Predation by carabid beetles on the invasive slug *Arion vulgaris* in an agricultural semi-field experiment

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Abstract

Arion vulgaris Moquin-Tandon 1855 is one of the most important invasive species in Europe, affecting both biodiversity and agriculture. The species is spreading in many parts of Europe, inflicting severe damage to horticultural plants and cultivated crops partly due to a lack of satisfactory and effective management solutions. Molluscicides have traditionally been used to manage slug densities, although the effects are variable and some have severe side-effects on other biota. Thus, there is a need to explore potential alternatives such as biological control. The nematode *Phasmarhabditis hermaphrodita* is the only biological agent that has been applied commercially so far. However, other biological control agents such as carabid beetles have also been found to be promising. In addition, some carabid species have been shown to feed on *A. vulgaris* in the field as well as in the laboratory. Two species in particular have been found to be important predators of *A. vulgaris*, and these species are also common in agricultural environments: *Pterostichus melanarius* and *Carabus nemoralis*. This study is the first to use semi-field experiments in a strawberry field, manipulating densities, to investigate how *P. melanarius* and *C. nemoralis* affect densities of *A. vulgaris* eggs and juveniles, respectively. Gut contents of *C. nemoralis* were analysed using multiplex PCR methods to detect DNA of juvenile slugs. Results show that both *P. melanarius* and *C. nemoralis* significantly affect densities of slug eggs and juvenile slugs under semi-field conditions and that *C. nemoralis* seems to prefer slugs smaller than one gram. *Carabus nemoralis* seems to be especially promising in reducing densities of *A. vulgaris*, and future studies should investigate the potential of using this species as a biological control agent.

Keywords: *Arion vulgaris*, *Pterostichus melanarius*, *Carabus nemoralis*, semi-field experiments, juveniles, eggs, predation

Introduction

Gastropods are important crop pests in temperate and tropical regions (Barker, 2002). Their pest status has increased in the last 30 years, alongside many agricultural changes, probably due to the increasing demand for food quantity and quality. In many parts of the world, slugs as pests were unknown until zero-tillage or reduced cultivation practices were adopted along with changes in cropping patterns, e.g. increased areas of oilseed-rape (Glen, 2002; Speiser *et al.*, 2001). In Europe and in North America, slugs are serious pests of arable and vegetable crops as well as domestic gardens (Moens & Glen, 2002; Port & Ester, 2002). In North America,

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slugs are pests of field corn and soybeans (Hammond & Byers, 2002); and, in California, snails are pests of citrus production (Sakovich, 2002). In Norway, the most important introduction of an exotic gastropod species has been the so-called Iberian slug *Arion vulgaris* (von Proschwitz & Winge, 1994).

Arion vulgaris, which has traditionally been regarded as *A. lusitanicus* 1868 (Anderson, 2005; Quintero *et al.*, 2005), has been used as a model for invasive species with negative impacts on biodiversity and ecosystems (Grimm & Paill, 2001). It is an important plant defoliator and competes with native slug species, due to its large size and its high population densities. It is one of the most notorious slug pests in Europe, damaging horticultural plants and cultivated crops in agriculture (von Proschwitz & Winge, 1994; von Proschwitz, 1992, 2008; Dolmen & Winge, 1997). Many reasons have been proposed to explain its success: large body size, rapid reproduction, food tolerance and catholic feeding, ecological tolerance, and lack of enemies (Kozłowski, 2007). Its dispersive capacity can explain the invasion on local scale and also on larger scales, but the mechanism of this expansion is still not fully understood (Grimm & Paill, 2001; Grimm & Schaumberger, 2002).

Many authors have reviewed the role of ground-beetles as pest enemies (Thiele, 1977; Allen, 1979), mainly based on laboratory observations. Carabids have been found to feed on aphids (Andersen, 1992; Holopainen & Helenius, 1992), on dipterans (especially on eggs), and on coleopterans and lepidopterans (Kromp, 1999). In total, 30 species of carabids are currently known to predate slugs (Symondson, 2004; Hatteland, 2010). Some ground-beetles have been found to be effective in controlling slugs such as *Deroceras reticulatum* (Müller) (Agriolimacidae) and *Arion intermedius* Normand (Arionidae) in enclosures, where predation experiments indicated that carabid beetles and birds were responsible for significant mortality of the two slug species (Barker, 1991). *Abax parallelepipedus* Piller & Mitterpacher and *Pterostichus madidus* F. have been found to be more effective than the molluscicide methiocarb (Asteraki, 1993) in slug control when tested in sward boxes sown with white clover. No slugs were recovered from the carabid treatments, while more than half were recovered from the methiocarb treatment. *Abax parallelepipedus* has also been tested as a potential biological control agent against slugs in polythene tunnel experiments. This species was found to be highly effective at reducing the number of slugs and hence crop damage (Symondson, 1994). Studies involving semi-field experiments to test the impact of predation by *P. melanarius* on the slug *D. reticulatum* and emulating field conditions in outdoor mini-plots, demonstrate that *P. melanarius* can reduce slug populations under simulated field conditions (McKemey *et al.*, 2003). The population growth of carabid species was positively correlated with slug abundance, based on a study in an arable field over a five-year period (Symondson *et al.*, 2002). Another study found direct and active predation by *P. melanarius* against its slug prey (Bohan *et al.*, 2001). In another experiment (Oberholzer & Frank, 2003), the number of living oilseed-rape plants were counted under different treatments: only slugs present, slugs and *P. melanarius* present, and slugs and *Pterostichus cupreus* present. The number of living oilseed rape-plants was significantly lower in the treatment with only slugs, while the number of slugs was significantly lower in the treatment with *P. melanarius*, indicating that slug predation took place. Other studies have tested the predation by *P. melanarius* larvae on *D. reticulatum* and *A. intermedius*,

finding that the larvae killed both slug species (Thomas *et al.*, 2009).

The main goal of this study is to investigate the role of the carabid beetles *P. melanarius* and *C. nemoralis* as natural enemies of *A. vulgaris* under semi-field conditions. The following objectives were addressed: investigate if and how *P. melanarius* affects eggs of *A. vulgaris* under semi-field conditions; investigate if and how *C. nemoralis* affects juveniles of *A. vulgaris* under semi-field conditions; detect slug-DNA of juveniles in gut contents of beetles collected after the experiments.

Material and methods

Semi-field experiments were carried out in a strawberry field, in September 2010 and in May 2011. Eggs and juveniles of *A. vulgaris* were supplied as prey, and adults of *P. melanarius* and *C. nemoralis* were used as predators. Densities were manipulated to assess the effect of beetles on slug density. Predation on *A. vulgaris* juveniles was studied by detecting slug-DNA in the gut contents of beetles.

Sampling

Beetles and eggs were collected from an uncultivated field in a rural area outside Bergen, during August and September 2010, and April and May 2011. The meadow (60°38′N, 5°34′E) is partly surrounded by deciduous trees and is mown once a year. Beetles were collected with pitfall traps. Pitfall trapping is a well-known method for collecting beetles and is still regarded as the most convenient and cost-effective method (Greenslade, 1964; Luff, 1975; Lang, 2000; Ward *et al.*, 2001). Pitfall traps were arranged in the meadow in lines of 20 traps, 1–2 m between traps, with a total of approximately 300 traps. Traps were plastic cups 9.5 cm deep with a 7 cm opening, dug into the soil and covered by a metallic roof a few centimetres above the rim. The traps were checked and emptied every third day and target beetles were collected and put into plastic boxes with vegetation. All beetles collected were recorded and identified to species, sex and date of collection. They were kept in a climate chamber (Sanyo Incubator, MIR-553) at 14°C with simulated light conditions of the field (16 hours of light and eight hours of darkness). After collection, beetles starved one week to get rid of eventual external DNA contamination due to the permanence in the pitfall trap. Subsequently, they were fed earthworms once a week until the start of the semi-field experiment. Only *C. nemoralis* and *P. melanarius* were collected, both of which have previously been found to feed on *A. vulgaris* (Hatteland *et al.*, 2010, 2011). Eggs of *A. vulgaris* were collected in the field by searching the vegetation above ground. They were kept at 3°C to avoid hatching, in plastic boxes with water to avoid drought. Juvenile slugs were collected from Sletten, Bergen, and kept in the same conditions as slug eggs. Juvenile slugs and eggs were identified as *A. vulgaris* after observation in the field. The identification of juveniles was done based on the external morphological features of *A. vulgaris* juveniles, while eggs were determined by the shape, colour and size of eggs and clutches.

Semi-field experiment with slug eggs

In this experiment, we investigate if and how the presence of *P. melanarius* can affect slug densities. The experiment was established in a strawberry field in Askøy, an island near

Bergen (60°28′N, 5°12′E). The experiment was carried out from the 14th of September to the 23rd of September 2010. The strawberry patch was 38.5 m × 9.6 m and contained seven rows of strawberry plants, of which three were used. Thirty 1 × 1 m plots were created using metallic fences with a height of 30 cm. The fences were positioned in correspondence to the strawberry rows in order to include the vegetation inside each plot. The fences were dug 10 cm into the soil and a special anti-slug glue (Antischneck-Gel, Neudorff, GmbH, Emmerthal, Germany) was put on the fences to avoid the escape and the entrance of slugs. No manipulation of the plots was done prior to the experiment to maintain the natural field conditions. In each plot, three clutches of eggs were arranged, with 30 eggs per clutch, yielding a total of 90 eggs per plot. *Pterostichus melanarius* has been shown to be one of the most common and well-adapted carabid species in agricultural landscapes (Lövei & Sunderland, 1996; Thomas *et al.*, 1998). Moreover, a positive relationship has been found between slug biomass and numbers of beetles (Symondson *et al.*, 1996), and its peak of activity is around the middle of August, partly corresponding with the egg-laying period of *A. vulgaris* (Levesque & Levesque, 1994; Matalin, 2004; Kozłowski, 2007). Treatments were randomly assigned to the plots: ten plots with two beetles per plot, one female and one male; ten plots with three beetles per plot, two females and one male; and ten plots without any beetles as control. The number of beetles was chosen to reflect the natural density based on earlier surveys (Hatteland, 2010). Beetles were fed earthworms once a week prior to the experiment and then starved for one week before being used in the experiment. The beetles were then marked with a white spot using a marking ink pen to distinguish them from other beetles potentially present inside the plots. At the end of the experiment, eggs were re-collected for later analyses in the laboratory. Eggs were examined by counting and checking their status: eaten, hatched or unaffected.

Semi-field experiment with juveniles

In this experiment, we investigate if and how the presence of beetles can affect slug densities. Semi-field conditions using *A. vulgaris* juveniles and *C. nemoralis* were applied to test this. The experiment was established in the same strawberry field used for the previous semi-field experiment and was carried out from the 11th of May to the 21st of May 2011 using the same experimental design as in September. Juvenile slugs were collected and divided into three size categories corresponding to weight: 0.1 to 0.3 g, 0.4 to 0.8 g, and 0.9 to 1.2 g. Altogether, 15 slugs were put inside each plot, using six, six, and three specimens from the three size categories, respectively. The size and number of slugs was chosen to represent what is typically found in the field in May (Hatteland, unpublished data). Treatments were randomly assigned to the plots: ten plots with two beetles per plot, one female and one male; ten plots with three beetles per plot, two females and one male; and ten plots without any beetles as control.

At the end of the experiment, all plots were thoroughly searched to check for the presence of introduced *C. nemoralis* and *A. vulgaris* juveniles, moreover alternative predators and prey (carabid beetles, slugs and earthworms) were searched and collected. Introduced slugs and non-introduced ones were collected, identified and weighed. Carabid beetles were collected, subsequently killed and stored at −80°C for DNA analysis of the gut contents to check what they had eaten.

Earthworms found on the surface of each plots were collected, counted and identified. The plots were checked for slugs and beetles for up to four days after the experiment, since it was not possible to collect all of them in one day. For this reason, the experiment ran longer in some plots. This factor was accounted for in the statistical analyses.

Dissection of beetles

The beetles were dissected and their foreguts were put into previously weighed microfuge tubes at −80°C. Dissection instruments were sterilized with 96% ethanol and flame between specimens. Afterwards, each microfuge tube was weighed to estimate the foregut weight.

DNA extraction

DNA was extracted from beetle foreguts using the DNeasy Blood & Tissue Kit (Qiagen, Oslo, Norway), following the manufacturer's instructions. Extraction negatives (no tissue) were included for all sets of extractions to test for possible contamination during the extraction process. Extractions were stored in elution buffer at –80°C.

PCR protocols and programmes

All samples were tested for the presence of amplifiable DNA using general invertebrate primers of the mitochondrial cytochrome oxidase I (COI) gene (Folmer *et al.*, 1994). PCRs were run in 25 µl reaction volumes, containing 12.5 µl of GoTaq (Promega, Oslo, Norway) Mastermix, 1.0 µl of each primer (2.0 µl in total of 10 µM), 2.0 µl of DNA and 8.5 of dH$_2$O. The thermo-cycling programme consisted of 94°C for 1.5 min, followed by five cycles of 94°C for 30 s, 45°C for 1.5 min, 72°C for 1 min, then 36 cycles of 94°C for 30 s, 51°C for 1.5 min, 72°C for 1 min, and finally 72°C for 5 min.

Samples were also tested with two multiplexes for the presence of multiple slug species. The first multiplex was the COI multiplex, which was used for all samples and amplifies fragments of the COI gene of *A. vulgaris*, *A. ater* and *A. rufus* (Hatteland *et al.*, 2011). The PCR was optimized by adding bovine serum albumin (BSA), which has been proved to be a potent PCR-facilitator, including in gut contents of insects within predator-prey analysis (Juen & Traugott, 2005, 2006). PCRs were run in 12.5 µl reaction volumes, containing 6.25 µl of GoTaq (Promega) Mastermix, 0.25 µl of each primer (1.25 µl in total), 0.125 µl of BSA, 1.0 µl of DNA and 3.875 µl of dH$_2$O. The thermo-cycling programme consisted of 94°C for 2 min, followed by 35 cycles of 92°C for 30 s, 51°C for 30 s, 70°C for 50 s, and a final cycle of 68°C for 5 min.

A second multiplex was also used, to test if beetles could have fed on other species of slugs; the 12S multiplex with primers for *D. reticulatum* and *Arion* spp. (Dodd, 2004; Harper *et al.*, 2005) amplifying the mitochondrial ribosomal RNA gene. PCRs were run in 10 µl reaction volumes, containing 5 µl of Qiagen PCR Mastermix, 0.25 µl of each primer (1.0 µl), 0.1 µl of BSA, 1.0 µl of DNA, and 2.9 µl of dH$_2$O. The thermo-cycling programme consisted of 95°C for 15 min, followed by 39 cycles of 94°C for 30 s, 53°C for 1.5 min, 72°C for 1.5. min and a final cycle of 72°C for 10 min. All PCRs included positive (with target prey DNA) and negative controls (with distilled water instead of DNA). Amplified DNA was checked on 1–2% agarose gel.
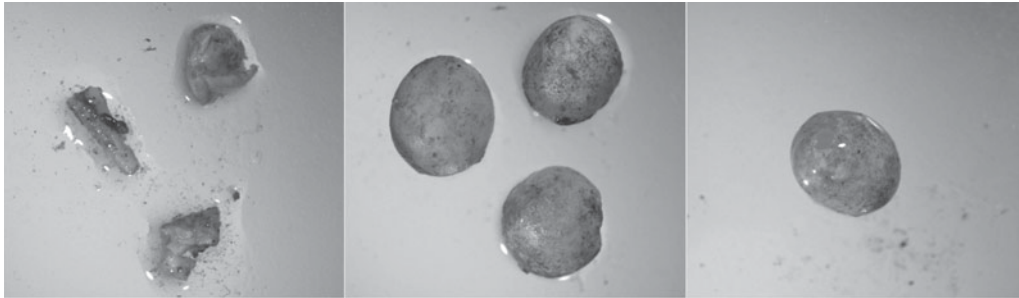
Fig. 1. Pictures taken under stereoscope showing features of collected eggs from the field (diameter of eggs is almost 4 mm). From left: eaten eggs, unaffected eggs and hatched eggs. The latter is visible due to the hole on the surface of the hatched egg.

Table 1. Egg status under the two treatment regimes and control.

	Control	T1 (2 beetles)	T2 (3 beetles)
Eggs unaffected	73%	69%	63%
Eggs hatched	13%	8%	10%
Eggs predated	11%	23%	23%

Statistical analyses

Statistical analyses were performed using the free software R (version 2.10.0) (R Development Core Team, 2008). Data from the semi-field experiments were treated by arcsine transformation. Linear mixed-effects models (LME) (Lindstrom & Bates, 1988) were applied to analyse the data from the semi-field experiments, to test if higher proportions of eaten eggs and juveniles found in treatments could be explained by the presence of beetles. The different plots were considered as random variables, since each plot was an independent event while the clutches of eggs were considered dependent events.

Results

Semi-field experiment with slug eggs

Eggs placed in the plots in the strawberry field were re-collected after the experiment but not all of them were found. The proportion of eggs found per clutch was calculated and a mean value derived for each treatment: 64% for treatment one (two beetles), 69% for treatment two (three beetles) and 75% for controls. The reduction in eggs found was significant (*t*-test: $P < 0.001$, $T = -19.35$) but did not differ significantly between treatments and control, suggesting that it was not due to the presence of the beetles (LME, $P = 0.4735$). Qualitative features of eggs were quite easy to identify, as the pictures in fig. 1 show; hatched eggs had a recognizable hole on the surface but kept their round shape, while unaffected eggs kept their round shape without showing any hole or damage on the surface, and they were still full of internal liquid. The surface of hatched and unaffected eggs generally did not show any signs of disturbance or bite marks. Eaten eggs showed a very disturbed surface, where bite marks were identifiable, and they lost their round shape and looked smashed and without internal liquids. Eggs eaten by beetles under laboratory conditions (Pianezzola, 2011) show the same shape, and we used them as a positive control to compare with eaten eggs from the field. Mean values of the proportions of all categories
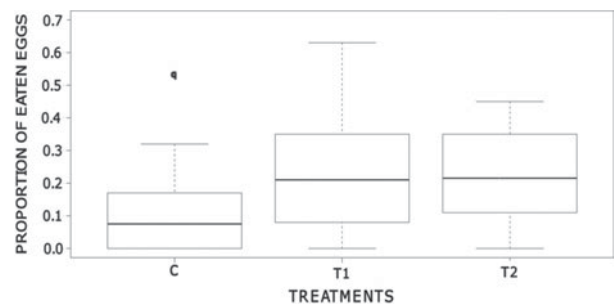

Fig. 2. Box plot of the mean values of proportions of eggs eaten under the three treatment regimes in the first semi-field experiment. C, control; T1, treatment one (two beetles); T2, treatment two (three beetles).

were calculated (table 1). A significant difference was found between the control and the two treatments (LME, $P = 0.0041$, $N = 90$) (fig. 2). However, there was no difference between treatments one and two (LME, $P = 0.7862$), showing that increasing beetle densities in the plots did not influence egg densities in this study.

Semi-field experiment with juveniles

A significant reduction of juveniles (*t*-test, $P < 0.001$) was found after the experiment (figs 3 and 4). Moreover, the reduction seemed to be due to the presence of beetles (LME, $P < 0.001$). In general, the weight of the slugs increased during the experiment (we added an additional category for slugs bigger than 1.2 g, as shown in fig. 3); and statistical analyses show that the two smallest size categories significantly differ between treatments and control, suggesting that beetles preferred slugs smaller than 1 g (LME, $P < 0.0001$, $N = 30$). Mean proportions of juvenile slugs found are 49% for control, 33% for treatment one (two beetles) and 23% for treatment two (three beetles) (fig. 4). A significant difference is found between the control and the two treatments (LME, $P < 0.001$, $N = 30$), suggesting that beetle presence affects slug densities. Total number of recollected *A. vulgaris* and non-introduced slugs is listed in table 2. Total number of collected earthworms is listed in table 3. Earthworms seemed to not affect the predation by the beetles (LME, $P = 0.8960$, $N = 30$).

In total, 46 specimens of *C. nemoralis* were recollected at the end of the experiment. They were tested for slug-DNA and eight of 46 beetles were positive for *A. vulgaris*, proving that at
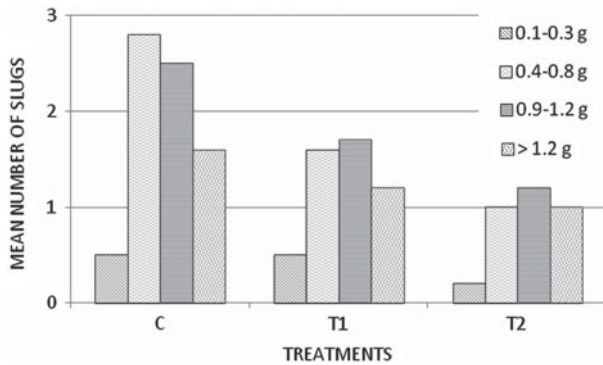
Fig. 3. Mean number of *Arion vulgaris* collected after the experiment divided into four weight categories. C, control; T1, treatment one (two beetles); T2, treatment two (three beetles).
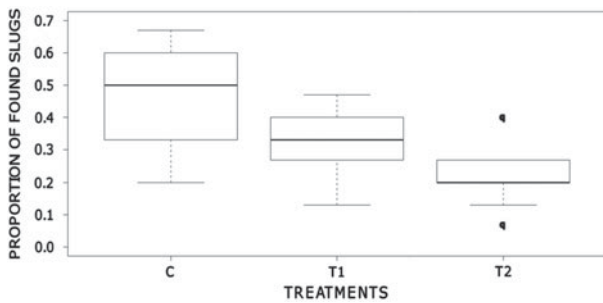

Fig. 4. Box plot of mean proportion of juvenile slugs in the second semi-field experiment. C, control; T1, treatment one (two beetles); T2, treatment two (three beetles).

least nearly 20% of the beetles had been feeding on *A. vulgaris* during the experiment. No other carabid species and no other alternative predators were found.

Discussion

Previous studies have shown that *C. nemoralis* and *P. melanarius* feed on eggs and juveniles of *A. vulgaris* (Hatteland *et al.*, 2010, 2011), and some authors have also tested these beetles in microcosms and mini-plots (Buckland & Grime, 2000; McKemey *et al.*, 2003). This study is the first to investigate prey-predation relations in semi-field conditions, by adding known densities of beetles, slug eggs and juvenile slugs to fenced plots. We found that *P. melanarius* and *C. nemoralis* can affect slug eggs and juvenile densities, respectively. Previous studies have shown that the egg-laying period is from August to November and that the juvenile phase is mainly from October to June for *A. vulgaris* in western Norway (Hatteland *et al.*, unpublished data; Roth *et al.*, unpublished data). The egg-laying period, thus, partly overlaps with the activity of *P. melanarius*, which was still present in late August and the beginning of September. The activity peak of *C. nemoralis* is in spring (Lindroth, 1985; Turin *et al.*, 2003), when the slugs are highly abundant as juveniles.

Our results demonstrate that *P. melanarius* may affect densities of slug eggs, but it is not dependent on the number of beetles put into the plots; there is no significant difference

Table 2. Total number of non-introduced slug species and *A. vulgaris* collected, for treatments and control.

Slug species	Control	T1 (2 beetles)	T2 (3 beetles)
Arion distinctus Mabille 1868	3	2	5
Arion fuscus (O. F. Müller, 1774)	2	2	1
Arion silvaticus Lohmander 1937	8	5	16
Arion vulgaris Moquin-Tandon 1855	74	50	34
Boettgerilla pallens Simroth 1912	3	1	1
Deroceras reticulatum (O.F. Müller 1774)	3	2	0

Table 3. Total number of earthworms collected, for treatments and control in the second semi-field experiment.

Earthworms species	Control	T1 (2 beetles)	T2 (3 beetles)
Allolobophora chlorotica (Savigny, 1826)	3	1	0
Aporrectodea caliginosa (Savigny, 1826)	9	3	11
Aporrectodea rosea (Savigny, 1826)	20	10	25
Dendrobaena octaedra (Savigny, 1826)	1	0	4
Lumbricus castaneus (Savigny, 1826)	0	3	0
Lumbricus rubellus (Hoffmeister, 1843)	20	26	15
Octolasium lacteum Linnaeus 1758	2	0	1
Total	55	43	56

between treatment one, with two beetles, and treatment two, with three beetles. We found a significant reduction of eggs not only in treatment plots but also in control plots. Statistical analyses suggest that this reduction is not due to the presence of beetles, and the reason for this reduction cannot be adequately explained. Lost eggs may have been eaten completely or they may have been removed a long way from the clutches by the beetles or other predators.

Personal observations under laboratory conditions (Pianezzola, 2011) show that both *P. melanarius* and *C. nemoralis* feed on slugs eggs with less voracity than they do on juvenile slugs and earthworms. Foreguts of *C. nemoralis* fed on slug juveniles have been found to be much heavier than the foreguts of beetles fed on slug eggs, which can suggest that predation on juveniles is higher than on eggs (Hatteland, unpublished data). Observations during the feeding trials suggested that physical features and composition of the eggs can affect their palatability. It is possible that the hard shell inhibits carabid beetles from eating eggs, although personal observations show that carabid beetles can easily break the eggshell.

Previous studies investigating the biochemical composition of the eggs of gastropods find that they are rich in proteins, carbohydrates, calcium, and other inorganic ions (Bayne, 1966; Meenakshi & Scheer, 1969). Regarding the possible toxicity of slug eggs, Schroeder *et al.* (1999) isolated

the defensive diterpene miriamin from *A. vulgaris* eggs, which deterred the coccinellid *Harmonia axyridis* from feeding on them. But other studies on carabid beetles feeding on slug eggs suggested that carabid beetles are not affected by this compound, since they actively fed on slug eggs without showing any mortality (Oberholzer & Frank, 2003; Hatteland *et al.*, 2010). It is reasonable to think that the nutritional features of slug eggs are not the reason for their lower susceptibility to predation, but their physical features. *Pterostichus melanarius* accepted slug eggs in the absence of other prey, while *C. nemoralis* seemed very reluctant to eat them (Pianezzola, 2011). Feeding experiments using multiple prey species are needed to determine the feeding preferences of carabid beetles.

The results of the semi-field experiment with *C. nemoralis* demonstrate that this carabid beetle may reduce slug densities by consuming juveniles. Moreover, the absence of other carabid species and alternative predators in the plots is further prove that *C. nemoralis* is the main reason fot the reduction in juveniles. The presence of alternative prey, such as other slug species and earthworms, seems not to affect the predation by beetles. In addition, the total number of slugs and earthworms did not differ significantly between treatments and control.

Compared with *P. melanarius*, *C. nemoralis* is bigger and needs to feed more. In previous studies, *C. nemoralis* has been found to consume juveniles up to 1.3 g, although it preferred slugs smaller than 1 g (Hatteland *et al.*, 2010). This is confirmed in the present semi-field experiment; of the four size categories of juvenile slugs, we found a significant reduction in the first two categories (0.1–0.3 g, 0.4–0.8 g). Moreover, after the semi-field experiment, we found a general increase in slug weight, showing that the slugs had grown or gained moisture and carabid beetles continued to prefer the smaller slugs.

Observations during the feeding trials with earthworms suggest that *C. nemoralis* is an active feeder; even under stressed conditions, when put into the Petri dish, it attacked the prey without hesitation and kept on feeding for the two hours it was allowed to feed. The same behaviour has been observed in previous studies with *C. nemoralis* feeding on slugs (Hatteland *et al.*, 2010).

Our results show that there is a trend between treatment one and treatment two in the second semi-field experiments; fewer slugs were found at the end of the experiment for treatment two than for treatment one (LME, $P = 0.0758$). Although the difference is not statistically significant, the trend suggests that increasing the number of carabid beetles involved may influence slug densities.

DNA analyses of gut contents of *C. nemoralis* showed that eight of 46 beetles were positive for slug-DNA of *A. vulgaris* and none of them were positive for the DNA of other analyses for *Arion* spp. and *D. reticulatum*. Even if DNA analyses only displays recent feeding activity and does not distinguish between direct predation and scavenging (Juen & Traugott, 2005; King *et al.*, 2008), for the aim of our research, DNA-based techniques were the most valuable approach to confirm that *C. nemoralis* fed on *A. vulgaris*.

The results of this semi-field experiment suggest that *C. nemoralis* actively search for juvenile slugs to feed on, possibly by detecting the mucus of the slugs (Digweed, 1994). Ayre (1995) showed that *C. nemoralis* can follow mucus of *D. reticulatum*. In the same way, *P. melanarius* can find juveniles and adult slugs by smelling them or detecting their chemical substances, as their larvae do (Thomas *et al.*, 2008), but there are no studies yet investigating the same for slug eggs. It is

reasonable to assume that *P. melanarius* feed on slug eggs by finding them at random and without active predation behavior.

Future studies should design semi-field experiments with carabid beetles and slug eggs and juveniles, focusing on multi-prey choice and controlling densities of alternative prey, vegetation type and structure, together with multiplex DNA analyses of the gut contents of the beetles. This would be possible under laboratory conditions or in an artificial arena. Moreover, feeding preference experiments under laboratory conditions need to be done, to investigate whether carabid beetles prefer eggs or juveniles, although our results suggest a preference for juveniles.

Future studies should also investigate how carabid beetles such as *C. nemoralis* can be used in greenhouses or small horticultural fields, where their densities can easily be controlled. Studies are also needed in open fields to investigate if maintaining the natural presence of these carabid beetles can aid in the control of *A. vulgaris*, for example by habitat management (Pickett & Bugg, 1998). In both cases, studies have to address the possible use of these carabid beetles as a biological control of *A. vulgaris*, as Symondson (2004) suggests, on the basis that biological control is the most realistic approach and best for conservation.

Acknowledgements

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