	

Note: The farmed snails were introduced in May 2011; however, samples were collected after 13 months, in June 2012.

Differences in shell diameter

Between the ‘source’ and ‘inhabited’ plots, for natural snails from the 2+ age group, which included the total specimens, the difference in shell diameter was significant (df=98; t=–2.56; p=0.012), the same as the difference for immature (df=72; t=–2.31; p=0.024) and mature (df=24; t=–2.34; p=0.028) 2+ aged snails: the snails from ‘source’ plot had smaller diameter than from ‘inhabited’ plot (Table 5). In the total group of farmed snails aged 2+, specimens from the ‘source’ plot had smaller shell diameters than those from the ‘inhabited’ and ‘empty’ plots, with highly significant differences (F=32.4; p=0.000; df=303) (Table 6). The same as above pattern was exhibited by snails in the immature farmed snails group (F=20.5; p=0.000; df=148).

Table 4. Percentages of mature and immature individuals of Roman snails (*Helix pomatia* L.) in the 2+ aged group

Degree of maturation 2+ aged snails	Source plot		Inhabited plot		Empty plot	
	number of individuals	proportion (%)	number of individuals	proportion (%)	number of individuals	proportion (%)
Natural snails						
Total	56	100.0	45	100.0	-	-
Mature	15	26.8	11	24.4		
Immature	41	73.2	34	75.6		
Farmed snails						
Total	54	100.0	43	100.0	210	100.0
Mature	13	24.1	21	48.8	88	58.1
Immature	41	75.9	22	51.2	122	41.9
Total: natural + farmed snails						
Total	110	100.0	88	100.0	210	100.0
Mature	28	24.4	33	37.5	88	58.1
Immature	82	74.6	55	62.5	122	41.9

Table 5. Comparison of the shell diameter (mm) between natural Roman snails in two research plots

State of maturation	Shell diameter (mm)		t-test		
	source plot	inhabited plot	t	p	df
1+	24.77	24.95	–0.19	0.852	24
2+ immature	28.48	29.68	–2.31	0.024	72
2+ mature	30.73	31.91	–2.34	0.028	24
2+ total	29.08	30.24	–2.56	0.012	98
3+	32.86	33.11	–0.55	0.582	61

Table 6. Comparison of the shell diameter (mm) between farmed Roman snails in three research plots

Degree of maturation	Plots	Bonferroni test			ANOVA	
		parameters of the post hoc Bonferroni test			F	p
		MS = 6.032; df = 148.00				
		{1}	{2}	{3}		
		26.98	29.14	29.96		
Farmed	Source {1}		0.003	0.000	20.541	0.000
immature	Inhabited {2}	0.003		0.501		
2+	Empty {3}	0.000	0.501			
		MS = 6.382; df = 303.00				
		{1}	{2}	{3}		
		28.12	30.41	31.22		
Farmed	Source {1}		0.000	0.000	32.416	0.000
total	Inhabited {2}	0.000		0.177		
2+	Empty {3}	0.000	0.177			

Table 7. Comparison of the shell diameter (mm) between Roman snails in the 2+ age groups originating from farmed and natural populations

Plots	State of maturation	Shell diameter (mm)		t-test		
		farmed population	natural population	t	p	df
Source	Immature	26.98	28.48	–2.61	0.011	80
	Mature	31.73	30.73	2.11	0.045	26 108
	Total	28.13	29.08	–1.78	0.078	
Inhabited	Immature	29.14	29.68	–0.79	0.432	53
	Mature	31.81	31.91	–0.16	0.876	29 84
	Total	30.42	30.24	0.33	0.740	

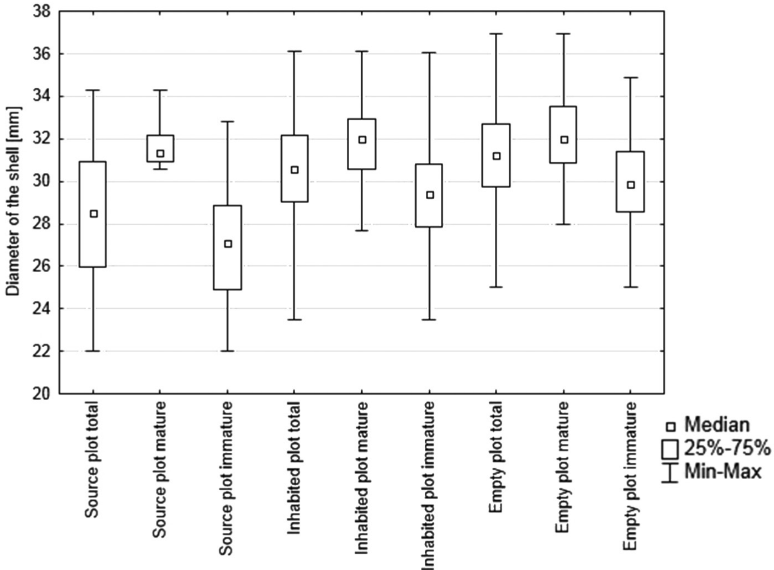

Figure 2. Median shell diameter in three populations of Roman snails (*Helix pomatia* L.) of farmed origin aged 2+

In the groups of mature snails from the three plots mentioned above, no statistically significant differences were found (F=32.4; p=0.60; df=152). For the 2+ age group, comparison was also made of the shell diameters of snails from the natural populations and farmed snails growing in the 'source' and 'inhabited' plots (Table 7). In the 'source' plot immature snails from the natural population had greater shell diameters than snails of farmed origin (t-test: df=80; t=-2.61, p=0.011). However, mature snails from the natural population of the 'source' plot had greater shell diameters than snails of farmed origin only on the marginally significant level (t-test: df=26; t=2.11; p=0.045). All the corresponding differences on the 'inhabited' plot were not significant (Table 7). When comparing the means for shell diameter in the three farmed groups of mature snails aged 2+ (Figure 2), it can be stated that mature snails were the most similar in the 'inhabited' and 'empty' plots.

Discussion

After thirteen months of observations, the higher density of a farmed originated, naturalised population in the 'empty plot' has been stated than in the total populations for the other two plots. In the same plot, the percentage of somatic and sexually mature farmed originated snails aged 2+ was found to be higher than in the same groups from the other two research plots. There were no statistically significant differences in the shell diameters of mature farmed snails in all the research plots. Compared to the first year of the study (Ligaszewski et al., 2014), Roman snails of farmed origin considerably extended their territorial range, maintaining their high percentage share in the local natural populations.

According to Garcia et al. (2006) the growth rate of *Helix aspersa* Müller is a good indicator of its welfare, the components of which are good environmental hygiene and relatively light stocking density. Both welfare conditions were met by the 'empty' plot where, due to the earlier absence of a native population, there was no overstocking following the introduction of the farmed snails and, therefore, neither snail feces nor ecto- and endoparasites. Sowing this plot a year earlier with seeds of a high nutritive value for snails (Ligaszewski et al., 2014) definitely had a beneficial effect on the snail metabolism during the first year (Jess and Marks, 1989; Iglesias and Castilleo, 1999). In addition, unlike the other two plots, this plot was not covered with trees, which provided longer daylight for the naturalised population. This, perhaps, is why the naturalised snails formed such a large, fast- growing and early-maturing population compared to the other research plots. The poorest results were obtained after introducing the farmed snails to the native population in the 'source' plot, which suggests less favourable welfare indicators due to overstocking of the population, shading of the plot and the low nutritive quality of the plants growing there. Ultimately, however, no significant differences were found in the shell diameters of the snails of farmed origin from all the research plots which had achieved complete shell maturity, since shell size was associated with the origin of Roman snail hatchlings from the same population. Similarly, as also in the case of other *Helicidae* species, a statistically significant impact of natural environment diversification on shell sizes of adult snails from *Marmorana* genus (Fiorentino et al., 2008) was not observed. In the first year of life the studied Roman snail hatchlings were reared in unified greenhouse conditions. This may not only cause the limited impact of shell diameter of the parents from the natural population on the growth speed of the Roman snail in its further development (Gołąb and Lipińska, 2009), but also the period of shell formation in environmental conditions characteristic for particular research plots was shortened by one greenhouse breeding season.

To conclude, in the thirteenth month of observations following the introduction of farmed Roman snail hatchlings aged 1+ into three different natural plots, we found this form of active protection of this snail species to be of relatively high effectiveness: in two research plots with their own natural populations, the introduction resulted in a 30–40% increase in the proportion of mature and maturing snails aged 2+, which is important for the dynamics of population reproduction. Particularly noticeable effects were obtained after introducing farmed Roman snails into the 'empty' plot, which had been emptied of its natural population; in this plot, in which the density of the naturalised population reached 0.21 specimens per m^2, the Roman snails of farmed origin laid eggs earlier in the season than snails from the other two research plots.

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