Growth and reproduction of the slug *Deroceras laeve* (Müller) (Pulmonata: Stylommatophora) under controlled conditions

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Abstract

The marsh slug *Deroceras laeve* (Müller) (Pulmonata: Stylommatophora) was recently introduced into the southeast of the Buenos Aires Province of Argentina, and has become one of the main pests of cropping systems under no-tillage management. The growth, survival, and reproduction of *D. laeve* were monitored at 12ºC and 20ºC to determine the most appropriate thermal conditions for its development. A sigmoid model $\{Y = A /[1 + e^{(B+Cx)}]\}$ was used to describe the relationship between postembryonic development and temperature. At the beginning of development, the growth rate was lowest at 12ºC but after the inflection point ($2^1/_2$ months) was lower at 20ºC than at 12ºC. The slugs showed a higher mean body weight at 12ºC (936.2 mg ± SE 18.96) than at 20ºC (409.4 mg ± SE 16.02). Significant differences were seen in the mean incubation period at the different temperatures, as well as in the hatching rate, the duration of the preoviposition and oviposition periods, the number of eggs per clutch, and longevity; no differences were seen, however, in terms of mean fecundity. The incubation period was 35.3 and 16.03 days and the hatching rate 89.2% and 66.4% at 12 and 20ºC respectively. At 20ºC, longevity was less than half (21.9 weeks) that recorded at 12ºC (54.4 weeks). At both temperatures the net reproductive rate (R_0) was similar, but at 12ºC the population increased 400 times in 33.1 weeks (T), while at 20ºC it increased 359 times in half that time ($T = 16.5$ weeks). These results show that, in the Buenos Aires Province, *D. laeve* produces two generations per year.

Additional key words: biological parameters, growth curve, life table parameters, temperature.

Resumen

**Análisis del crecimiento y reproducción de *Deroceras laeve* (Müller) (Pulmonata: Stylommatophora)
bajo condiciones controladas**

Deroceras laeve (Müller) (Pulmonata: Stylommatophora) es una especie introducida que, en el sudeste de la provincia de Buenos Aires, Argentina, ha sido reconocida como una plaga en sistemas de cultivo bajo siembra directa. Se estudió el crecimiento, la supervivencia y la reproducción de *D. laeve* a 12ºC y 20ºC para determinar cual es la temperatura más apropiada para su desarrollo. Para describir la relación entre la tasa de crecimiento y la temperatura se utilizó el modelo sigmoideo $\{Y = A / [1 + e^{(B+Cx)}]\}$. Al comienzo del desarrollo, la tasa de crecimiento fue más baja a 12ºC y después del punto de inflexión ($2^1/_2$ meses), la tasa de crecimiento fue más baja a 20ºC que a 12ºC. Las babosas criadas a 12ºC tuvieron un peso promedio mayor (936,2 ± 18,96 mg) que a 20ºC, alcanzando éstas aproximadamente la mitad del peso (409,4 ± 16,02 mg). Se hallaron diferencias significativas tanto en el período de incubación como en la fertilidad, períodos de preoviposición y oviposición, número de huevos por desove y la longevidad; sin embargo, no hubo diferencias significativas en la fecundidad. El período de incubación fue de 35,3 y 16,03 días, con una fertilidad de 89,2% y 66,4% a 12ºC y 20ºC, respectivamente. La longevidad fue menos de la mitad a 20ºC (21,9 semanas) que a 12ºC (54,4 semanas). R_0 fue similar en ambas poblaciones, pero a 12ºC la población aumentó 400 veces en 33,1 semanas (T), mientras que a 20ºC incrementó 360 veces en la mitad del tiempo ($T = 16,5$ semanas). Teniendo en cuenta estos datos, en la provincia de Buenos Aires *D. laeve* se aproxima a una fenología bivoltina.

Palabras clave adicionales: babosa, curva de crecimiento, parámetros biológicos, parámetros de la tabla de vida, temperatura.

Introduction

The marsh slug *Deroceras laeve* (Müller) is a species native to North America, where it is most widespread (Hammond, 1996), although it has been introduced into England, Europe, Russia, New Zealand (South, 1992) and South America (Cazzaniga, unpublished).

Adequate moisture is crucial for slugs, which have a permeable integument and high body water content (78-92% of its shell-free body weight). Their survival depends in large part on their behaviour, but also on their remarkable ability to tolerate dehydration (Luchtel and Deyrup-Olsen, 2001).

When land is managed under conservation tillage, in which there is no mechanical disturbance of the soil, the retention of litter or residue and the very moist habitat that may develop favours slug survival and reproduction (Costamagna *et al.*, 1999). In the southeast of the Buenos Aires Province (Argentina), *D. laeve* is recognized as a pest of crops where conservation tillage is practised. As this tillage system has increased in popularity, wheat, sunflower, corn and soybean crops have all been damaged. The species feeds on seeds and seedlings, causing a reduction in plant density (LISEZA, 1997).

In contrast with other terrestrial slugs (e.g., *D. reticulatum*), there is little published information on the growth and reproduction of *D. laeve*. The determination of its development rate at different temperatures is vital before integrated pest management can be attempted. The aim of the present study was to monitor the development and reproduction of this species under two thermal conditions.

Material and Methods

The study was conducted from April 2004 to October 2005. Seventy mature specimens of *D. laeve* were collected from a sunflower crop (*Helianthus annuus* L.) at Balcarce, in the southeast of the Buenos Aires Province (37º 45' S, 58º 18' W). They were placed on moist soil in plastic boxes (5,819 cm³) with holes made in the lid (which allowed for air circulation while ensuring a moist atmosphere). They were fed dry rabbit food. These boxes were placed in a rearing chamber at 20 ± 1ºC with a 12 h dark and light period (LD:12/12 h) for one week.

On April 27, 2004, two groups of 20 slugs were taken at random and assigned to rearing conditions of 12ºC (LD: 8/16 h) or 20ºC (LD: 12/12 h). The slugs were placed in individual plastic boxes (385 cm³) for egg production. The eggs in the clutches were counted, and then removed and placed in individual boxes (385 cm³) with damp filter paper. These were then returned to the rearing chambers of their parents and examined daily. A total of 387 eggs (in three cohorts, i.e., those eggs laid on the same day) were collected at 20ºC and 259 eggs (four cohorts) at 12ºC for the determination of the incubation period and egg fertility.

To study slug growth, 112 individuals were selected as the eggs at 20ºC hatched (six cohorts, i.e., animals born on the same day), along with another 90 individuals (10 cohorts) from those that hatched at 12ºC. Each cohort (the number of individuals differed between cohorts) was placed in a separate 385 cm³ boxes and examined weekly, at which times the boxes and food were changed. The slugs were weighed at one month intervals using an electronic analytical balance (accurate to 0.01g) according to South (1982) and Hommay *et al.* (2001). The same person carried out each weighing at the same time of day in order to be as consistent as possible.

To study slug reproduction, 38 individuals were kept at 20ºC, and 40 at 12ºC. Weekly examinations were made when their boxes and food were changed. After the slugs started to reproduce, their eggs were counted and removed.

The life history variables determined were incubation period (days), hatching rate (%), duration of the preoviposition and oviposition periods (weeks), fecundity (eggs/slug), number of eggs per clutch, and longevity (weeks). Data were recorded until the slugs died.

Life tables were constructed on the basis of adult survival and the reproduction rate of the 38 individuals at 20ºC and 40 individuals at 12ºC recorded.

To describe the growth of the slugs, the corresponding data were fitted to a sigmoid curve with the equation:

$$Y = A / [1 + e^{(B+Cx)}] \qquad [1]$$

where Y = weight (mg) at time x (month), A = asymptote, and B and C are variables that affect the curvature.

The inflection point (the mean body weight at which the growth rate started to decrease) was calculated from $(-B)/C$. The growth curve was plotted using the R.2.1.1 program (R Development Core Team, 2005).

The data for the biological variables at the two temperatures were analysed by general linear models

using SAS v.8 software (SAS, 2001). The duration of the oviposition period and longevity fitted a negative binomial distribution (McCullagh and Nelder, 1989). Differences in fertility were examined using the Chi-squared test. Survival and fecundity curves were constructed as described by Birch (1948). Survival, l_x, is the probability at birth of a slug being alive at age x; and age-specific fecundity, m_x, is the mean number of offspring produced per unit time by a slug aged x. Both l_x and m_x were established at weekly intervals.

The population variables estimated were: net reproduction rate (R_0; i.e., the rate of multiplication in one generation) and generation time (T, in weeks; the time required for the population to increase R_0 times) (Birch, 1948).

Results

The growth data for the 12ºC and 20ºC experiment were entered into Eq. [1]. Table 1 summarizes the values for variables A, B and C.

At the beginning of development, when slugs were one month old, the growth rate was lower at 12ºC than at 20ºC (Fig. 1). At the latter temperature, the inflection point was reached when they were 2.5 months old and the mean body weight was 204.7 mg. At the same age at 12ºC the body mass was 132.9 mg. At the lower temperature the growth rate increased with time until the animals were 4.5 months old, when they weighed

Table 1. Sigmoid equation parameters at the two temperatures. In parenthesis, standard error

Conditions	A	B	C
12°C	936.2 (18.96)	3.9521 (0.29)	–0.8647 (0.07)
20°C	409.4 (16.02)	1.8911 (0.13)	–0.7629 (0.07)

468.1 mg. However, at the same age at 20ºC the body weight was 340.4 mg, 86% of the maximum mean body weight and near to the asymptote (Fig. 1). In addition, slugs reached a greater mean body weight at 12ºC (936.2 mg ± SE 18.96) than at 20ºC (409.4 mg ± SE 16.02, i.e., approximately half the weight of the slugs kept at 12ºC).

Table 2 indicate the effects of being kept at 12ºC and 20ºC on reproduction and survival. Significant differences were seen for all of the biological variables examined (p < 0.0001), except for mean fecundity (eggs/slug) (p = 0.1653). Eggs were laid either in clutches or singly, mostly below the soil surface. The incubation periods were 35.3 and 16.03 days at 12ºC and 20ºC respectively; 89.2% of the eggs hatched at 12ºC and 66.4% at 20ºC. Longevity was significantly lower at 20ºC than at 12ºC (p < 0.0001) (Table 2).

At both temperatures development was completed, but longevity was more prolonged at the lower temperature (54.4 weeks at 12°C and 21.9 weeks at 20ºC) (Table 2). This greater longevity at 12ºC was related to
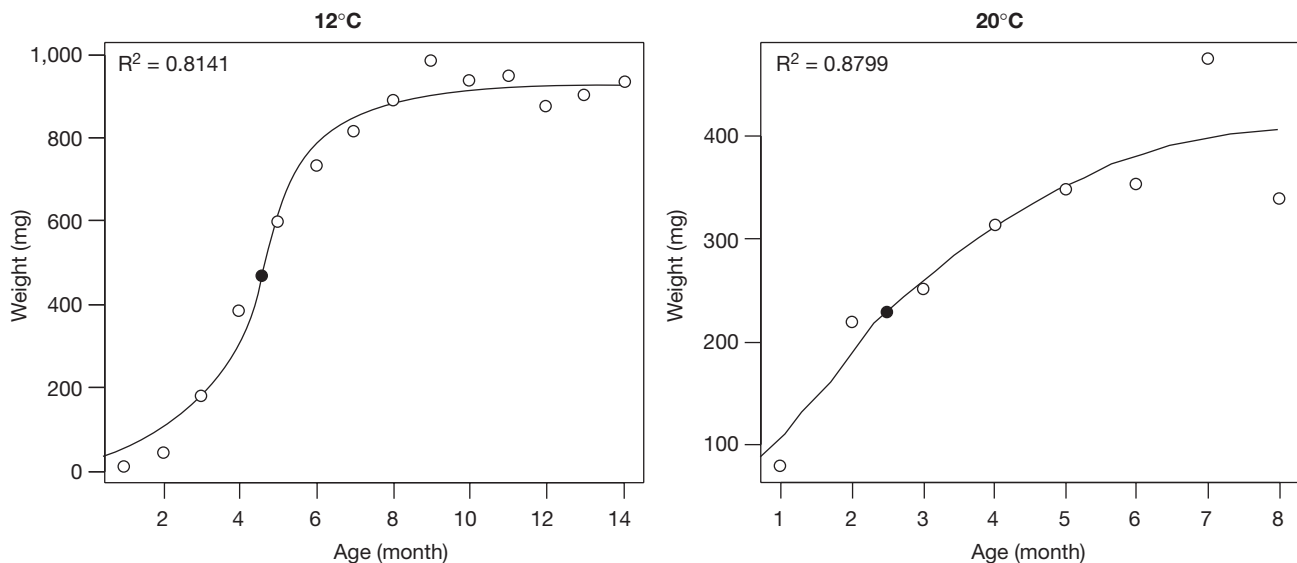

Figure 1. Growth curves of *D. laeve* at 12°C and 20°C. Black circles represent inflection points.

Table 2. Mean ± SD of *D. laeve* life cycle variables at 12°C and 20°C

Biological parameters	12°C	20°C	Degrees of freedom	p-value
Incubation period (days)	35.3 ± 2.1a	16.03 ± 1.6b	486	< 0.0001
Fertility (%)	89.2 a	66.4 b	CST	0.001
Preoviposition period (weeks)	18 ± 1.0a	8.5 ± 1.0b	77	< 0.0001
Oviposition period (weeks)	31 ± 10.4a	13 ± 4.6b	77	< 0.0001
Fecundity (eggs/slug)	407.6 ± 184.4a	352.9 ± 164.4a	78	0.1653
Number of eggs per clutch (eggs/clutch)	5.05 ± 1.0b	6.58 ± 1.4a	76	< 0.0001
Longevity (weeks)	54.4 ± 11.2a	21.9 ± 5.1b	78	< 0.0001

Means in each column followed by the same letter are not significantly different (p > 0.05). SD: standard deviation. CST: Chi-squared test.

the beginning of oviposition, which was delayed for 10 weeks. In addition, the reproductive period was 18 weeks longer at 12°C than at 20°C.

The survival curve characteristics matched those of the Type 1 curve proposed by Deevy (1947 in Rabinovich, 1980) (Fig. 2). A rapid decline in survival was seen at approximately 48 weeks under the 12°C conditions, and at 20 weeks under the 20°C conditions, when population survival reached 70% (Fig. 2).

The midpoint of survival was attained at week 53 and 23 for those reared at 12°C and 20°C respectively; the greatest longevity was 69 weeks and 33 weeks respectively under these conditions respectively (Fig. 2).

The maximum daily egg production per slug and week (m_x) was lower at 12°C (20.25 eggs/slug) than at 20°C (62.58 eggs/slug). Although at 12°C fecundity was low, the time to egg laying was longer and the fecundity rate was stable (unlike at 20°C) (Fig. 2). The
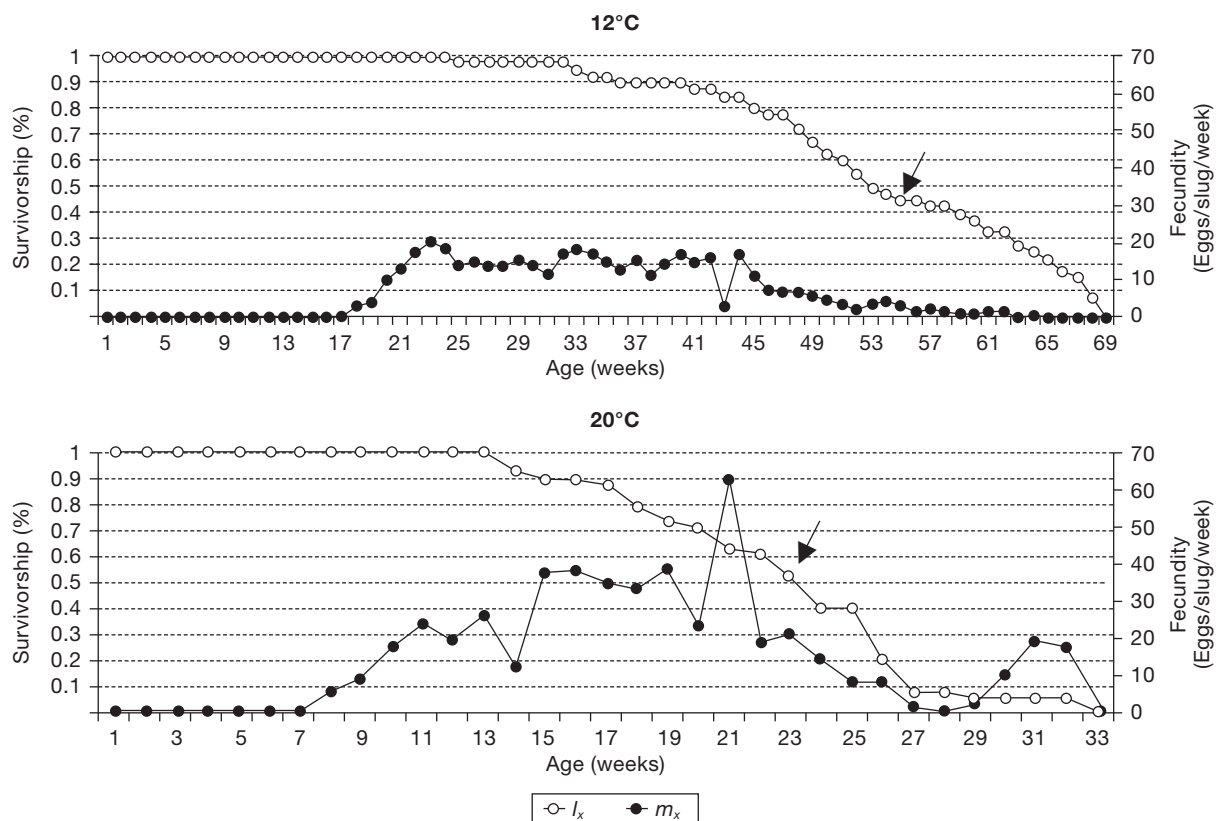

Figure 2. Age-specific survival (l_x, y axis on left) and age-specific fecundity (m_x, y axis on right) of *D. laeve* at 12°C and 20°C. The arrow indicates 50% survival.

maximum daily fecundity rates were attained when a large percentage of the population was alive.

Both populations had a similar net reproductive rate (R_0), but their mean generation time was different. At 12ºC the population increased 400 times in 33.1 weeks, while at 20ºC it increased 359 times in just 16.5 weeks.

Discussion

The literature contains no studies on the biology of *D. laeve* in Argentina; indeed there is little information on this species growing under controlled conditions from anywhere in the world. Although slugs are not always subject to constant temperatures in nature, this study provides valuable insight into the effects of 12ºC and 20ºC on the development time, survival, longevity and fecundity of this species.

Probably the most significant difference between this study and others made on the related species *D. reticulatum*, is the analysis of postembryonic development. Abeloos (1944, cited in South, 1992) and South (1982) found that under constant conditions of temperature, a logarithmic plot of body weight showed a linear increase with time. The mean slug body weight was plotted against time for each breeding condition and regression lines were fitted for each part of the growth curve corresponding to a different phase. However, more realistic results are obtained by fitting the data to a sigmoid model. The sigmoid curve not only helps explain the growth of these slugs, it provides an excellent tool for estimating the changes of their growth rate over the life cycle. Such a curve can also exactly determine the pre-reproductive and reproductive periods.

According to Abeloos, British populations of the genus *Deroceras*, such as those of the species *D. laeve*, are characterized by two growth phases: juvenile and mature. However, the present results suggest that a general sigmoid model reflects the growth pattern of the slug. The present results establish that 50% of the maximum mean body weight (the asymptote) is achieved at the inflection point, when the slugs start to lay eggs. Under these circumstances, the growth curve for *D. laeve* has two phases: a juvenile phase of pre-oviposition, and a mature phase of oviposition during which the slugs lay their eggs.

Self-fertilization is the normal breeding system in *D. laeve,* although related species, such as *D. reticulatum,* rarely produce self-fertilized eggs and have been noted for their ability to outcross (McCraken and Selander, 1980). The present study confirms that isolated individuals of *D. laeve* produce fertile eggs. This is an important observation since the type of breeding system used is related to colonizing ability; self-fertilizing species appear to be disproportionately successful in colonizing different habitats (Foltz, 1984, in South, 1992).

The eggs of *D. laeve* showed similar hatching times to those reported by Taylor (1902-1907, cited in South, 1992), about 20-40 days. Carrick (1942, in South, 1992) reported 18-22 days at 20 ± 1ºC for *D. reticulatum*.

At 20ºC, fertility was low (66.4%), and showed discrepancies with data for other species such as *D. reticulatum* (84.4%). However, at 12ºC fertility is similar in both (89.2% for *D. laeve* and 91.1 for *D. reticulatum*) (N. L. Clemente, unpublished).

Longevity decreased as temperature increased, from 54.4 weeks at 12ºC to 21.9 weeks at 20ºC. At 12ºC, *D. laeve* appears to have a life cycle similar to that of *D. reticulatum*, which is completed in 50.07 weeks, while at 20ºC the life cycle lasted 34.92 weeks (N. L. Clemente, unpublished data). This explains why *D. reticulatum* is distributed over much of the world; it adapts well to a wide temperature range.

Just before the slugs died their bodies became harder and darker, a process that continued after their death. According to Szabó (1935, in South, 1992) this is also common in *D. agreste*, while *D. reticulatum* tends to become translucent shortly before death (South, 1982). This information on slug health is vital for forecasting and pest management.

Finally, Burenkov (1977, cited in South, 1992) described a univoltine phenology for *D. laeve* in the Moscow region, while Boag and Wishart (1982, cited in South, 1992) found that in the Alberta region of Canada it was an annual species which over-wintered as immature slugs. However, generation time data and field observations recorded in the southeast of the Buenos Aires Province (P. L. Manetti, unpublished) show *D. laeve* approximates to a bivoltine phenology. Individuals reproduce only once during their lifetime, either in autumn or spring. The reason for this may lie in the small seasonal changes in the temperate regions of South American countries, where *D. laeve* is now apparently widely distributed (Cazzaniga, unpublished).

Acknowledgements

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