RESEARCH NOTE

Egg production in *Arion vulgaris*: density dependence in *A. vulgaris* and interspecific effects of *Limax maximus*

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Egg production in terrestrial slugs is a key factor that influences population development. Both density-dependent and density-independent effects on egg production may therefore control population growth (South, 1992). Furthermore, interspecific competition may mediate niche segregation, habitat exclusion or local extinction (Watz & Nyqvist, 2022). In terrestrial slugs, exploitation competition is probably rare, whereas there are several examples of interference competition, both direct via aggressive behaviour (Wellington & Rollo, 1979; Cook, 1981; Rollo, 1983) and indirect via mucus trails (O'Hanlon *et al.*, 2020). One slug species that has been shown to behave aggressively towards conspecifics and heterospecifics is *Limax maximus* (Linnaeus, 1758), and its behaviour may negatively affect the reproduction of other large slugs (Rollo, 1983). These slugs likely compete for day shelters as well as suitable egg-laying habitat, and may feed on each other's eggs.

In private gardens (and to some extent in commercial agriculture), *Arion vulgaris* (Moquin-Tandon, 1855) has, during the last decades, emerged as one of the most serious invasive mollusc pests throughout large parts of Northern Europe (von Proschwitz, 1997; Kozłowski & Kozłowski, 2011; Zając *et al.*, 2017). Measures have been developed to control this species (Adomaitis *et al.*, 2022; Watz & Nyqvist, 2021). In many areas, *A. vulgaris* lives sympatrically with other large gastropods, and there are anecdotes circulating among gardeners that different species, such as *Helix pomatia* and *L. maximus*, may reduce the density of *A. vulgaris* (Ørmen *et al.*, 2010; Dörler *et al.*, 2021). Clear evidence for a negative effect of interspecific competition on *A. vulgaris* populations is, however, lacking.

In this study, we investigated how the egg laying of *A. vulgaris* was affected by the addition of conspecifics and heterospecific *L. maximus*, using replicated mesocosms. We hypothesized that (1) there would be a negative density-dependent effect on egg production and that (2) the effect would be stronger when the competitors were *L. maximus* than when the competitors were conspecifics. Specifically, we predicted that the numbers and total mass of eggs per slug would be higher in mesocosms with low than high slug density, and that the lowest mass and numbers would be found in mesocosms where *L. maximus* had been added.

The experiment was carried out in a private garden in Kristinehamn, Sweden (WGS 84: N59.32°, E14.11°), during the egg-laying season between 5 August and 21 September 2021, and shielded from sunlight under a roof. We collected slugs in the neighbouring area, and 48 *A. vulgaris* (mean ± SD = 11.7 ±

4.0 g) and 12 *L. maximus* (10.1 ± 2.3 g) were used in the experiment. The mesocosms consisted of plastic containers (length × width × height = 58 × 35 × 39 cm). The bottom of the containers was lined with moss, fern leaves and birch bark, which created a suitable egg-laying habitat. Slugs were fed pieces of apple throughout the experiment. Temperature varied between 6 and 22 °C.

Eggs from *A. vulgaris* are opaque (Kozłowski, 2000), whereas eggs from *L. maximus* are semitransparent (Fig. 1) and thus easily distinguished. On five occasions (23 August and 1, 7, 13 and 21 September), we searched the mesocosms carefully for eggs. *Limax maximus* eggs were weighed, whereas *A. vulgaris* eggs were weighed and counted. In addition, we recorded the number of egg clutches and whether the eggs in a clutch were untouched or spread out (the latter is an indication of egg predation). All eggs found at these five occasions were removed from the mesocosms, along with any slugs that were found dead.

We manipulated the slug density in the mesocosms by using three different treatments in the experimental design: (1) two individuals of *A. vulgaris*, (2) four of *A. vulgaris* and (3) two each of *A. vulgaris* and *L. maximus*. Each treatment was replicated six times, resulting in a total of 18 mesocosms. Thus, we had one base level treatment with low density and two treatments with higher density, either by adding conspecifics or heterospecifics. Dead slugs were removed but not replaced.

We analysed three response variables: (1) the number of *A. vulgaris* eggs averaged per individual found in each mesocosm, (2) total egg mass averaged per individual and (3) mean mass of individual eggs (i.e. egg size). The mean individual egg mass was calculated as the total egg mass divided by the number of eggs. Normality and homogeneity of variances, respectively, were tested using Shapiro–Wilk's tests ($P > 0.05$) and Levene's tests ($P > 0.05$). To investigate the effects of treatments on the response variables 1–3, we used an ANCOVA, where the logarithm of the mean mass of the *A. vulgaris* in each mesocosm was included as a covariate. In addition, we tested if *A. vulgaris* mortality (proportion of deceased individuals) differed among treatments using a Kruskal–Wallis test.

We found in total 53 egg clutches from *A. vulgaris*. The mean number of eggs per individual was 51 (mass = 1.6 g) and the mean number of egg clutches per individual was 1.1. Seven clutches (13%) had scattered eggs; six of these were found in mesocosms with two *L. maximus* and two *A. vulgaris*, whereas one scattered egg
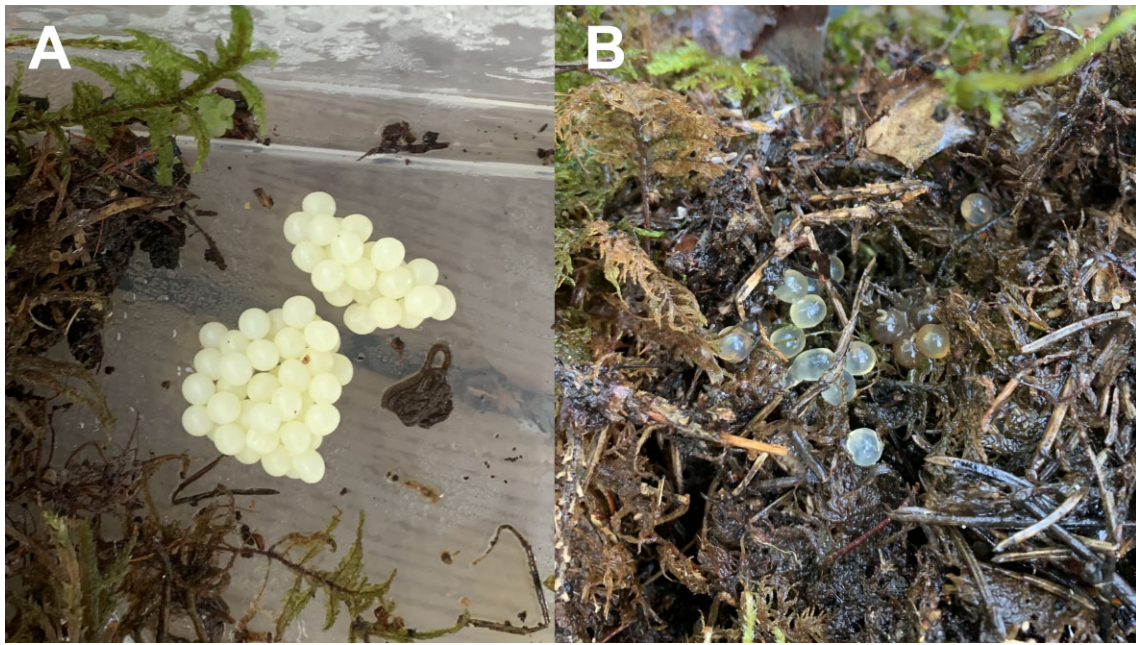
Figure 1. Eggs of *Arion vulgaris* (**A**) and *Limax maximus* (**B**). Data on egg laying was collected from mesocosms between 5 August and 21 September 2021.

clutch was found in a mesocosm with four *A. vulgaris*. No eggs were scattered in mesocosms with two *A. vulgaris*. During the experiment, 33 *A. vulgaris* died (69%). This mortality was expected because this species dies in the autumn after egg laying (Kozłowski, 2000; Slotsbo *et al.*, 2013). Two individuals of *A. vulgaris* (4%) died without any eggs found in their mesocosms; these two mesocosms also contained *L. maximus*. We observed bite marks on some *A. vulgaris*, but these injuries were not quantified. Density treatment did not affect *A. vulgaris* mortality ($H_2 = 1.442$, $P = 0.486$, $\varepsilon^2 = 0.085$).

There was a nonsignificant trend that more *A. vulgaris* eggs per individual were found in mesocosms with a low density of *A. vulgaris* than in those with a high density ($F_{2,14} = 3.356$, $P = 0.064$, $\eta_p^2 = 0.324$; Fig. 2A). With a similar pattern and a significant effect, total egg mass per *A. vulgaris* individual was higher in low- than high-density mesocosms ($F_{2,14} = 4.184$, $P = 0.034$, $\eta_p^2 = 0.384$; Fig. 2B). Treatment did not affect the mean egg size (i.e. mean mass of individual eggs; $F_{2,14} = 2.473$, $P = 0.126$, $\eta_p^2 = 0.292$; Fig. 2C). There was a significant positive effect of $\log_{10}$-transformed *A. vulgaris* mean body mass on both number of eggs ($F_{1,14} = 4.856$, $P = 0.045$, $\eta_p^2 = 0.258$) and total egg mass per individual ($F_{1,14} = 7.322$, $P = 0.017$, $\eta_p^2 = 0.343$), as well as on egg size ($F_{1,14} = 9.449$, $P = 0.010$, $\eta_p^2 = 0.441$). The mean total egg mass laid per *L. maximus* individual was ($\pm$ SE) 3.9 ± 0.9 g.

There was a negative effect of slug density on the egg laying of *A. vulgaris*, but whether the added slugs in the high-density treatments were conspecifics (*A. vulgaris*) or heterospecifics (*L. maximus*) played a minor role. The effect of density was large, both on the number of eggs and egg mass, but not on egg size. Although only the analysis of the effect on egg mass resulted in a statistically significant difference between treatments, the high egg mass observed in the low-density treatment was likely caused by an increase in eggs laid rather than an increase in egg size. Our results do not provide clear evidence for our prediction that *L. maximus* would negatively influence the reproduction of *A. vulgaris*. On the other hand, scattered egg clutches, indicating the occurrence of egg predation, were almost exclusively found in mesocosms with *L. maximus*. Previous studies of the interspecific effects of *L. maximus* on other large slugs (*Arion ater* and *Ariolimax columbianus*) using field cages have shown that *L. maximus* can reduce both the number of eggs laid and egg size in other species (Rollo, 1983), as well as hindering access to suitable

egg-laying habitat (Rollo & Wellington, 1979). These studies were carried out without replication of the treatments, but they nevertheless demonstrate that the aggressive behaviour of *L. maximus* can influence the reproduction of arionid slugs. Based on the findings in our study, however, it is unlikely that adding *L. maximus* to a locality with high densities of *A. vulgaris* can reduce the population of the latter species through negative effects on egg production. Although there was evidence for a negative density-dependent (intra- and interspecific) effect on egg laying, the species composition of the competing slugs seemed to be of minor importance.

We used relatively high slug densities in our mesocosms (10 or 20 individuals m^{-2}, depending on the treatment). The invasive *A. vulgaris* can, however, occur at such densities in the wild. In a study of crop damage by *A. vulgaris*, for instance, Kozłowski & Kozłowski (2011) found between 10 and 21 individuals m^{-2} in more than 70% of their sites. In our study, there should have been many opportunities for direct and indirect (via mucus trails) interference, and the possibility to flee was limited. We expected that *L. maximus* would behave aggressively towards *A. vulgaris* in our mesocosms (Rollo, 1983). Indeed, the only mortality of *A. vulgaris* in mesocosms where no eggs were found (indicating another cause of death than that of the normal post-egg-laying mortality) occurred in mesocosms containing *L. maximus*. This circumstance, however, did not have any major effects on the overall pattern of egg production, and mortality rates did not differ among treatments.

The negative effect of density on the number or mass of eggs could either have been caused by fewer eggs laid or by egg predation (or egg cannibalism). Egg production in terms of the number of eggs and clutches, as well as egg size and timing, is under endocrine control (South, 1992). Individual slugs seem to be able to adjust these reproductive features to both biotic factors, such as competition (Rollo, 1983; Watz & Nyqvist, 2022), and the physical environment (South, 1992). Our study design did not allow us to identify the mechanism behind the negative effect. There was some evidence that supported the occurrence of egg predation from *L. maximus* (as indicated by scattered egg clutches), and less so of egg cannibalism. Nonetheless, there was no clear difference between the number of eggs between treatments with four individuals of *A. vulgaris* vs those with two of *A. vulgaris* and two of *L. maximus*; if egg predation played a major role, we would have expected to
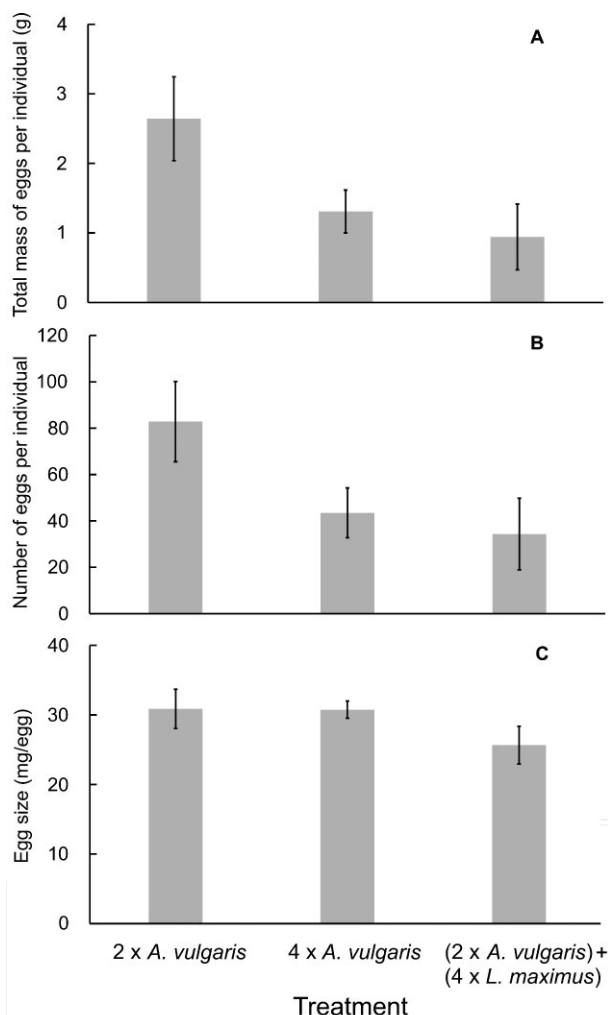
Figure 2. Reproduction of *Arion vulgaris* in mesocosms inhabited by two individuals of *A. vulgaris*, four of *A. vulgaris* or two each of *A. vulgaris* and *Limax maximus*. **A, B.** Mean number (**A**) and total mass (**B**) of eggs per individual. **C.** Egg size. Error bars indicate ± 1 SE.

observe a clear difference between these two treatments. We do not know, however, if the effect of *L. maximus* would have been larger if we had not removed the eggs found during the experiment. Egg predation might play a greater role if the slugs had more time to consume eggs, and our study thus addresses only one possible constraint in *A. vulgaris* reproductive output. Possibly energy allocation to egg production was influenced by slug density, perhaps as an effect of competition for egg-laying habitat. Most energy reserves for egg production were likely accumulated before the experiment started, but interactions with other slugs may influence egg resorption (Heller, 2001).

Future work might further address the mechanisms causing the reduced reproductive success evident in our high-density treatments, for example by combining direct observations of interference (Rollo & Wellington, 1979) with quantification of egg production and egg survival. Incorporating hatching and recruitment of juvenile slugs in a long-term mesocosm study would also address the potential accumulated egg predation over time, an aspect that we did not investigate in the current study. To study further how *L. maximus* may influence the egg laying of *A. vulgaris*, tracking the movements of the two species in a garden (Nyqvist *et al.*, 2020; Watz *et al.*, 2021) would potentially shed more light on interspecific interaction in a setting where displacement is also possible.

Egg production may constitute a key factor in the development of slug populations over time (South, 1992). A negative density dependence in egg production may to some extent control the development of arionid slug populations. Introducing another alien slug species to control the invasive *A. vulgaris* will, however, likely not be effective, at least in terms of a potential reduction in the reproductive success of *A. vulgaris*.

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