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REPRODUCTION OF *ARION LUSITANICUS* MABILLE, 1868 (GASTROPODA: PULMONATA: ARIONIDAE) INTRODUCED IN POLAND

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ABSTRACT: Reproduction of *Arion lusitanicus* Mabille was studied in the field, in a region of the slug's mass occurrence, and in laboratory, at temperatures of 10, 15, 20 and 25°C. Places of egg-laying, fecundity and development rate were determined. In the field, *A. lusitanicus* mated in the third decade of July and started laying eggs early in August. The eggs were laid in clutches on or in the soil. In horticultural and agricultural crops the slug laid the most numerous eggs on field edges and baulks. An individual egg production was ca. 400, from 5 to 190 eggs per clutch. The first juveniles hatched at the end of October. In laboratory a close correlation was found between the duration of incubation period and the percentage of hatched eggs. The incubation period was the shortest at 20°C, the mean percentage of hatched eggs being the highest at 15°C. No eggs hatched at 25°C.

KEY WORDS: Poland, slugs, *Arion lusitanicus*, reproduction, introduced species

INTRODUCTION

Arion lusitanicus Mabille, 1868 originates from the Iberian Peninsula (VON PROSCHWITZ 1994); much later it was recorded from Great Britain (QUICK 1952, 1960, ELLIS 1965). For the last 30 years the species has spread over a large part of C and W Europe (Bulgaria, the Netherlands, Switzerland, Austria, Germany), as well as in Sweden and Norway (SCHMID 1970, VAN REGTEREN ALTENA 1971, RIEDEL & WIKTOR 1974, DAVIES 1987, DE WINTER 1989, VON PROSCHWITZ 1992, 1994, VON PROSCHWITZ & WINGE 1994). For several years *A. lusitanicus* has been present also in SE Poland, in the region of Albigowa, Łańcut and Rzeszów, the first observation on its occurrence in Poland dating from 1993 (KOZŁOWSKI & KORNOBIS 1994, 1995, KOZŁOWSKI 1995). The slug has adapted very well to the local conditions; it is very abundant, especially in gardens and parks, as well as in thick bushes on river banks. It is a serious pest of many crops, mainly vegetables; it damages horticultural crops, ornamental plants and dicotyledon weeds.

Most studies on *A. lusitanicus* concerned first of all its morphology, distribution, dispersal and its significance as pest (SCHMID 1970, DAVIES 1987, DE WINTER 1989, VON PROSCHWITZ 1992, 1994, VON PROSCHWITZ & WINGE 1994). Considerably less information is available on its life cycle (QUICK 1960, DAVIES 1987, BRINER & FRANK 1998). This study was aimed at ascertaining the basic reproductive parameters of *A. lusitanicus* in Poland.

MATERIAL AND METHODS

FIELD OBSERVATIONS

The field studies were carried out in 1997–1998, in the region of the slug's mass occurrence in SE Poland (Albigowa, Wysoka, Rzeszów). From spring till late autumn the slug development, dates of mating and egg-laying were observed, and places of egg-laying sought for in cultivated fields (vegetables, agricultural crops) and outside them (fruit bush plantations, shrubs). In these habitats the number and size of clutches were determined on random plots of 1 m^2 (70 such plots in the fields and 60 outside them). During the observations, both the soil surface and the soil (to the depth of 20 cm) were examined in plant-covered and bare places.

FIELD EXPERIMENTS

In order to determine fecundity of *A. lusitanicus* under field conditions, the slugs were reared in closed containers in Poznań in 1998. The cages, of 50 × 70 × 50 cm, were made of metal angle bars, perforated metal sheets, and had additional walls made of mill gauze. Each cage had a partition that divided it in two equal chambers. The cages were dug into the ground to half their height and filled with 20-cm soil layer; oat and bran flakes, cabbage leaves and hen eggshells were provided as food. Ten pairs of immature slugs (one pair per cage) were placed in the containers to observe the reproduction from copulation to laying of the last eggs. Seven of the pairs reproduced. The resulting clutches were successively transferred to plastic containers lined with damp tissue paper. The clutches and eggs in clutches were counted twice a week. To determine the dates of the beginning and end of egg-laying, the slugs were reared in 1997–1998 also in Rzeszów, i.e. in the region of their mass occurrence. Ten immature slugs were placed per container dug into the ground; following copulation, dates of egg-laying were noted. The distribution of the obtained values was analysed.

LABORATORY EXPERIMENTS

The slug fecundity was estimated also under laboratory conditions (temperature 17–19°C, RH 93–95%, 16 h daylight). Juvenile slugs, collected in the field, were transported to Poznań in thermobags, joined into pairs (10 pairs) and placed in plastic containers (20 × 15 × 25 cm) filled with moist soil. The slugs were fed with oat and bran flakes, cabbage leaves and hen eggshells. Following copulation, the slug pairs were separated by transferring each individual into a separate container. The eggs laid by the slugs were successively transferred into Petri dishes lined with damp tissue paper. The dates of egg-laying, hatching and the number of hatched eggs were recorded thrice a week. The distribution of values of the studied parameters was analysed.

Observations of the rate of egg development were conducted in complete darkness, at the temperature of 10, 15, 20 and 25°C, RH 95%. Eggs laid by 16 slugs (joined in pairs before copulation) kept in separate containers (17–19°C, RH 93–95%, 16 h daylight) were collected in the second week of egg-laying and placed in plastic containers lined with damp tissue paper; the containers were kept at the above, respective temperatures. The eggs were checked daily for advancement of their development; the date and number of hatched eggs were noted. Four hundred eggs (100 eggs x 4 containers) were examined at each of the studied temperatures. The dependence between the number of days elapsing from the beginning of development and the number of hatched eggs was analysed. For the temperature of 10, 15 and 20°C, regression function was determined. The empirical distribution of the number of hatched eggs was checked for agreement with BERNOULI's distribution and with normal distribution (n = 400) by using limiting theorems. The rate of egg development for each temperature from the first day of hatching was compared in the first and second decades, by comparing tangent of the angle of inclination of the regression lines determined separately for these decades. Data on the percentage of hatched eggs (4 replications), transformed according to arcsin function (sqrt(k%)) were subject to variance analysis, and the generated hypothesis was verified with FISHER-SNEDECOR's test. TUKEY's test (significance level 0.05) was applied to verify detailed hypotheses.

RESULTS

OBSERVATIONS AND FIELD EXPERIMENTS

Under field conditions *A. lusitanicus* started mating (Fig. 1) in the third decade of July. The mating period lasted until the end of September in 1997 and until mid-October in 1998. No copulation was observed when the air temperature dropped below 5°C. At the beginning of the second decade of August, the slugs started laying eggs, the peak of oviposition falling on the second half of September. Subzero air tem-

Fig. 1. Copulating *A. lusitanicus* (Photo Author)

peratures in November interrupted the oviposition, however single eggs were still laid in December during daytime, at temperatures above zero (2–6°C, December 3, 1997). A large percentage of the eggs wintered over, and juneviles did not hatch till next spring. From the eggs laid in August, the first young hatched in the second decade of September, and a month later they were already 1.0 cm long. By the end of November, as a result of temperature decrease below 0°C, the slugs started retreating to their winter shelters in the litter, compost or soil, where they remained till the end of February. During winter months (December-February) the slugs became active whenever temperature increased; they fed on humus, plant remnants, roots and tubers, and then returned to their shelters. They resumed their regular activity in March, at temperatures above 5°C, and continued their growth.

The eggs of *A. lusitanicus* were laid mostly in clutches forming irregular but compact egg masses (Fig. 2). The eggs were spherical or oval, shiny, milky white, measuring on an average 4.2 × 3.5 mm. Eggs of major diameter exceeding 5 mm were also encountered. Older eggs were often yellowish.

The slugs laid eggs in gardens, on arable fields and their overgrown edges, meadows, baulks, in orchards, on plantations of fruit bushes, in dense shrubbery, on river and stream banks, as well as in parks, cemeteries, in cellars and irrigation ditches. Clutches were found on the surface of bare and vegetation-covered soil, e.g. under leaves of annual cultivated plants, among perennial weeds (couch-grass, grasses) and crop plants (perennials, clover, alfalfa), under objects lying on the ground (bricks, planks, metal sheets, polyethylene foil), and in the soil, to a depth of 2–10 cm, depending on the vegetation cover. In bare places (newly ploughed fields, mole hills, river banks) the eggs were laid in deeper soil layers (5–10 cm), in cracks, clefts and under clods. However, in vegetation-covered soil, the eggs were most often found at a depth of 2–5 cm, close to roots. Besides, eggs were found in compost (15 cm deep), in holes bitten out in roots of carrots or beets and in potato tubers. Clutches were found on 54.3% studied field crop areas, 70% being laid on field edges and baulks. On fruit bush plantations and among shrubs, clutches occurred on 33.3% area examined. The number of clutches per 1 m^2 ranged from 1 to 9. The number of eggs per clutch ranged from 12 to 124 (Table 1). In plant crops the highest number of clutches (mean 5.1/m^2) occurred in the soil, while the highest number of eggs per clutch (mean 67.3) was found in compost and on the surface of vegetation-covered soil (mean 53.5), especially among perennial weeds growing on field edges and baulks. Among dense shrubs and on fruit bush plantations (raspberry, currant,

Fig. 2. A clutch of eggs of *A. lusitanicus* on the soil in a container for field culture (Photo Author)

blueberry) the highest egg number per clutch occurred in bare soil (mean 48.7). The highest number of clutches per 1 m^2 was found on vegetation-covered soil in those habitats (mean 5.6). Some clutches among fruit bushes and under weed turf on baulks were distributed close to each other and joined to form "nests" of 10–15 cm^2. Such joint clutches included from 300 to 500 eggs. Four nests with 298, 348, 385 and 497 eggs were observed. Generally, among arable fields the slugs laid the highest number of eggs in

Table 1. *Arion lusitanicus*. Sites of egg-laying: Łańcut, Albigowa, Wysoka

Site	Number of clutches	Minimum number of clutches per m^2	Maximum number of clutches per m^2	Mean number of clutches per m^2	Minimum number of eggs per clutch	Maximum number of eggs per clutch	Mean number of eggs per clutch
Field crops							
On bare soil	35	1	3	1.3	14	26	20.4
On vegetation-covered soil	55	1	8	3.3	24	124	53.5
In bare soil	25	1	4	2.6	19	56	37.5
In vegetation-covered soil	50	1	6	5.1	18	64	43.6
In compost	25	2	3	2.3	50	104	67.3
Bush thickets							
On bare soil	25	2	3	2.7	12	46	26.4
On vegetation-covered soil	30	3	8	5.6	16	58	27.5
In bare soil	40	1	5	2.5	15	121	48.7
In vegetation-covered soil	35	3	9	4.7	14	67	31.2

Table 2. Characteristics of reproduction of *Arion lusitanicus* under field conditions – Poznań 1998

Trait	N	Minimum	Maximum	Median	Mean	SD
Eggs/indiv.	14	243.0	541.0	401.0	405.0	114.0
Clutches/indiv.	14	3.0	7.5	4.5	5.1	1.5
Eggs/clutch	71	5	185	64	67.5	46.8
Egg-laying days	7	24	65	43	44.1	14.5

unarable places such as field edges and baulks. This was especially visible during ploughing, when in places on baulks hundreds of eggs were scattered on the soil surface.

In the field cultures in Rzeszów, the slugs laid eggs from August 18th till December 13th 1997, and from August 10th till November 7th 1998. A shorter period of egg-laying in the second year of observations resulted from subzero air temperatures persisting from the second decade of November till the end of December. The first slugs hatched on September 19th in 1997 and on September 7th in 1998. In both seasons hatching continued till the temperature dropped to about 5°C.

A single individual of *A. lusitanicus* laid, on an average, 405 eggs (slugs reared in Poznań, Table 2) in 5 clutches. The clutches included from 5 to 185 eggs, on an average 68 eggs per clutch.

LABORATORY EXPERIMENTS

Under laboratory conditions (17–19°C, RH 93–95%, 16 h daylight) individual slugs laid eggs 6 to 11 times, the egg-laying period lasting from 18 to 57 days (mean 38.1, Table 3). An individual laid from 3 to 95 eggs at a time, sometimes adding them to those previously laid. The total number of eggs laid per slug varied from 122 to 382 (mean 265.6). The eggs were laid in clutches of 5–190 (mean 60.2). Following oviposition, ca. 75% slugs died, the first individuals (15%) died as early as 10 days after laying the last egg, while the remaining ones died within the next 15 days. The time from egg-laying to hatching of the first young (Fig. 3) amounted to 26–49 days (mean 36.3, Table 3). The values of the presented parameters: time from egg-laying to hatching and percentage of hatched eggs, varied widely.

The rate of egg development in the laboratory (Table 4, Fig. 4), at a constant temperature of 10°C was significantly slower than that at 15 and 20°C; the average time from egg-laying till hatching at 15°C was significantly longer than at 20°C. At the temperature of 25°C the eggs became mould-covered and none of them hatched. The mean percentage of hatched eggs was significantly lower at 10°C (30.5%) than at 15°C (85.5%). The percentage of hatched eggs did not differ significantly between the temperatures of 15 and 20°C, and amounted to 54 and 85.5%, respectively. The temperature of 15°C seems to be favourable for the egg development.

The rate of hatching at the three mentioned temperatures is presented as regression equations (Fig. 4), the regression being rectilinear for 10°C ($p = 0.000$, determination coefficient $R^2 = 77.0\%$) and curvilinear for 15 and 20°C ($p = 0.000$, $R^2 = 71.0\%$, and $p = 0.000$, $R^2 = 79.8\%$). The dependence between the number of days elapsed from egg-laying and the percentage of hatched eggs at each temperature was determined for a corresponding period of hatching of 18–21 days (Fig. 4, Table 4). Despite a good adjustment (satisfactory values of critical significance level p and determination coefficient R^2) of the obtained regression functions with the experimental data for the first days of hatching (at 10 and 15°C) these functions assume negative values. Similar negative values were obtained at the adjustment of exponential, logarithmic, logistic and other functions, and the adjustment was worse. The increment rate of the number of hatched eggs varied between the temperatures. In the first decade of hatching period, the number of hatched young increased the most rapidly at 20°C and in the second decade at 15°C (tangent of the angle of inclination of regression lines determined for the first and second part of a two-decade period of hatching at each temperature).

Table 3. Characteristics of reproduction of *Arion lusitanicus* under laboratory conditions

Trait	N	Minimum	Maximum	Median	Mean	SD
Eggs/indiv.	20	122.0	382.0	265.5	265.6	100.3
Clutches/indiv.	20	3.0	5.5	4.3	4.3	0.8
Eggs/clutch	85	5.0	190.0	50.0	60.2	42.0
Egg-laying days	20	18.0	57.0	35.0	38.1	13.0
Days to hatching	20	26.0	49.0	36.5	36.3	7.8

Fig. 3. Eggs and hatchlings of *A. lusitanicus* on a Petri dish (Photo Author)

Table 4. Number of days from egg-laying to hatching and percentage of hatched eggs of *Arion lusitanicus*, and results of TUKEY's test at $\alpha = 0.05$

Trait	Temperature	N	Minimum	Maximum	Meadian	Mean	SD
Number of days	10°C	61	44	65	55	54.5 a	4.7
Number of days	15°C	171	31	52	45	46.0 b	3.9
Number of days	20°C	108	25	43	29	30.3 c	3.4
Percentage hatched	10°C	4	6	42	37	30.5 b	16.5
Percentage hatched	15°C	4	78	96	84	85.5 a	7.7
Percentage hatched	20°C	4	20	70	63	54.0 ab	23.6

DISCUSSION

A. lusitanicus starts mating in the second half of July. The mating period lasts for 2.5–3 months, its duration being determined chiefly by the air temperature. Temperatures below 10°C are likely to limit the mating activity, whereas a further decrease in temperature to 5°C precludes copulation. In Glasgow (UK), *A. lusitanicus* copulates from the end of July till the beginning of September (DAVIES 1987) i.e. for about a month. Egg clutches of *A. lusitanicus* are laid directly on the ground or under the soil surface, both in bare and vegetation-covered places. They are found under stones, stumps, branches, in compost and under various objects lying on the ground (DAVIES 1987, VON PROSCHWITZ 1994, KOZŁOWSKI 1995).

Observations on the occurrence of slug eggs in plant cultivations indicate that the most numerous clutches are laid on field edges and baulks, i.e. places rarely disturbed during the vegetation season. The information may be of importance when searching for methods to control this pest. Individuals of *A. lusitanicus* reared under field conditions laid over 400 eggs. Similar numbers of eggs per slug were reported by VON PROSCHWITZ (1992, 1994). Under laboratory conditions (17–19°C, RH 93–95%, 16 h daylight) the

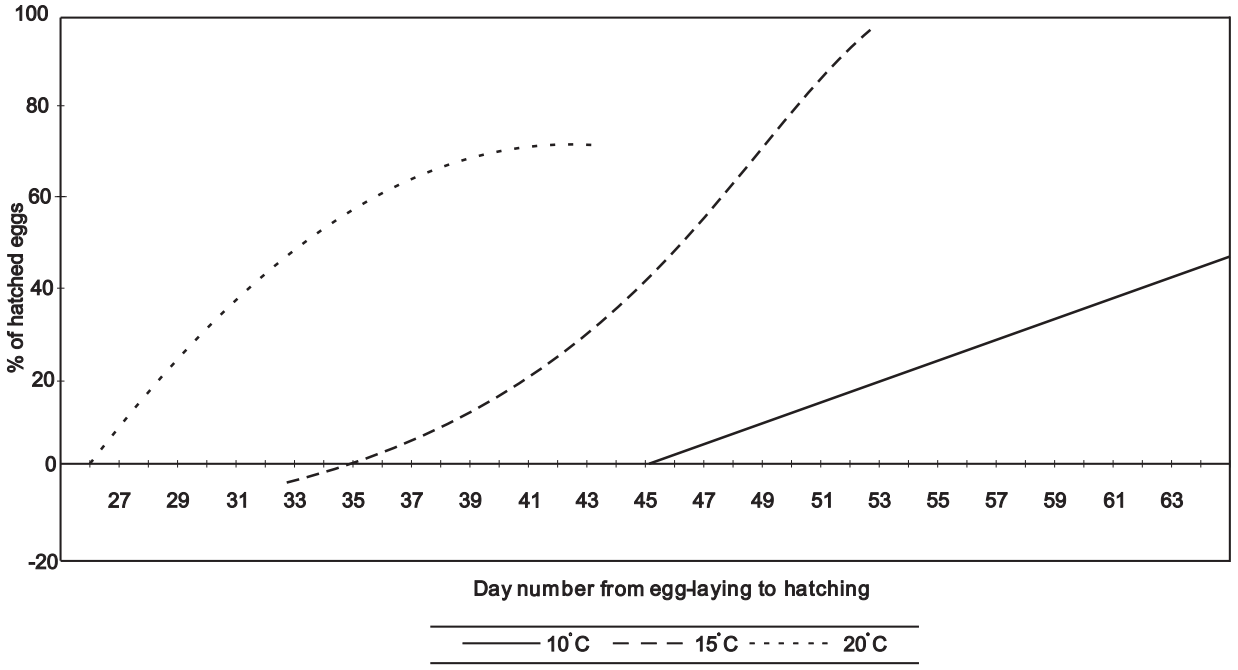

Fig. 4. Hatching rate of *A. lusitanicus* at three temperatures. Regression equations: $y_{10} = -96.01 + 2.14x$; $y_{15} = 170.74 - 11.80x + 0.20x'$; $y_{20} = -333.43 + 19.25x - 0.23x'$

fecundity was lower (mean 266 eggs) than in the field. Perhaps the transport of slugs to Poznań, where they were kept in a warm laboratory, and the effect of containers (unfavourable soil conditions, reduced air circulation, limited space etc.) had an adverse effect on their condition, thus reducing the number of laid eggs. According to DAVIES (1987) rearing this slug in laboratory is very difficult and often fails. This imposes certain limitations on attempts at tracing of egg-laying and development. Eggs of *A. lusitanicus* observed in Poland are spherical or oval, of 4.2 × 3.5 mm. According to QUICK (1952) eggs of *A. lusitanicus* are smaller (3.1 × 2.8 mm). In cannot be excluded that the egg size reported by QUICK (1952) pertains to another form of *A. lusitanicus* (sensu QUICK = *A. flagellus* Collinge (DAVIES 1987). DAVIES (1987) reports that the major diameter of eggs of *A. lusitanicus* exceeds 4 mm, i.e. like in *A. lusitanicus* from Poland. Both the observations from Poland and the results obtained by DAVIES (1987) indicate that most slugs die after egg-laying. Studies on the rate of egg development at four temperatures (10, 15, 20 and 25°C) reveal that the time from egg-laying till hatching of the first slugs is the shortest (mean 30.3 days) at 20°C. At 17–19°C young hatched 6 days later, and at 15°C – 16 days later. The percentage of hatched eggs was the highest (85.5%) at 15°C. It seems thus that temperatures of 15–17°C are the most favourable for the development of the slug eggs. To draw further conclusions, the egg development at temperatures below 10°C should be examined. This is especially important, since many eggs of *A. lusitanicus* winter over, the young hatching only next spring, which indicates that they may develop sucessfully also at lower temperatures. The obtained values of the time from egg-laying to hatching and the percentage of hatched eggs, especially at 15–17°C, are much variable. This indicates that under natural conditions both the fecundity and hatching of slugs may vary depending on various abiotic and biotic factors. According to DAVIES (1987) the egg viability and the size of clutch of *A. lusitanicus* are variable. She often found clutches with only infertile, or with mixed, fertile and infertile eggs. Some individuals fail to lay eggs which accumulate in their oviduct, the slug dying as a result of the pressure (DAVIES 1987). Despite this, *A. lusitanicus* shows a high biological potential and spreads in countries of Western and Central Europe, in Great Britain, Scandinavia (DAVIES 1987, VON PROSCHWITZ 1992, 1994) and Poland (KOZŁOWSKI 1995, KOZŁOWSKI & KORNOBIS 1995).

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