

**The Role of *Pterostichus madidus* and *Nebria brevicollis*
as Predators of the Slug *Deroceras reticulatum*.**

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A thesis submitted for the degree of
Doctor of Philosophy
of
The University of Newcastle Upon Tyne

May 2000

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Acknowledgements

First of all I would like to thank my supervisor Dr. Gordon Port for all his advice, encouragement and understanding during my PhD. Many thanks to Alan Craig for helping me collect the slugs and beetles and for the endless stories. Thanks to Jackie Hodgson and Alan White for all their technical support.

A huge thank you to everyone who gave me support throughout my Dad's illness and after his death. There is no way I would have made it this far without your help and understanding. Thanks especially to Rachel Morris, Ewan Keir, Jackie Hodgson and Sally Singh.

Finally a huge thank you to David for everything, especially putting up with me and my family over the past few years.

Dedicated to my dad, John Mair who died of cancer on
11th September 1998 aged 53.

'Ach mairidh gaol.'

Abstract

Slugs are important pests in many agricultural crops and potential biological control agents are being studied as an alternative to molluscicide application. The role of the carabids *Pterostichus madidus* (Fabricius) and *Nebria brevicollis* (Fabricius) as predators of the slug *Deroceras reticulatum* (Müller) was examined in the laboratory.

These generalist beetle species were only capable of killing small, healthy slugs (<0.11g) as they were unable to overcome the defence mucus production of larger slugs. Dead slugs were scavenged in preference to killing healthy slugs. The relatively high proportion of positive serological results from field caught carabids may reflect a high scavenging rate rather than actual predation on live slugs.

Slugs are difficult prey items for generalist beetles to overcome due to their defence mucus production. Results suggest that few slugs will be consumed in the presence of alternative prey which are less difficult for beetles to overcome. Slugs which could no longer produce defence mucus were readily attacked by both beetle species.

Although beetles killed few healthy slugs the presence of beetles influenced slug behaviour with slugs of all sizes foraging for shorter periods of time. Any reduction in slug activity on the soil surface would in turn lead to a reduction in seedling damage.

The results suggest that the role of potential predators in pest control can only be evaluated fully with a detailed understanding of their behaviour.

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1. General introduction

1.1 Pest status of slugs

Slugs are important pests of agriculture and horticulture in areas of the world that have a moist temperate climate (Port & Port, 1986). Over recent decades the pest status of slugs has increased due to farming practices e.g. growing monocultures of crops, susceptible to slug damage, in both arable fields and horticultural glasshouses with cultural techniques that provide increased protection from predation and desiccation. Glasshouses also provide winter warmth and summer moisture (Triebkorn *et al.*, 1996).

A number of agricultural crops are affected. In the UK slugs are a serious pest of wheat, oilseed rape and potatoes. They are perceived by farmers to be the primary pest in wheat (especially following oilseed rape) and secondary to aphids as pests of barley (Glen, 1989). Severe damage is caused to autumn-sown cereals due to hollowing of the grain and eating the stems of young seedlings, killing the plant before or just after emergence (Glen, 1989). The leaves of emerged plants are also grazed but this is considered less important than seed or seedling damage (Anon, 1984). Horticultural crops ranging from lettuce and brassicas to flowers e.g. chrysanthemums and soft fruits e.g. blackcurrant are all susceptible to slug damage (Martin & Forrest, 1969).

Thirty three species of slug are found in the UK (South, 1992). Of these, *Deroceras reticulatum* (Müller), *Arion hortensis* (Férussac) and *Milax budapestensis* (Hazay) are the most economically important (South, 1992).

1.2 Slug Control

Slugs are important pests, but the density at which crops are damaged can be variable i.e. high slug densities in some crop types causing little damage and relatively low densities causing damage when crops are at a sensitive growth stage (Hunter, 1978).

The majority of slug control is carried out by treating with molluscicide baits although -alteration of planting/harvesting dates and growing less/non-susceptible crop varieties is also employed (Hunter, 1978).

1.2.1 Cultural Control

Changes in farming practices i.e. less intensive, integrated arable farming systems are likely to cause an increase in slug numbers. Non-inversion tillage improves the soil structure reducing the risk of erosion and leaching as well as preserving beneficial soil organisms such as predatory insects and earthworms (Kendall *et al.*, 1994). However, non-inversion tillage does not provide the physical disturbance of ploughing, which has been shown to reduce slug numbers, and leaves stubble on the surface which provides a food source for slugs. A reduction in tillage has also been shown to lead to increased abundance and activity of *D. reticulatum*, *Deroceras agreste*

Mörch, *Arion distinctus* Mabilie and *Arion fasciatus* Nilsson in winter oilseed rape in Germany (Voss *et al.*, 1998).

The presence of cover crops provides slugs with food and shelter over the autumn and winter. This results in an increased risk of damage to spring sown crops following the cover crop (Glen *et al.*, 1994).

Straw burning was shown to reduce slug populations with a resulting reduction in damage to seeds and seedlings (Martin & Kelly, 1986). This practice was banned in 1993 and this is thought to have led to an increase in slug damage (Glen *et al.*, 1994).

The risk of damage to wheat seeds is directly related to slug biomass and inversely related to depth of drilling and percentage of fine soil aggregates (Glen, Milsom & Wiltshire, 1989). Large slug numbers, therefore do not necessarily cause a high percentage of seed loss. Large slug numbers in less intensive, integrated farming systems can be tolerated if cultural measures are taken before, during and after cereals are sown (Glen *et al.*, 1994).

Seedbed conditions also affect pest status. Slugs move through the soil, but a fine, firm seedbed makes it difficult for slugs to penetrate and therefore find seeds (Glen *et al.*, 1994).

1.2.2 Less / Non- Susceptible Crop Genotypes

Port & Port (1986) suggested that wheat cultivars which had a high total nitrogen content were attacked more often than cultivars with a lower total nitrogen content. Laboratory experiments have been carried out to determine the susceptibility of wheat cultivars to slug damage (Spaull & Davies, 1992). Ten cultivars of winter wheat were tested (Apollo, Avalon, Boxer, Galahad, Longbow, Mercia, Motto, Norman, Parade and Slejpner) with *D. reticulatum* adults. The total water soluble sugar content of the seeds and the exuded water soluble sugars were measured. C.v. Avalon was damaged most with c.v. Parade being attacked least. The cultivars preferred by slugs had a larger sugar content than those that were damaged least. Similar experiments carried out by Evans & Spaull (1996) also showed the most susceptible cultivars to be Avalon, Mercia and Riband (the most popular cultivars of winter wheat grown in the UK recently) with least damage to c.v. Parade, Brigadier, Hussar, Hunter and Buster. Again the damage is related to sugar content in the ungerminated seed and the release of sugars and other solutes during germination.

Potato tubers are damaged by slugs and susceptibility appears to be due to substances exuded from actively growing tubers with levels of sucrose, certain amino acids and chlorogenic acid being important (Stephenson & Bardner, 1976). Atkin (1979) found that potato varieties with a low total protein content were preferred. Levels of starch, glycoalkaloid and phenolic acid content appear to alter preference to slugs (Storey, 1985; Johnston *et al.*, 1989).

Oilseed rape cultivars are now being grown with low concentrations of glucosinolates which has made them more prone to slug attack (Glen, Jones & Fieldsend, 1990).

1.2.3 Chemical Control

Molluscicides are a widely used control measure for slugs. Control is influenced strongly by the timing of molluscicide application, attractiveness, palatability and toxic properties (Kelly & Martin, 1989). Molluscicides were originally designed from inorganic, caustic, desiccating or narcotic substances e.g. copper sulphate, calcium cyanide and salts of aluminium, boron and sodium (Kelly & Martin, 1989).

A mixture of copper sulphate and kainite (an anthelmintic) was first used to control slug damage in sugar beet crops in the Vale of York, England (Anderson & Taylor, 1926). This treatment, however, required direct contact with slugs and significant control was only achieved when large amounts were applied at night, when foraging slugs contacted the compound.

The two most commonly used molluscicides in the UK at present are metaldehyde and methiocarb (Garthwaite & Thomas, 1996).

Metaldehyde (polymerised from acetaldehyde) was originally used as a molluscicide in 1934 in South Africa. It is an effective molluscicide provided a sufficient quantity of the bait is consumed to cause poisoning. This chemical causes excessive mucus secretion and dehydration leading to death

by desiccation under the correct environmental conditions (Martin & Forrest, 1969). Triebkorn & Ebert (1989) have shown that after ingesting metaldehyde there is an initial increase in mucus production by the skin and digestive tract (depleting slug energy reserves), followed by damage to the mucus cell membranes and organelles, destroying the mucus producing system.

It was not until 1960 that metaldehyde became a commercially important molluscicide (Heim *et al.*, 1996). Metaldehyde continued to be the most effective molluscicide for thirty years until the development of the carbamate methiocarb in 1968 (Martin & Forrest, 1969).

Methiocarb was originally synthesised as an insecticide, but proved to be an effective molluscicide (Frain, 1982). This molluscicide is broad spectrum, acting on the central nervous system by blocking the neurotransmitter cholinesterase causing paralysis and loss of muscle tone.

Molluscicides are generally applied in pellet form. Commercial formulation of methiocarb bait (Draza® in the UK) contains 4% methiocarb, a blue dye, a conserving agent and an attractive bait material (bran) (Anon., 1995). The pellets contain a blue dye in order to deter birds (Hermann, 1964). Recent field studies have shown that both methiocarb and metaldehyde pellets remain toxic for approximately ten days in moist conditions and longer in dry conditions (Chabert, 1996). In the field, however, the highest slug mortality occurs in the first four days after application (Glen *et al.*, 1996).

Metaldehyde in the UK is sold as pellet baits usually containing 4% active ingredient combined with bran or another cereal (South, 1992).

Under baited traps following a night's foraging, Martin & Forrest (1969) found slugs poisoned by metaldehyde had secreted large amounts of mucus over the pellets and their immediate surroundings and, although unable to move in a co-ordinated manner, were writhing in an attempt to crawl away. Slugs poisoned by methiocarb were almost motionless under the traps, many apparently not having moved from the position in which they consumed the pellets and there was no excessive mucus secretion. In more recent studies, Howling (1989), found that slugs poisoned by metaldehyde were more likely to remain on the soil surface and those poisoned by methiocarb were more likely to move underground before dying.

Molluscicides are most effective on warm and humid nights when slug activity is greatest. Their success relies on slugs actively foraging for and consuming a lethal dose (Kelly & Martin, 1989). Even when slugs do eat the bait, irritancy and lack of palatability due to the toxic ingredient often prevents the consumption of a lethal dose and results in recovery from temporary poisoning.

1.2.3.1 Molluscicide use today

Since the 1970's there has been a 38-fold increase in molluscicide usage. In the UK in 1995, over 800,000 hectares of agricultural and horticultural crops were treated with molluscicides. This area of land requires the application of

4,800 tonnes of molluscicide, of which 250 tonnes are active ingredient (Garthwaite & Thomas, 1996). It has been estimated that each year ten million pounds is spent on slug control in agriculture and horticulture (not including the cost of application).

Of the molluscicides applied, metaldehyde accounts for 55%, methiocarb 40%, thiodicarb 5% and metal sulphates <1%. In 1994, 61% of the total area of molluscicides applied to arable crops was applied to wheat (83% broadcast application with 17% incorporated at drilling), 15% to oilseed rape, 9% to winter barley and 8% to potatoes (Garthwaite & Thomas, 1996).

1.2.3.2 Non-target effects-

The increased use and broad spectrum of activity of some molluscicides could pose a serious problem in relation to adverse side effects on non-target species. A number of studies have been carried out to determine the effects of molluscicides on a range of organisms.

The results from earthworm tests are inconclusive. Initial earthworm tests indicated that a small percentage (< 5%) was affected by methiocarb pellets, due to accidental contact on the soil surface. Small numbers of other organisms including Tipulidae (leatherjackets), Phalangidae (harvestmen) and Isopoda (woodlice) were killed (Martin & Forrest, 1969). The effects of both metaldehyde and methiocarb on *Lumbricus terrestris* have been studied in laboratory trials. Bieri *et al.*, (1989) found that earthworms did not suffer any effects when exposed to metaldehyde pellets. When exposed to methiocarb,

L. terrestris showed an increased mortality rate (23% mortality over the experimental period). Sub-lethal effects included reduced activity leading to a reduction in body weight. More recent studies (Wellmann & Heimbach, 1996) have shown that methiocarb pellets applied at the normal agricultural rate (40 pellets/m²) have no effect on *L. terrestris* mortality, behaviour or activity and suggest that the laboratory experiments carried out by Bieri *et al* used a much higher application rate (400 pellets/m²) and this accounted for the observed toxic effects.

Wiltshire & Glen (1989) found no significant effect of either metaldehyde or methiocarb on millipedes, centipedes or Diptera. The numbers of carabid and staphylinid beetles were significantly reduced in methiocarb treated plots.

In laboratory and semi-field trials evaluating the toxicity of molluscicides to carabid beetles methiocarb caused high mortality whilst metaldehyde rarely showed any toxic effect. Methiocarb mortality rates ranged from 66-100% for *Carabus granulatus* L., *Poecilus cupreus* L. and *Harpalus rufipes* De Geer. A lower mortality rate of 25% was found in *Pterostichus melanarius* Illiger (Büchs *et al.*, 1989).

The effect of repeated applications of methiocarb pellets has been shown to reduce carabid beetle populations in the field. Methiocarb applications in late autumn reduced the number of winter-active carabids. Broadcast application reduced carabid activity to <5% whilst drilling reduced activity to 10-15% in comparison to untreated plots. Spring and summer active species (i.e. not

active at the time of molluscicide application) were unaffected (Purvis & Bannon, 1992).

Further laboratory experiments have shown that the carabids *P. melanarius*, *Abax parallelepipedus* Piller & Mitterpacher and *Nebria brevicollis* Fabricius will feed on both metaldehyde and methiocarb pellets. Both pellets appeared to be equally attractive to motivated beetles (i.e. starved with no alternative food). No lethal or sub-lethal effects were observed after the ingestion of metaldehyde. Beetles which consumed methiocarb pellets displayed sub-lethal (lying on their backs with legs twitching) and lethal effects (Kennedy, 1990).

Both metaldehyde and methiocarb pellets can be lethal to cats and dogs with baits being consumed directly off the ground or from storage areas (Studdert, 1985).

1.2.4 Need for Biological Control

Classical biological control has been defined as ‘the suppression of pest insects and weeds by introduction and establishment of specific natural enemies from their area of origin’ (Waage, 1999). In this type of biological control predators continually regulate the pest population and both exist in an equilibrium below the economic threshold. In open, agricultural systems with annual disruption this method of control is difficult to maintain. However, it may be possible to use indigenous natural enemies to regulate

the populations of indigenous pests, if the natural enemy populations can be enhanced by inundative releases or by conservation.

Modern farming practices are moving towards integrated pest management, which involves the co-ordination of cultural, chemical and biological control. Integrated Pest Management is the use of all possible methods, including natural factors to control pests and diseases on crops and therefore promotes the use of highly specific agrochemicals in pest control to avoid harm to beneficial and non-target organisms (Bieri *et al.*, 1989).

In order to determine whether biological control of a pest, in this instance slugs, could play an important role existing control mechanisms have to be evaluated.

Cultural control methods are not very effective at controlling crop damage caused by slugs. Chemical control of slugs can give very variable levels of control depending on environmental factors. As previously mentioned (Section 1.2.3) molluscicides are most effective in periods of high slug activity, as successful control requires slugs to actively forage for and consume the bait (Kelly & Martin, 1989). Slug survival, post ingestion of a molluscicide dose is enhanced in humid conditions (South, 1992). The application of baits should be timed to achieve greatest success although baits can remain toxic for up to 12 days after application (Chabert, 1996). Modelling to predict when slug activity will be greatest and therefore the best time to apply molluscicide is being developed (Shirley *et al.*, 1998).

The effects of molluscicides on non-target organisms, including potential predators are a concern. Molluscicides have been shown to cause mortality in carabid species that are known to consume slugs (Büchs *et al.*, 1989).

1.2.5 Biological Control

1.2.5.1 Non- carabids

Seven species of Sciomyzid fly (genus *Tetanocera* and *Euthycera*) are known to kill slugs. Adult flies lay eggs on plants and decaying organic matter at ground level. Larvae hatch and pass through three larval instars over a three to four month period. Overwintering occurs as a third instar larva. In spring larvae may actively seek hosts, but most remain still on vegetation and wait for a prey item to pass by. Larvae attach themselves to slugs using mouth hooks and then enter the slug's body. They feed on tissue and secretions and leave the host after its death. Each larva can kill up to 25 slugs during its growth period (Reidenbach *et al.*, 1989).

The slug species *Laevicaulis alte* (Férrusac) is a serious agricultural and horticultural pest in India, Indonesia and the Pacific islands. The native mite *Fuscuropoda marginata* (Koch) has been shown to reduce the numbers of *L. alte* in the field (Raut & Panigrahi, 1991). Soil moisture is an important factor and *F. marginata* were found to be most effective at a temperature range of 25-35°C when the soil moisture was in the range 46-65% of field capacity. Further studies are being carried out to evaluate *F. marginata* as a potential biological control agent of *L. alte* (Raut, 1996).

The rhabditid nematode, *Phasmarhabditis hermaphrodita* (Schneider) was discovered parasitising *D. reticulatum* in 1988 (Wilson *et al.*, 1993). Infective nematode larvae enter the slug shell cavity via a small pore at the posterior end of the mantle. Inside the shell cavity they shed their outer cuticle and grow into reproductive adults, causing the mantle to swell (Wilson *et al.*, 1993). Nematode reproduction in the shell cavity results in the death of the slug. Slugs killed by *P. hermaphrodita* are not found on the soil surface therefore it is suggested that infected slugs move down into the soil before they die (Glen *et al.*, 1996). The commercial product, Nemaslug® was launched in 1994 to control slugs in the home garden market (Glen *et al.*, 1994b). Since then the role of *P. hermaphrodita* in a range of crops has been investigated. It has been shown to kill all the major pest species of slug, and the garden snail *Helix aspersa* L. (Coupland, 1995). Field studies indicate that there is no threat to non-target molluscs in habitats adjacent to treated arable areas (Wilson *et al.*, 1995).

Commercial production of *P. hermaphrodita* is carried out in liquid fermenters from which infective larvae are harvested. Larvae can be stored in cold conditions for several months. A spray or drench of infective larvae is applied to the soil at a recommended rate of $3 \times 10^9 \text{ ha}^{-1}$. Nematodes are most effective when applied just before the stage when crops are susceptible. Following infection, slug feeding is greatly reduced providing rapid protection to the crop. Soil treated with *P. hermaphrodita* appears to repel slugs (Glen *et al.*, 1996). At present the cost of nematode application is a

major constraint on its use. With improved formulation and application methods the use of *P. hermaphrodita* will become a more cost-effective strategy.

Trials to determine the efficiency of *P. hermaphrodita* in organic farms in Switzerland indicate that the nematode can protect oilseed rape plants from slug damage with a success rate higher than that of metaldehyde treated plots (Speiser & Andermatt, 1996).

Experiments indicate that *P. hermaphrodita* could be a successful biological control agent although trials are required to determine the optimum environmental conditions for maximum control.

1.2.5.2 Carabids

The association between predatory carabid beetles and molluscs has been well documented for a number of years. Some species of carabid feed principally on molluscs (these include beetles from the genus *Cychrus*, *Scaphinotus* and *Carabus*). These beetles are fluid-feeders (i.e. secrete fluid containing digestive enzymes onto their prey then subsequently ingest the fluid from the partially digested prey) and possess elongated mandibles, heads and abdomens with a powerful cibarial-pharyngeal pump which sucks the liquified food into the digestive system (Evans & Forsythe, 1985). These beetles are relatively slow runners as they are generally heavier, bulkier and stronger than other cursorial ground beetles and it is suggested that this bulk

may enable them to attack large, slow prey e.g. molluscs, worms and caterpillars (Forsythe, 1991).

Previous authors have identified several species of polyphagous carabid beetles as mollusc predators. Early studies relied on the identification of mollusc remains in dissected beetle crops e.g. *P. madidus* Fabricius, *Pterostichus melanarius* Illiger, *Pterostichus diligens* Sturm, *Pterostichus strenus* Panzer, *Pterostichus vernalis* Panzer, *A. parallelepipedus*, *H. rufipes*, *Agonum viduum* Panzer, *Agonum obscurum* Herbst (Davies, 1953; Dawson, 1965; Cornic 1973; Luff, 1974). Slugs, however have few hard structures to allow identification.

Many beetles, however, are fluid feeders and therefore no solid mollusc remains would be identified leading to the degree of slug predation being underestimated.

Serological techniques have since been developed to determine beetle predation on molluscs. Tod (1973), using a precipitin technique, identified 14 species of carabid as mollusc predators i.e. *Carabus violaceus* L., *Carabus catenulatus* (= *problematicus*) Herbst, *Carabus nemoralis* Müller, *Pterostichus niger* Schaller, *Pterostichus anthracinus* Illiger, *P. madidus*, *P. melanarius*, *Cychrus caraboides* L., *N. brevicollis*, *Nebria glyllenhali* Schönherr, *Calathus piceus* Marsham, *Calathus fucipes* Goeze, *Calathus micropterus* Duftschmid and *Calathus melanocephalus* L..

More recently enzyme-linked immunosorbent assay's (ELISA's) using both monoclonal and polyclonal antibodies have been developed. Initial tests used polyclonal antisera. Slug haemolymph was used as the antigen as the main component, haemocyanin, gives a strong immunological response and its molecular structure in molluscs is very different to that found in arthropods e.g. spiders, woodlice, centipedes and millipedes. Initially this antiserum was used to determine antigen decay rates in two predators, *A. parallelepipedus* and *P. madidus* (slug consumption at the same site at the same time). *A. hortensis* and *D. reticulatum* were fed to starved beetles then the beetle crop contents were weighed and then ELISA tested. The rate of antigen decay was slower in *P. madidus* (detection up to 94h) than in *A. parallelepipedus* (detection up to 35h) (Symondson & Liddell, 1993a). This polyclonal antiserum was then used to detect slug predation in the field. The crop contents of *A. parallelepipedus* and *P. madidus* were analysed. Of the *A. parallelepipedus* caught 89.5% tested positive for slug proteins (females had significantly higher concentrations and mass of slug proteins than males). Of the *P. madidus* caught 42% tested positive. These results indicated that even in the presence of alternative prey a large proportion of the diet of *A. parallelepipedus* was made up of slugs. Slugs made up a smaller proportion of the diet of *P. madidus* as a lower percentage tested positive and slug haemolymph concentrations were lower (Symondson & Liddell, 1993b). These authors also suggested that the actual percentage of the population which had consumed slugs was higher as beetles that were

likely to be caught in pitfall traps may have been highly motivated due to hunger (Symondson & Liddell, 1993b).

Ayre (1995) also developed a double-sandwich ELISA to determine predation on slugs by carabids. This polyclonal test reacted against the slugs *D. reticulatum*, *M. budapestensis*, *A. hortensis* & *D. caruanae*. Commonly caught carabids from three field sites in northern England were tested. *N. brevicollis* was found to feed most frequently on slugs (37% of captured individuals tested positive) with only 10% of *P. madidus* and 9% of *P. melanarius* testing positive. The same beetle species were found to feed on slugs to differing extents between the three field sites.

Recently, monoclonal antibodies have been developed to determine predation on molluscs. These antibodies are highly specific, capable of identifying genus, species and life history stage e.g. eggs. A monoclonal antibody (MAb) was first developed to detect the remains of Arionid slugs (*A. subfuscus*, *A. ater*, *A. hortensis* and *A. intermedius*) in carabid beetles. MAb's have a shorter detection period, only capable of detecting predation over the previous night's foraging period (Symondson & Liddell, 1993c). Species specific MAb's have also been developed against *D. reticulatum* (Symondson & Liddell, 1996). Slug remains could be detected for up to 60h in the crops of *P. melanarius*. Feeding on alternative prey after the consumption of a slug extended the detection period to 89h (Symondson & Liddell, 1995).

Serological techniques can not distinguish scavenging and actual predation, consuming injured prey, wounding, partial consumption or determine prey size preference. Positive serological results may also be obtained as a result of 'secondary predation' i.e. a secondary predator may have consumed a primary predator which consumed the prey item (Sunderland, 1996).

Extra-intestinal digestion takes place in many larval and adult carabid beetles. This leads to a low consumption of detectable antigens in beetle crops. In these instances the level of predation would be underestimated using serological techniques (Sunderland, 1996).

Behavioural observations of carabid and slug interactions are therefore required to determine the role of beetles as potential biological control agents.

Work has been carried out in semi-field and field conditions to determine the potential of carabid beetles as control agents of slugs. Experiments in grass/clover sward boxes have shown that the presence of *A. parallelepipedus* (density 4/m²) and *P. madidus* (8/m²) reduced the damage caused by *D. reticulatum* to clover as effectively as the application of methiocarb baits. Slugs weighed 0.18 - 0.2g and were present at a density of 20/m² (Asteraki, 1993).

A. parallelepipedus has been shown to control the numbers of *D. reticulatum* in lettuce plots inside a polythene tunnel (6 beetles and 30

slugs/m²) and lead to a reduction in crop damage. Beetles were more effective at controlling slugs in immature lettuce plots as beetles were not able to kill slugs feeding within the folded leaves of mature plants. In simple laboratory trials both male and female beetles were shown to have similar kill rates (0.3 and 0.22 slugs/day respectively, slug weight 0.3-0.6g) but females ate significantly more than males (Symondson, 1989). *A. parallelepipedus* is not present in large numbers in arable fields, but has been shown to have the potential for mass rearing which would make it suitable as a control agent in polythene tunnels and glasshouses (Symondson, 1994).

Field trials have found a positive relationship between the biomass of slugs and the numbers of *P. melanarius* caught in pitfall traps and it is suggested that *P. melanarius* preferentially feeds on slugs in these areas (Symondson *et al.*, 1996).

More detailed behavioural experiments carried out in the laboratory have shown that both generalist and specialist carabid beetles show altered locomotory behaviour in the presence of slug mucus. On soil containing mucus, beetles foraged longer at slower speeds and also showed a higher degree of turning and time spent stationary (Ayre, 1995). The mollusc specialist *Carabus nemoralis* has been shown to orientate to *D. reticulatum* mucus and earthworm mucus in an orientation chamber (Digweed, 1994).

Laboratory studies have also shown that a number of generalist beetle species can consume newly hatched slugs and that in some instances the

presence of beetles can cause an increase in slug mortality even when no predation occurred (Ayre, 1995).

Carabid beetles are known to be important natural control agents for many other pest species in agricultural systems. Predation on cereal aphids by generalist beetle species has been well documented (Sunderland, 1975; Sunderland & Vickerman, 1980; Chiverton, 1986; Chiverton, 1987; Sunderland *et al.*, 1987; Chiverton 1988). Laboratory studies have shown that *P. melanarius* is able to control leatherjackets (*Tipula* spp.) (Chapman, 1994). Other work has shown that carabids prey on cabbage root fly (Coaker & Williams, 1963) wheat bulb fly (Burn, 1982) and winter moth pupae (Frank, 1967).

Polyphagous carabid beetles could play an important role in integrated pest management. Carabids are abundant in agricultural fields (Lövei & Sunderland, 1996) and although they are not specialists they may be important in prolonging the period between pest outbreaks. Their efficiency may be increased by using 'habitat islands' to act as refuges and colonisation areas (Thomas *et al.*, 1991; Lys, 1994; Chapman & Armstrong 1996).

Agricultural land is disrupted on an annual basis, which would destroy the equilibrium between specialist predators and their prey. Polyphagous predators are considered poor control agents due to the fact that they feed on a variety of prey items and their population dynamics are not synchronised with the pest. In an agricultural system, however, polyphagy may enable the

predator to remain in the habitat when the pest is no longer present and when pest numbers increase the predator can switch to this prey type (Murdoch *et al.*, 1985).

1.3 Project Outline

The aim of this work was to determine the role of two carabid species, *P. madidus* and *N. brevicollis* (commonly found in arable crops in northern England) in slug control. These two species have previously been identified using serological techniques and some behavioural experiments, as predators of slugs. More detailed behavioural studies were carried out in this work to determine the importance of slug prey for these beetle species and in turn to determine their potential as biological control agents.

The particular objectives addressed in each chapter of the thesis are:

(i) 'Effect of size and condition of the slug *Deroceras reticulatum* on predation by the carabid beetles *Pterostichus madidus* and *Nebria brevicollis*.' The objective of this study was to determine the size of slugs consumed by these generalist beetle species and to determine whether there was a preference shown for healthy, injured or dead slugs.

(ii) 'The effect of the presence of *Pterostichus madidus* (Coleoptera, Carabidae) on the activity of the slug *Deroceras reticulatum*.' Slug behaviour was examined with and without the presence of a predator to determine whether slug activity was altered in the presence of this beetle species.

(iii) 'Predation on the slug *Deroceras reticulatum* by the carabid beetles *Pterostichus madidus* and *Nebria brevicollis* in the presence of alternative prey.' Generalist beetle species, by definition, feed on a variety of prey items. The objective of this study was to determine the numbers of slugs consumed when beetles were presented with alternative prey types.

(iv) 'The influence of mucus production by the slug *Deroceras reticulatum* on predation by *Pterostichus madidus* and *Nebria brevicollis* (Coleoptera: Carabidae).' The objective of this study was to determine the ability of generalist beetle species to overcome the defence mucus produced by slugs and whether slugs which could no longer produce defence mucus were more suitable prey items.

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2. Effect of size and condition of the slug *Deroceras reticulatum* on predation by the carabid beetles *Pterostichus madidus* and *Nebria brevicollis*.

2.1 Abstract

- 1 Slugs are important pests in many agricultural crops and molluscicides are commonly used to control slugs, however, these affect non-target organisms. Encouraging biological control may help to reduce molluscicide use, but the efficiency of potential natural enemies needs to be investigated.
- 2 Serological tests have shown that certain carabid species consume slugs. These techniques, however, do not distinguish between scavenging and true predation nor do they provide information on the size or other characteristics of the prey consumed. The study reported here was undertaken to establish whether scavenging of dead slugs might be an important factor contributing to positive serological test results.
- 3 Both *Pterostichus madidus* (Fabricius) and *Nebria brevicollis* (Fabricius) consumed *Deroceras reticulatum* (Müller) under laboratory conditions. Dead slugs were scavenged in preference to injured or healthy slugs.
- 4 Only small, live slugs (<0.11g) were killed by both beetle species which may be incapable of killing larger slugs
- 5 These generalist beetle species appeared unable to overcome the defence mucus produced by slugs. The data suggest that positive

serological results from field collected beetles may reflect scavenging rather than predation on live or injured slugs.

2.2 Introduction

Slugs attack a wide range of crops including wheat, oilseed rape and potatoes and are important agricultural pests in moist, temperate climates (Port & Port, 1986). Of the thirty three species found in the UK (South, 1992), *Deroceras reticulatum* (Müller) is considered to be one of the most economically important (South, 1992).

The majority of slug control is achieved by treating crops with molluscicide baits (containing either metaldehyde or carbamates) and their success relies on slugs actively foraging for the bait and consuming a lethal dose (Kelly & Martin, 1989). Even when slugs do eat the bait, irritancy and lack of palatability caused by the active ingredient often prevents the consumption of a lethal dose and slugs often recover from temporary poisoning. Juvenile slugs may also be less well controlled than adults due to differences in activity or foraging patterns (Kelly & Martin, 1989).

Molluscicide baits have been shown to affect non-target organisms. Laboratory experiments have shown that the carabids *Pterostichus melanarius* (Illiger), *Abax parallelepipedus* (Piller & Mitterpacher) and *N. brevicollis* will feed on both metaldehyde and methiocarb pellets. No lethal or sub-lethal effects were observed after the ingestion of metaldehyde but

beetles which consumed methiocarb pellets displayed sub-lethal (lying on their back with legs twitching) and lethal effects (Kennedy, 1990). In laboratory and semi-field trials evaluating the toxicity of molluscicides to carabid beetles methiocarb caused high mortality whilst metaldehyde rarely showed any toxic effect. Methiocarb mortality rates ranged from 66-100% for *Carabus granulatus* Linneus, *Poecilus cupreus* Linneus and *Harpalus rufipes* (DeGeer). A lower mortality rate of 25% was found in *P. melanarius* (Buchs *et al.*, 1989). Methiocarb applications in late autumn reduced the numbers of winter active carabids (95% reduction in total carabid activity) whilst spring and summer active species (i.e. not active at molluscicide application) were unaffected (Purvis & Bannon, 1992).

The role of potential natural enemies of slugs has been studied in another approach to management of these pests and, if effective, may lead to a reduction in molluscicide usage. Whilst some species of carabid beetle are specialist mollusc feeders these are relatively uncommon in arable crops (Ayre, 1995; Kromp, 1999), however several species of more common polyphagous carabid have also been identified as mollusc predators. Laboratory studies have shown that these carabids attack slugs (Stephenson, 1965) and studies of field collected material used the presence of mollusc remains in dissected beetle crops to confirm consumption (Davies, 1953; Luff, 1974; Sunderland, 1975). However, as many carabids are fluid feeders solid remains were observed in very few beetles.

More recently, serological techniques have been used to determine predation on molluscs by carabids in the field. Early studies employed polyclonal antisera and precipitation techniques to detect invertebrate prey remains in polyphagous predators (Tod 1973; Sunderland, 1988). These tests, however, used macerates of whole slugs and therefore contained a large number of antibodies which would react with a number of prey species. More sensitive techniques have since been used, such as ELISA using polyclonal antisera (Symondson & Liddell, 1993; Ayre, 1995) or using monoclonal antibodies (Symondson & Liddell, 1996). Whilst some of these serological studies have shown that a significant proportion of carabid beetles may have consumed slug tissue (Symondson *et al.*, 1996) others have indicated that the proportion varies between carabid species and, more importantly, within species between sites (Ayre & Port, 1996). In a study in Northumberland using ELISA testing Ayre, (1995) found that 37% of *N. brevicollis* collected from pitfall traps had fed on slugs, but only 10% of the *P. madidus* tested positive.

Preliminary behavioural studies of carabids as slug predators have been carried out (Symondson 1993; Ayre, 1995), however, more detailed studies are required as serological techniques do not distinguish between scavenging and true predation nor do they provide information on the size or other characteristics of the prey consumed (Sunderland, 1996). In the investigation reported here, interactions between the carabids *P. madidus* and *N. brevicollis* and the slug *D. reticulatum* were investigated to assess beetle predatory behaviour in the presence of different prey types. Experiments

were carried out to determine the range of slug sizes consumed by both beetle species and to assess the importance of predation and scavenging.

2.3 Materials and methods

2.3.1 Experimental animals

2.3.1.1 Beetles

Adult *P. madidus* and *N. brevicollis* were collected from woodland and grassland habitats at Close House, Heddon on the Wall, Northumberland (Grid Reference NZ 1265) using dry pitfall traps (8 cm diameter x 10.5 cm depth). Traps were emptied three times per week. Prior to use in experiments, beetles were maintained individually in 9 cm diameter petri dishes with a disc of moist blotting paper in the base. Petri dishes were placed in batches in plastic bags to maintain high humidity and kept in a controlled environment room with a light : dark cycle of 16:8 and at a temperature of approximately $12 \pm 2^\circ\text{C}$. Beetles were maintained by feeding with chopped blowfly larva/pupa (*Calliphora* sp.) provided *ad lib*. All beetles were held for a minimum of one week prior to experiments.

2.3.1.2 Slugs

Slugs of a single species, *D. reticulatum*, were collected from beneath shelter traps (40 x 40cm squares of hardboard) at Close House. Slugs were maintained at $12 \pm 2^\circ\text{C}$ in 20 cm x 20 cm x 12 cm tubs containing moist roll (Kimwipe) and fed a constant supply of Layers Mash (Heddon Garage,

Heddon on the Wall, Northumberland). The moist environment ensured that slugs for use in the experiments were fully hydrated.

2.3.2 No-choice trials

After three days starvation individuals of both species of beetle were given access to a single slug. Three classes of slug were used (i) healthy - slug was weighed (ii) injured - slug was stabbed in the tail with a syringe needle then weighed (iii) dead - slug was weighed, frozen to kill it, and defrosted before use. Slug weight ranged from 0.131 - 0.324g (mean 0.199g \pm 0.008 SE) with no significant differences in weight between treatments. Experiments were carried out in petri dishes (9 cm diameter) with a layer of moist blotting paper in the base with a 16:8 light:dark cycle and a temperature of 12 \pm 2°C. One slug was added to each petri dish containing a single beetle. There were 18 replicates. The numbers of slugs consumed were recorded daily for ten days. Results were analysed using the contingency χ^2 test.

2.3.3 Choice trials

Interactions between *P. madidus* and healthy, injured and dead *D. reticulatum* were investigated to assess beetle's predatory behaviour in the presence of different prey types. Experiments were carried out in a 15 cm x 38 cm plastic arena, 8 cm deep, filled to a depth of 2 cm with soil. Large clods of soil were broken up and all visible plant material and small organisms removed. The soil was moistened thoroughly at the start of the experiment and water sprayed onto the surface at regular intervals to maintain a high soil moisture content. A single beetle was placed in the arena

with one slug from each class (i.e. healthy, injured or dead). Each beetle was only used once. Slug weight was recorded at the start of the experiment. A band of Fluon® (polytetrafluoroethylene) (Whitford Plastics, Cheshire, England) 3 cm wide, was painted around the rim of the arena to prevent slugs and beetles escaping. Beetle and slug activity was recorded for two nights using a video camera (Baxall CD9242/IR sensitive to low light levels and infra-red illumination from an array of light emitting diodes) and a Panasonic AG-6040 time-lapse video recorder. Beetles were starved for either 3 or 7 days prior to the experiment. As *P. madidus* and *D. reticulatum* are nocturnal only night time behaviour was examined. Foraging behaviour over both nights was considered. Experiments were carried out with a 16:8 light:dark cycle and a temperature of $12 \pm 2^\circ\text{C}$.

In these experiments the components of *P. madidus* behaviour were examined and classified as follows:

- (i) time spent active on the soil surface
- (ii) time spent attacking/feeding on prey
- (iii) total number of attacks/contacts with slugs over each foraging period (a contact was classed as any time a beetle touched a slug).
- (iv) total number of slugs killed
- (v) number of contacts with a slug before a kill
- (vi) beetle speed (sampled at instants)

Controls consisted of a single beetle in the arena. There were 5 replicates in each class except *P. madidus* females starved for one week ($n = 6$). Analysis

of activity was carried out using the Micromeasure® program (tracking of beetle movement and speed). Differences between speeds and activity periods (arcsine transformed percentages) were analysed using the Students *t*- test.

2.3.4 Size preference

Both *P. madidus* and *N. brevicollis* were presented with a single live slug from one of three size classes. Size classes used were :

1. Small - 0.01 - 0.11g
2. Medium - 0.12 - 0.22g
3. Large >0.23g

Experiments were carried out in 9cm diameter petri dishes with a disc of moist blotting paper in the base with a 16:8 light to dark cycle and a temperature of $12 \pm 2^{\circ}\text{C}$. Controls consisted of 10 slugs in each size class (maintained individually in 9cm diameter petri dishes with moist blotting paper). Beetles were starved for three days prior to experiments which were run over seven days with predation being scored daily. There were 10 replicates. Results were analysed using the contingency χ^2 test.

2.4 Results

2.4.1 No-choice trials

The results in Table 2.1 indicate that both species of beetle consumed slugs under laboratory conditions. When all three prey types are considered, beetles killed or scavenged 17.5% of the prey available. When only healthy and injured slugs are considered, 4.6% were attacked over the experimental period whereas 44.4% of dead slugs were scavenged. No healthy slugs were killed by *N. brevicollis* (0%), one injured slug was consumed by a female (5.5%) and four dead slugs were scavenged (22.2%). Two healthy slugs (11.1%) were killed and eaten by *P. madidus* individuals, no injured slugs were killed (0%) and eleven dead slugs were consumed (61.1%).

Both beetle species showed a significant preference for dead slugs over injured and healthy slugs (*N. brevicollis*: $\chi^2 = 7.875$, $P < 0.05$; *P. madidus*: $\chi^2 = 20.871$, $P < 0.001$).

2.4.2 Choice Trials

Whilst the details of behaviour were the prime objective of this experiment the results in terms of slugs attacked are also of interest. Tables 2.2 and 2.3 show the numbers of *D. reticulatum* killed and/or consumed in choice trials by *P. madidus* females and males.

Female *P. madidus* killed or scavenged more slugs than males. Female beetles which had been starved for 3 days did not consume any slugs over the experimental period. Female beetles which had been starved for 1 week scavenged five dead slugs on the first night i.e. it appears dead slugs were scavenged in preference to healthy or injured prey. One third of the beetles

attacked slugs on the second night killing one injured and one healthy slug (these individuals had scavenged a dead slug on night1). One beetle did not scavenge or kill any class of slug over the experimental period. The resulting predation rate (i.e. killing healthy or injured slugs) for females starved 1 week was 0.16 slugs/beetle/night.

Male *P. madidus* which had been starved for 3 days did not consume any slugs on the first night. On the second night 3 dead slugs were scavenged. This indicates that males show the same preference as females i.e. dead slugs were scavenged in preference to healthy or injured individuals. Two beetles did not scavenge or kill any class of slug over the experimental period. The resulting predation rate (i.e. killing healthy or injured slugs) for males starved 3 days was zero slugs/beetle/night. Males which had been starved for 1 week did not kill/scavenge any slugs over the experimental period.

Table 2.3 shows data (number of contacts, time elapsed before a kill and handling time) for beetles which killed/scavenged slugs over the experimental period. Handling time in this experiment was considered to be the time from beetle contact with the slug to the beetle commencing foraging after cessation of feeding. Eighty three percent of female *P. madidus* which had been starved for one week killed/scavenged a slug on the first night. Eighty percent of these eighty three percent consumed a dead slug on first contact which occurred from less than one minute up to twenty six minutes after the start of foraging (mean 11.4 min \pm 4.3 SE). Over the second foraging period (i.e. night two) one third of the beetles killed/scavenged a slug. Again the

number of contacts before consumption was low - the injured slug being attacked and killed on first contact and the healthy slug being attacked and killed on the second contact. These slugs had been contacted and rejected on the previous night i.e. the injured slug was contacted once on the first night and the healthy slug was contacted 17 times on the first night. The time elapsed before the start of consumption was longer on the second night (40 to 326 minutes). The time spent feeding on a slug (handling time) ranged from 5-94 min (mean $52.6\text{min} \pm 13.8\text{ SE}$) over both nights. None of the slugs attacked/scavenged were totally consumed by *P. madidus* during the first feeding period.

Male *P. madidus* which had been starved for three days did not kill/scavenge any slugs on the first night. Sixty percent of beetles consumed a dead slug on the second night and all the attacks on dead slugs occurred on the first contact. These dead slugs had been contacted between one and three times on the first night. The duration of first contacts ranged from 30-65minutes (mean $45.0\text{ min} \pm 10.4\text{ SE}$). The time spent feeding ranged from 30 seconds to 57 minutes (mean $22.5\text{ min} \pm 17.5\text{ SE}$). There were no significant differences in handling times between males and females (night 1 females and night 1 males $t = 1.04$, $P = 0.34$, $DF = 5$; night 2 females & night 2 males $t = 1.33$, $P = 0.31$, $DF = 4$).

After each attack on a healthy or injured slug (which produced thick defence mucus), beetles of both sexes underwent a period of mandible cleaning

lasting approximately 10-15 seconds. There was no apparent mandible cleaning after attacks on dead slugs.

Female *P. madidus* which had been starved for three days and males which had been starved for one week did not consume any slugs over the experimental period.

Figure 2.1 shows the mean number of contacts with slugs over both nights (each time a beetle touched a slug was classed as a contact). The number of contacts ranged from 0-40 over each 8h foraging period. In all classes of beetle, the number of contacts was greatest on night 1, and was significantly so for both classes of female ($P < 0.01$). Both sexes of beetle showed a higher number of contacts with healthy and injured slugs than with dead slugs although few healthy or injured slugs were killed (Table 2.4).

The mean speed of beetles over the experimental period is shown in Figure 2.2. All classes of beetle i.e. starved 3d/1wk control and starved 3d/1wk plus slugs, for both male and female beetles showed a distinct pattern. Speed during the night i.e. over the foraging period, was significantly slower than speed during the day (in all classes $P \leq 0.0005$). Within each class of beetle, both sexes showed no significant differences in speed between night 1 and night 2 and day 1 and day 2 ($P > 0.05$ in all classes - except *P. madidus* females starved 3d controls where day 1 speed was significantly faster than day 2 speed ($t = 2.68$, $P = 0.031$, $DF = 4$)). There were no significant differences in speeds between beetles which had access to slugs and their

control group ($P > 0.05$ in all classes). Beetle activity during the day was minimal with beetles rapidly moving from one refuge to another whilst night time activity consisted of foraging behaviour.

Figures 2.3 & 2.4 show the total activity (i.e. time spent active on the soil surface) over the whole experimental period. Both male and female beetles were active for a large proportion of the period. Females which had been starved for 1 week + slugs were least active ($46.6\% \pm 7.1$) and the most active were males which had been starved for 3 days + slugs ($76.0\% \pm 7.7$). Female beetles which were starved for 1 wk + slugs were significantly less active than the control group ($t = 3.25, P = 0.011, DF = 4$).

2.4.3 Slug size

The numbers of slugs in each size class consumed by *P. madidus* and *N. brevicollis* are shown in Table 2.5. *P. madidus* consumed 40% of the small slugs (0.01 - 0.11g) but no medium (0.12 - 0.22g) or large (>0.23g) slugs. Only one small slug was consumed by *N. brevicollis* and no medium or large slugs. *P. madidus* individuals showed a significant preference for small slugs over medium and large slugs ($\chi^2 = 9.231, P = 0.01$). There was no size preference shown by *N. brevicollis* due to the low numbers of slugs consumed ($\chi^2 = 2.069, P > 0.05$).

There was no slug mortality in all three size classes of control slugs over the experimental period.

2.5 Discussion

Both *P. madidus* and *N. brevicollis* killed and consumed, or scavenged *D. reticulatum* individuals in laboratory trials. In no-choice trials both species scavenged significantly more dead slugs than killed healthy or injured individuals. From the data presented in choice trials, dead slugs are scavenged in preference to injured or healthy slugs. It appears that female *P. madidus* kill/scavenge more slugs than males as females killed 18.1% and scavenged 45.4% of the slugs presented with males killing 0% and scavenging 27.2%.

Female *P. melanarius* which had consumed slugs in the field, were also found to have a greater crop weight than males and it was suggested that females may consume double the number of slugs than males (Symondson *et al.*, 1996). In laboratory trials both male and female *A. parallelepipedus* were shown to have similar kill rates (0.3 and 0.22 slugs/day respectively, slug weight 0.3-0.6g) but females ate significantly more than males (Symondson, 1989). Female beetles require increased nutrition for egg production, with the numbers of eggs laid correlated with the amount of food ingested (van Dijk, 1994). *P. madidus* females are slightly larger than males and may be able to overcome slug defence mucus production.

P. madidus and *N. brevicollis* consumed more dead than either healthy or injured slugs. Dead slugs no longer produce mucus whereas injured and healthy slugs begin producing thick defence mucus at the onset of a beetle

attack. Observations indicated that beetles attacking both injured and healthy slugs were hampered and deterred by both general mucus and defence mucus causing the mouthparts and forelegs to become fouled following an attack. As dead slugs no longer produce mucus they may be more suitable prey items due to a reduced handling time. Dead slugs in this study were generally scavenged on first contact. Pakarinen (1994) also showed that mucus produced by *D. reticulatum* aided survival when individuals were attacked by generalist beetle species and that generalist beetles were more likely to avoid attacking slugs if alternative prey were available.

Scavenging of dead slugs is possible throughout the year as slugs have a fairly constant mortality rate. The consumption of poisoned, dead slugs by generalist beetle species could have an impact on beetle behaviour and survival as a large number of dead slugs available to be scavenged are those that have been killed by molluscicides. Howling (1989), found that slugs poisoned by metaldehyde were more likely to remain on the soil surface and those poisoned by methiocarb were more likely to move underground before dying. The consumption of metaldehyde poisoned slugs is unlikely to have any sub-lethal or lethal effects on carabid beetles (Büchs *et al.*, 1989; Kennedy, 1990). Methiocarb poisoned slugs have been shown to be an attractive food source for carabids and the consumption of slugs killed by methiocarb has been shown to sub-lethally affect species including *P. melanarius*, *N. brevicollis* and *A. parallelepipedus* (Kennedy, 1990).

Both sexes of beetle showed a higher number of contacts with healthy and injured slugs than with dead slugs although there were few healthy or injured slugs killed. Contacts with dead slugs were generally followed by consumption whereas those on healthy or injured slugs were aborted due to defence mucus production.

Beetles use their mandibles for prey capture and the forces created by the mandible tips are used to hold prey and pierce the integument (Wheater & Evans, 1989). A positive correlation has been found in five species of Pterostichini (including *P. madidus*), between the size of the mandible gape and the maximum size of prey killed (Wheater, 1988). Hengeveld (1980) stated that generalist species seem to eat what they can swallow, whereas specialist species choose their prey. *P. madidus* consumed significantly more small slugs (<0.11g) than medium or large slugs. *N. brevicollis* has been found to attack a range of prey up to 4mm in length (Penny, 1966) which would include slugs from the smallest size class in this study. This suggests that these generalist beetle species are only capable of killing small slugs. Hagley et al., (1982) found that *Amara aenae* DeGeer, was only able to attack the first larval instar of the codling moth and *H. rufipes* DeGeer, was effective at consuming the first two larval instars of *Pieris rapae* Linnaeus.

Data from slug population studies in Northumberland (Shirley *et al.*, unpublished data) indicated that slugs in the smallest size class are present in wheat crops from autumn (September) through to late spring (April/May) although this is dependent on weather conditions. In Northumberland,

overwintering *P. madidus* females become active in late spring, by summer there is a peak in numbers of newly emerged beetles of both sexes and by late summer the majority of active beetles are male. In the autumn female beetles are active and eggs are laid from August to November (Luff, 1973). Beetles are therefore active and available to consume small slugs in the autumn and in early spring. Although adult beetles are not active over the winter months the low temperatures at this time also reduce slug activity and feeding damage to crops. *N. brevicollis* adults have two activity peaks, one from May to July and the second peak from September to November (Penny, 1966) and this also coincides with the presence of small slugs.

Serological analysis of field caught beetles has found a number of species testing positive for slugs remains. Symondson & Liddell (1993), found that 89.5% of *A. parallelepipedus* and 42% of *P. madidus* tested positive. In this study 29.8% of *P. madidus* (no-choice trials plus choice trials) would have given positive ELISA's, however only 5.1% had attacked and killed live slugs. Ayre (1995) found a number of beetle species in Northumberland gave positive ELISA's and of the generalists *N. brevicollis* fed on slugs most frequently with 37.4% of beetles testing positive, 10.1% of the *P. madidus* tested were positive and 9.2% of the *P. melanarius*. Ayre (1995), did take into account the mean weight of slugs in different fields with the lowest numbers of beetles testing positive in fields where the largest slugs were found. From the data presented in this study 11.1% of *N. brevicollis* would have tested positive but only 1.8% would have killed slugs.

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Video observations showed that on all occasions slugs were only partially consumed on the first attack. Several individual beetles could therefore consume slug tissue from the same dead slug, each giving a positive ELISA. This could be interpreted as separate kills for each beetle, overestimating their efficiency as biological control agents. Symondson *et al.*, (1996), using polyclonal antibodies found that 84% of field caught *P. melanarius* tested positive. Bohan *et al.*, (2000) suggested that populations of *P. melanarius* and slugs were dynamically associated with each other and that beetle predation on slugs was direct and dynamic with predation by *P. melanarius* causing significant changes in local slug density (around 50% reduction in 30 days) and distribution. Bohan *et al.*, (2000) found eleven percent of the trapped beetles tested positive for slug tissue (using monoclonal antibodies). In both cases pitfall traps were emptied twice per week (beetles trapped over 3 nights). After consuming slug tissue beetles test positive for up to 2.5 days with monoclonal antibodies and up to 4 days with polyclonal antibodies (Symondson & Liddell, 1996b). Simple calculations can thus be made to estimate beetle densities which would cause such a reduction in slug numbers.

Symondson *et al.*, (1996) found 84% of beetles tested positive. As beetles were trapped over 3 nights the percentage of beetles estimated to have eaten in the last 24h is 21% (84% divided by the length of time slug proteins can be detected i.e. 4 days). If beetles can reduce slug populations by approximately 50% in one month and if there are initially 100 slugs/m², 2 slugs must be

eaten each night. With 21% of beetles eating slugs each night, the beetle density would be $6/m^2$. However, Bohan *et al.*, found only 11% of beetles tested positive meaning that only 4.4% of beetles fed on slugs each night. Repeating the above calculations, to reduce slug populations by approximately 50%, the beetle density would need to be $45/m^2$. Densities of *P. melanarius* in the field have been found to range from 0.25 - $11/m^2$ (Thiele, 1977; Scheller, 1984; Ayre, 1995; Kromp, 1999), therefore a beetle density of $6/m^2$ is possible, however, a density of $45/m^2$ is unrealistic indicating that the 50% decline in slug populations is unlikely to be due to beetles alone.

The data presented suggest that the relatively high proportion of positive serological results from field caught carabids (Ayre, 1995; Symondson & Liddell, 1996) may reflect a high scavenging rate rather than actual predation on live or injured slugs. This brings into question the efficiency of generalist beetle species as potential biological control agents of slugs. Whilst these predators undoubtedly contribute to reducing pest numbers their true importance can only be evaluated through a combination of direct (behavioural) and indirect (serological) studies.

2.6 Acknowledgements

This work was funded by the Perry Foundation and the University of Newcastle upon Tyne. We would like to thank Alan Craig for his help with collecting the experimental animals and Mark Shirley for helpful discussion.

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Table 2.1

Numbers of healthy, injured and dead *Deroceras reticulatum* eaten by *Pterostichus madidus* and *Nebria brevicollis* in no-choice trials (n=18).

	Eaten
<i>P. madidus</i> + healthy slug	2
<i>P. madidus</i> + injured slug	0
<i>P. madidus</i> + dead slug	11
<i>N. brevicollis</i> + healthy slug	0
<i>N. brevicollis</i> + injured slug	1
<i>N. brevicollis</i> + dead slug	5

Table 2.2

Numbers of *Deroceras reticulatum* killed and/or consumed in choice trials by each class of *Pterostichus madidus* over the experimental period.

Class	No. of Replicates	Slugs attacked	
		Night 1	Night 2
Females			
Starved 3 days	5	0	0
Starved 7 days	6	5*	2 ⁺
Males			
Starved 3 days	5	0	3*
Starved 7 days	5	0	0

* - dead slugs scavenged

+ - 1 injured & 1 healthy slug eaten

Table 2.3

The number of contacts, the time elapsed between the start of foraging (night) and an attack resulting in a kill, and the time taken to consume a slug (handling time = T_h) observed in interactions between *Pterostichus madidus* and *Deroceras reticulatum* . N1 = night one N2 = night two

Class	No. Contacts Before		Time Elapsed Before		Time Spent Feeding On Kill	
	Kill		Kill (min)		(Th) (min)	
	N1	N2	N1	N2	N1	N2
Female						
Starved 1 week						
Beetle 1	1	1	<1	326	94	38
Beetle 2	1	-	8	-	29	-
Beetle 3	1	2	6	40	90	86
Beetle 4	2	-	26	-	26	-
Beetle 5	-	-	-	-	-	-
Beetle 6	1	-	16	-	5	-
Male						
Starved 3 days						
Beetle 1	-	1	-	65	-	0.5
Beetle 2	-	-	-	-	-	-
Beetle 3	-	-	-	-	-	-
Beetle 4	-	1	-	30	-	10
Beetle 5	-	1	-	40	-	57

Table 2.4

Percentages of healthy, injured and dead *Deroceras reticulatum* contacted by *Pterostichus madidus* in choice trials. N1 = night one N2 = night two (n = 5 for all classes except females starved 3 days n = 6)

	Healthy		Injured		Dead	
	N1	N2	N1	N2	N1	N2
Females						
Starved 3 days	54	14	21	79	25	7
Starved 1 week	48	24	23	76	29	0
Males						
Starved 3 days	41	53	52	26	7	21
Starved 1 week	29	26.5	41	47	30	26.5

Table 2.5

Percentages of small, medium and large *Deroceras reticulatum* slugs consumed by *Pterostichus madidus* and *Nebria brevicollis* beetles in no-choice trials. Slug weights were (i) small 0.01-0.11g (ii) medium 0.12-0.22g (iii) large >0.23g (n=10)

	Small slugs	Medium slugs	Large slugs
<i>Pterostichus madidus</i>	40%	0%	0%
<i>Nebria brevicollis</i>	10%	0%	0%

Figure 2.1

Mean number of contacts between *Pterostichus madidus*, starved either for three days or one week, and *Deroceras reticulatum* over the experimental period. * = significant difference ($P < 0.01$) (n = 5 for all classes except females starved 3 days n = 6)

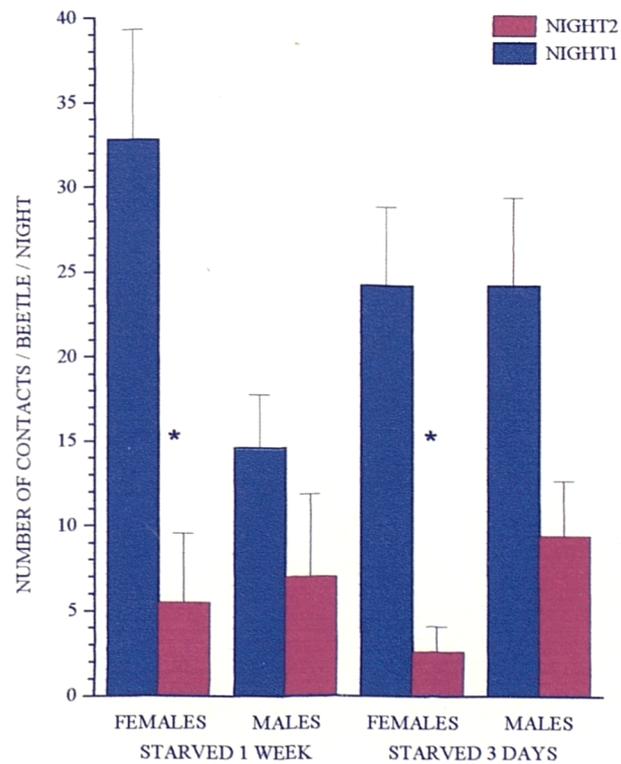


Figure 2.2

Mean speed of male and female *Pterostichus madidus* by night and day during the experimental period. (n = 5 for all classes except females starved 3 days n = 6)

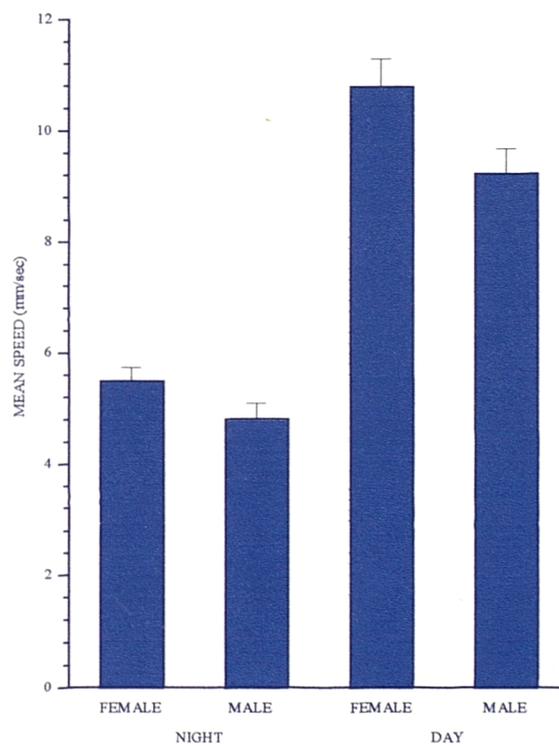
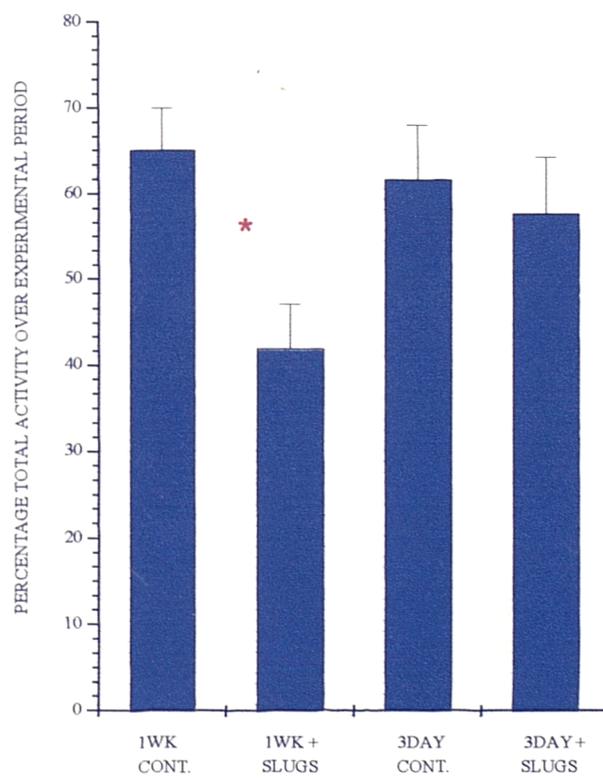


Figure 2.3

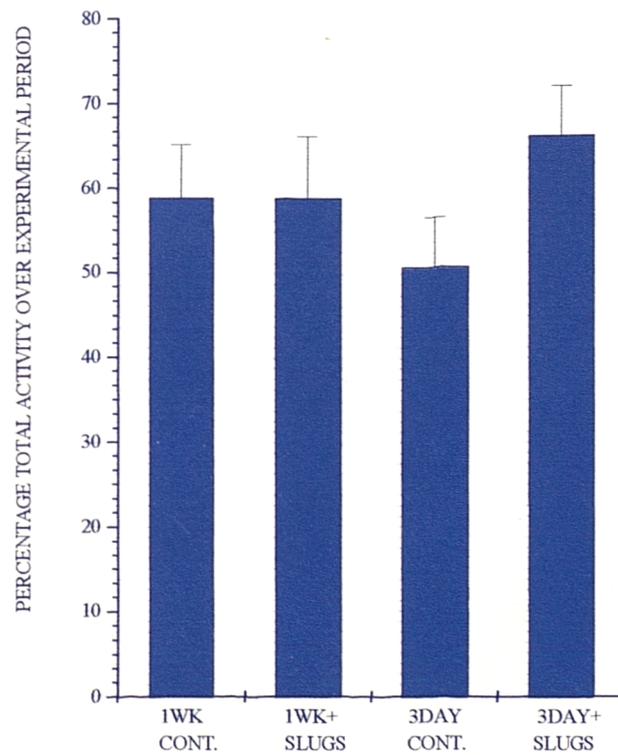
Percentage of the experimental period *Pterostichus madidus* females were active. Beetles were observed with and without *Deroceras reticulatum* prey and with after being starved for either three days or one week. (n = 5 for all classes except females starved 3 days n = 6)



* Significant difference ($P < 0.005$)

Figure 2.4

Percentage of the experimental period *Pterostichus madidus* males were active. Beetles were observed with and without *Deroceras reticulatum* prey and with after being starved for either three days or one week. (n = 5 for all classes except females starved 3 days n = 6)



3. The effect of the presence of *Pterostichus madidus* (Coleoptera, Carabidae) on the activity of the slug *Deroceras reticulatum*.

3.1 Abstract

- 1 Slugs are important pests in many agricultural crops and molluscicides are commonly used to control slugs, however, these affect non-target organisms. Encouraging biological control may help to reduce molluscicide use, but the efficiency of the potential natural enemies needs to be investigated.
- 2 Interactions between the pest slug *Deroceras reticulatum* (Müller) and a potential natural enemy, the carabid beetle *Pterostichus madidus* (Fabricius), were investigated in laboratory conditions.
- 3 The presence of *P. madidus* influenced slug behaviour. When beetles were present slugs spent significantly less time actively foraging on the soil surface. When slugs were active their speed was significantly greater when beetles were present.
- 4 Although small slugs (0.01-0.1g) were used in the experiments, none were preyed upon by *P. madidus* reflecting the low kill rate on live slugs.
- 5 Although *P. madidus* may not be an efficient slug predator, their presence could reduce the time slugs spend actively foraging and in turn lead to a reduction in seedling damage.

3.2 Introduction

In moist, temperate climates slugs are perceived to be a major pest of a wide number of agricultural and horticultural crops (Port & Port, 1986). Of the thirty three species found in the UK (South, 1992), *Deroceras reticulatum* (Müller) is considered to be one of the most economically important (South, 1992).

If the environmental conditions are suitable i.e. adequate soil moisture and air temperatures between 0°C and 20°C, slugs are active during the night (Young *et al.*, 1993). This night time activity is greatest in summer and autumn and, as many arable crops are sown at this time of year, there is a high probability of crop damage.

The majority of slug control is achieved by treating crops with molluscicide baits (containing either metaldehyde or carbamates) and their success relies on slugs actively foraging for the bait and consuming a lethal dose (Kelly & Martin, 1989). Even when slugs do eat the bait, irritancy and lack of palatability caused by the active ingredient often prevents the consumption of a lethal dose and slugs often recover from temporary poisoning. Furthermore molluscicide baits have been shown to affect non-target organisms. Molluscicide baits containing metaldehyde are often implicated in poisoning of vertebrates, whereas the carbamates are more often the cause of invertebrate poisoning. Laboratory experiments have shown that the carbamids

Pterostichus melanarius (Illiger), *Abax parallelepipedus* (Piller & Mitterpacher) and *Nebria brevicollis* (Fabricius) will feed on both metaldehyde and methiocarb pellets. Kennedy (1990), found no lethal or sub-lethal effects after the ingestion of metaldehyde, but beetles which consumed methiocarb pellets displayed sub-lethal and lethal effects. In laboratory and semi-field trials evaluating the toxicity of molluscicides to carabid beetles methiocarb caused high mortality whilst metaldehyde rarely showed any toxic effect. Methiocarb mortality rates ranged from 66-100% for *Carabus granulatus* Linneus, *Poecilus cupreus* Linneus and *Harpalus rufipes* (DeGeer). A lower mortality rate of 25% was found in *P. melanarius* (Buchs *et al.*, 1989). Methiocarb applications in late autumn reduced the numbers of winter active carabids (95% reduction in total carabid activity) whilst spring and summer active species (i.e. not active at the time of most molluscicide application) were unaffected (Purvis & Bannon, 1992).

The role of potential natural enemies of slugs has been studied in another approach to management of these pests and, if effective, may lead to a reduction in molluscicide usage. Some species of carabid beetle are specialist mollusc feeders e.g. beetles from the genera *Cychrus*, *Scaphinotus* and *Carabus*, however these are relatively uncommon in arable crops (Ayre, 1995; Kromp, 1999). Other beetle species, which are generalist feeders, will also feed on molluscs and can survive in the crop when there are no molluscs present by feeding on alternative prey. They are therefore available to attack slugs when slug numbers increase.

Several species of polyphagous carabids have been identified as mollusc predators. Early studies relied on the identification of mollusc remains in dissected beetle crops (Davies, 1953; Luff, 1974; Sunderland, 1975), but as many carabids are fluid feeders no solid remains were observed. Laboratory studies have also shown that carabids attack slugs (Stephenson, 1965).

More recently, serological techniques have been used to determine predation on molluscs by carabids in the field. Early studies employed polyclonal antisera and precipitin techniques to detect invertebrate prey remains in polyphagous predators (Tod 1973; Sunderland, 1988). These tests, however, used macerates of whole slugs and therefore contained a large number of antibodies which would react with a number of prey species. More sensitive techniques have since been used, such as ELISA using polyclonal antisera (Symondson & Liddell, 1993; Ayre, 1995) or using monoclonal antibodies (Symondson & Liddell, 1996). These investigations have shown that carabid species from a number of genera will feed on slugs and the study reported here is part of a body of work being done to elucidate their role in the management of slug pests.

Pterostichus madidus (Fabricius) is a generalist carabid species which is common in arable crops in Northumberland. Serological tests (Ayre, 1995) and preliminary behavioural studies (Asteraki, 1993) have suggested that this species may be an important slug predator. The predatory behaviour by *P.*

madidus on slugs is described elsewhere (Mair & Port, 1999; 2000), but in the course of these studies it became clear that there were more subtle interactions between *P. madidus* and *D. reticulatum* and we report here the effect of beetle presence on slug behaviour.

3.3 Materials and methods

3.3.1 Experimental animals

3.3.1.1 Beetles

Adult *P. madidus* were collected from woodland and grassland habitats at Close House, Heddon on the Wall, Northumberland (Grid Reference NZ 1265) using dry pitfall traps (8 cm diameter x 10.5 cm depth). Traps were emptied three times per week. Prior to experimentation, beetles were maintained individually in 9 cm diameter petri dishes with a disc of moist blotting paper in the base. Petri dishes with beetles were placed in batches in plastic bags to maintain high humidity and kept in a controlled environment room with a light : dark cycle of 16:8 and a temperature of 10°C ($\pm 2^\circ\text{C}$). All experiments were carried out under the same conditions. Beetles were maintained by feeding with chopped blowfly larva/pupa (*Calliphora* sp.) *ad lib*. All beetles were held for a minimum of one week prior to experimentation.

3.3.1.2 Slugs

Slugs of a single species, *D. reticulatum* were collected from beneath shelter traps (40 x 40 cm squares of hardboard) at Close House. Slugs were

maintained in 20 x 20 x 12 cm tubs containing moist roll (Kimwipe) and fed a constant supply of Layers Mash (chicken food). They were kept in the same environmental conditions as the beetles.

3.3.2 Experimental protocol

Beetles and slugs were kept under the experimental light:dark and temperature regime for a minimum of one week prior to the experiments. Beetles were starved for seven days, but Layers Mash was available to slugs until the start of the experiment. Slugs used in experiments were from the smallest size class (0.01-0.1g) that *P. madidus* had been observed to attack (Chapter 2).

Experiments were carried out in a 15 x 38 x 7 cm plastic arena filled to a depth of 2 cm with soil. Large clods of soil were broken up and all visible plant material and small organisms removed. The soil was moistened thoroughly at the start of the experiment and water sprayed onto the surface at regular intervals to maintain a high soil moisture content. A band of Fluon® (polytetrafluoroethylene) (Whitford Plastics, Cheshire, England) 3 cm wide, was painted around the rim of the arena to prevent slugs escaping. A refuge for beetles (5 x 5 cm cardboard square) was also placed in the arena. This was simply laid on top of the soil surface.

A single beetle was placed in the arena with two slugs. Beetle and slug activity were recorded using a camera (Baxall CD9242/IR- sensitive to low

light levels and infra-red illumination from an array of light emitting diodes) and a Panasonic AG-6040 time-lapse video recorder. Time-lapse video recordings were made on 180 min VHS format video tapes at 48 h speed. Experiments were recorded for 24 hours. As *D. reticulatum* and *P. madidus* are nocturnal foragers (video observations showed activity commencing within 20 min of the start of the dark period) only night time behaviour was examined. Experimental animals were placed in the arena during the day and the recording started. This allowed for a settling period of approximately 5 h before the dark period. Control experiments consisted of 2 slugs in the experimental arena with no beetles present (n=10). Different slugs were used in each trial, controls and experiments were recorded on alternate nights. Experiments with beetles were divided into *P. madidus* females (n=5) and *P. madidus* males (n=5). Each beetle was only used once.

3.3.3 Analysis

Beetle and slug behaviour was analysed using the Micromasure® programme to view time lapse video tapes.

A number of parameters were recorded for each animal:

- (i) total time slug was active on soil surface
- (ii) total time beetle was active on soil surface
- (iii) total time slug was inactive on soil surface
- (iv) number of contacts between beetles and slugs and resulting slug behaviour (retreating under refuge/leaving refuge).

(v) mean slug speed over experimental period

(vi) interactions between slugs

Statistical analysis was done using MINITAB. The data that approximated to a normal distribution were analysed using a Students *t*-test (duration of beetle & slug activity and slug speed) and non-parametric data (slug inactivity and beetle contacts) were analysed using the Mann-Whitney test.

3.4 Results

3.4.1 Slug activity on soil surface

Although small slugs (0.01 to 0.1g) were used, no slugs were attacked over the experimental period.

Time active on the soil surface for slugs in each class is shown in Figure 3.1. Slugs in the control group i.e. without any beetles had a mean activity period of 226.9 ± 18.9 min (\pm standard error in all cases, $n=10$) over the 24 hour experiment. Slugs with *P. madidus* present were significantly less active on the soil surface with a mean activity period of 125.5 ± 27.8 min and this difference was significant ($t = 3.01$, $P < 0.01$, $DF = 38$).

When the data were divided according to beetle sex, slugs with female *P. madidus* present had a mean activity period of 169.8 ± 41.2 min which was not significantly different to the activity time in the control group ($t = 1.26$, $P > 0.05$, $DF = 28$). Slugs with male *P. madidus* present had a mean activity

period of 102.9 ± 25.0 min. which was significantly less active than the control group ($t = 3.95$, $P < 0.001$, $DF = 28$).

3.4.2 Beetle activity on soil surface

Female *P. madidus* were active for 715.8 ± 56.0 min. Male *P. madidus* were active for 778.0 ± 110.0 min. There was no significant difference in surface activity between the two sexes ($t = 0.51$, $P > 0.05$, $DF = 8$).

3.4.3 Slug inactivity on soil surface

Slugs spent some time inactive on the soil surface in all experiments. Figure 3.2 shows slug inactivity over the experimental period in the control group and with both sexes of beetle. In the control group the median duration of inactivity was 1.4 min. over the 24 hour experiment. When beetles were present the median duration of inactivity was significantly longer (46.0 min) ($W = 298.0$, $P = 0.001$). The presence of female beetles led to a median inactivity period of 55.5 min and when males were present slugs were inactive for a median of 31.5 min. In both experiments the slugs were inactive for significantly longer than in the control ($W = 256.0$, $P < 0.01$ and $W = 252.0$, $P < 0.05$, respectively). Periods of inactivity occurred in response to the presence of beetles (without contact) and also following contacts with beetles.

3.4.4 Beetle contacts with slugs and resulting behaviour

The number of contacts between each beetle and slug was recorded. A contact was simply classed as any time a beetle touched a slug. Slug behaviour following contact with a beetle was also observed. The number of times a slug left a refuge (under cardboard refuge/clump of soil) following contact was recorded as was the number of times, post contact, a slug retreated under a refuge. The results for each replicate are shown in Table 3.1.

Female beetles contacted slugs significantly more than males ($W = 139.0$, $P = 0.011$). Some contacts caused slugs to move under a refuge: 1.5% of contacts with a female beetle and 2.8 % of contacts with a male beetle. Contacts with slugs under a refuge forced them to become active on 3.8% of contacts with female beetles and 22.5% of contacts with male beetles.

3.4.5 Slug speed

Slug speed over the experimental period was recorded in mm/sec. Figure 3.3 shows slug speeds over the experimental period in the control group and when beetles were present. Slugs with no beetles present had a mean speed of 0.256 ± 0.006 mm/sec. When beetles were present mean speed increased significantly to 0.469 ± 0.007 mm/sec ($t = 21.07$, $P < 0.0001$, $DF = 38$). The presence of female beetles led to a speed of 0.461 ± 0.01 mm/sec and when males were present slug speed was 0.477 ± 0.01 mm/sec, both being significantly faster than the speed in the control (males: $t = 17.87$, $P < 0.0001$, $DF = 29$; females: $t = 16.31$, $P < 0.0001$, $DF = 29$).

3.4.6 Interactions between slugs

There were few interactions between slugs. In the control group, two pairs of slugs contacted each other once. The presence of beetles did not alter the rate of slug contacts, again two pairs of slugs contacted each other on one occasion.

3.5 Discussion

P. madidus did not kill any *D. reticulatum* over the course of the experiment even though slugs of the smallest size class were used. This reflects the low kill rate by this generalist beetle on slugs (Mair & Port, in press).

The presence of *P. madidus* reduces the time *D. reticulatum* spends actively foraging on the soil surface as the slugs spend more time inactive. However, this effect may be counteracted by the slugs travelling faster when they do move. It would be interesting to extend this type of study to assess the effect of beetle activity on plant damage by slugs, even where the beetles were not attacking the slugs. A small percentage of contacts caused an active slug on the soil surface to move under a refuge (1.7%). This response will decrease further the amount of time slugs are active, albeit by a small amount. When slugs were contacted whilst under a refuge a fifth of contacts from males caused these slugs to become active on the soil surface. This may force slugs to become active at times when the environmental conditions are not ideal

for slug activity e.g. low moisture levels leading to desiccation of active slugs.

Although both sexes of beetle were active for a similar period female beetles contacted slugs significantly more often than males. Male *P. madidus* have been found to be less efficient predators of slugs than females (Mair & Port, 1999). Female *P. melanarius* which had consumed slugs in the field were also found to have a greater crop weight than males and it was suggested that females may consume double the number of slugs than males (Symondson *et al.*, 1996). In laboratory trials both male and female *A. parallelepipedus* were shown to have similar kill rates (0.3 and 0.22 slugs/day respectively, slug weight 0.3-0.6g), but females ate significantly more than males (Symondson, 1989). Female beetles require increased nutrition for egg production, with the numbers of eggs laid correlated with the amount of food ingested (van Dijk, 1994). *P. madidus* females are slightly larger than males and may be able to overcome slug defence mucus production. The results presented here indicated that although males do not kill as many slugs as females the presence of male beetles has a more significant effect on slug behaviour causing a reduction in the time slugs spend foraging on the soil surface. Any reduction in slug activity on the soil surface would in turn lead to a reduction in seedling damage.

In Northumberland overwintering *P. madidus* females become active in late spring, by summer there is a peak in numbers of newly emerged beetles of

both sexes and by late summer the majority of active beetles are male. In the autumn female beetles are active and eggs are laid from August to November (Luff, 1973). Due to the climate in northern England slug activity is greatest in summer and autumn, coinciding with the sowing of winter cereals (Shirley *et al.*, 1999) Beetle numbers are therefore at their highest when slugs are likely to be a problem.

D. reticulatum individuals have been shown to detect the odour of the generalist beetle *Pterostichus melanarius* (Dodds *et al.*, 1997). Air containing beetle defensive pygidial secretions were shown to induce rapid signalling in the slug olfactory nerve. It was suggested that although slugs move relatively slowly compared to their predators there could be an advantage for slugs to be able to detect potential predators. The detection of generalist beetle species may allow slugs to flee or to prepare for production of defensive mucus secretions which usually requires up to four seconds after the onset of an attack (Dodds *et al.*, 1997). The results presented here show that *D. reticulatum* may also be able to detect semiochemicals from *P. madidus* and alter its behaviour to reduce the chances of an attack. This may be achieved by remaining under a refuge or by being inactive on the soil surface primed for defence mucus production.

Although *P. madidus* may not be an efficient slug predator, killing only a small percentage of small slugs (<0.1g) (Mair & Port, 1999), the influence of beetle presence on slug behaviour could affect slugs of all sizes. Even if slugs

were too large to be attacked and killed the beetles would still be able to exert some control by altering slug foraging behaviour.

As carabid beetles influence slug behaviour, increasing beetle numbers in crops could lead to a decrease in slug foraging and a resulting decrease in crop damage. Numbers of beetles in crops can be enhanced by various methods including crop diversification (increasing crop heterogeneity and weediness), intercropping and the presence of crop boundaries such as grassy field margins and conservation headlands (Kromp, 1999). Wildflower strips sown to encourage beneficial arthropods, however, have been shown to act as refuges for slugs which then move into the adjacent crop causing increased damage (Frank, 1996). Kirkland *et al.*, (1999) have shown that the use of semiochemical dispensers (*Phacelia tanacetiflora* flower extract and (E)- β -farnesene) placed in a winter wheat crop significantly increased the numbers of carabids in the crop. Using this method carabids can be lured into crops without providing refuges for slugs.

3.6 Acknowledgements

This work was funded by the Perry Foundation and the University of Newcastle Upon Tyne.

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Table 3.1

The number of contacts between *Pterostichus madidus* beetles and *Deroceras reticulatum* slugs when confined in experimental arenas over a single night. The number of contacts that lead to the slug moving under a refuge and the number of times a slug was contacted whilst under a refuge and then became active are also shown.

Slug	Contacts	Move under refuge following contact	Leave refuge following contact
Female Beetles			
1	63	0	0
2	45	0	0
3	24	0	2
4	27	0	2
5	22	0	3
6	31	0	0
7	33	2	2
8	6	0	1
9	1	1	0
10	11	1	0
Male Beetles			
1	2	0	2
2	0	0	0
3	7	0	0
4	1	0	1
5	8	0	3
6	4	0	2
7	2	0	0
8	19	1	3
9	11	0	3
10	17	1	2

Figure 3.1

Time spent active by *Deroceras reticulatum* over the experimental period with and without the presence of *Pterostichus madidus* (both sexes, n = 10; females and males n = 5).

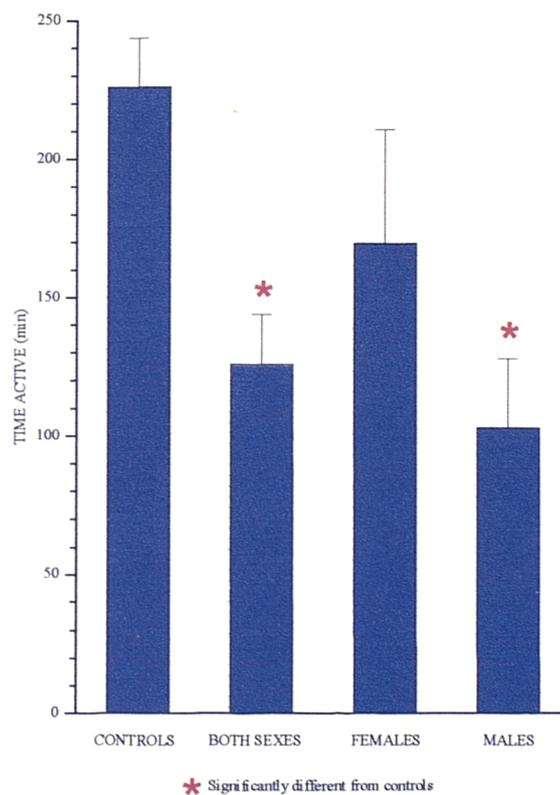


Figure 3.2

Amount of time spent inactive each night by *Deroceras reticulatum* either with or without *Pterostichus madidus* males or females . (n = 10 for controls and 5 for male and female experiments)

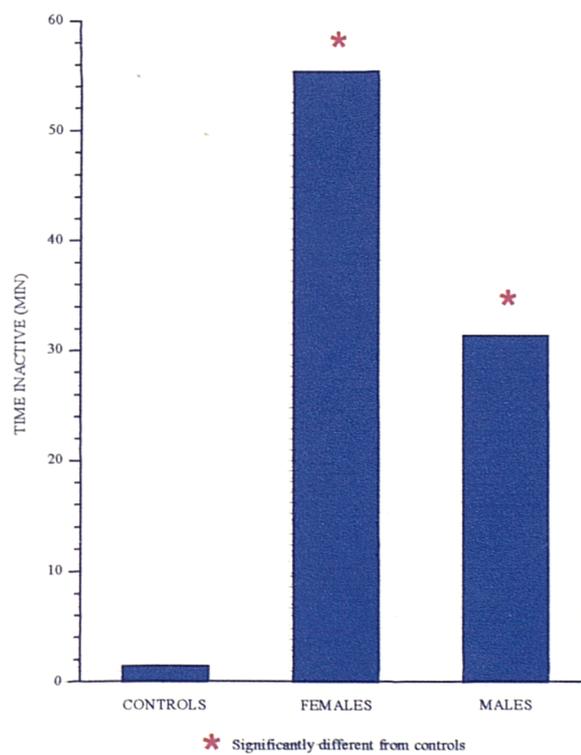
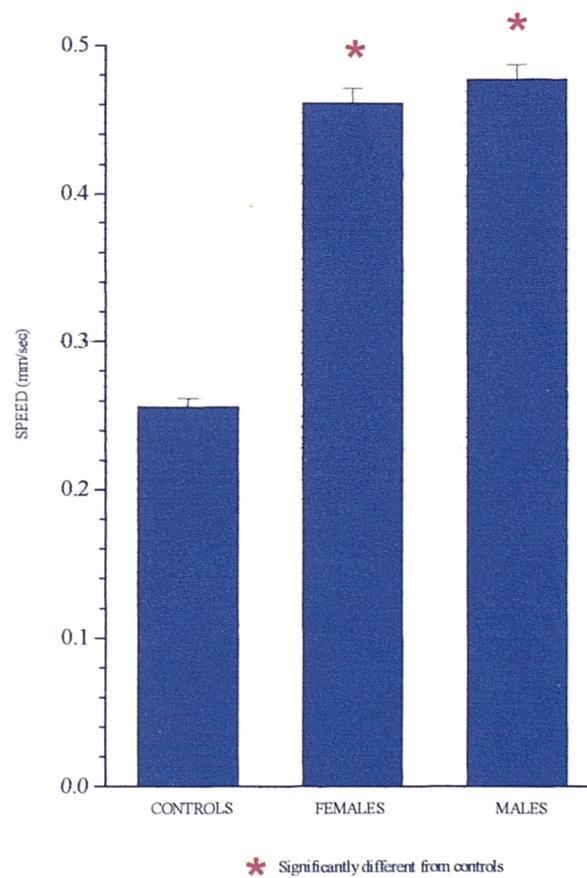


Figure 3.3**Speed of *Deroceras reticulatum* over the experimental period.**

4. Predation on the slug *Deroceras reticulatum* by the carabid beetles *Pterostichus madidus* and *Nebria brevicollis* in the presence of alternative prey.

4.1 Abstract

- 1 Slugs are important pests in many agricultural crops and potential biological control agents are being studied as an alternative to molluscicides. Carabid beetles may be able to reduce slug populations, but their role as control agents may be influenced by the presence of alternative prey.
- 2 Attacks on the pest slug *Deroceras reticulatum* (Müller) by the carabid beetles *Pterostichus madidus* (Fabricius) and *Nebria brevicollis* (Fabricius) were investigated in the presence of alternative prey (earthworms and *Calliphora* fly larvae). Consumption of slug eggs and aphids was also investigated.
- 3 All five prey types were consumed to varying degrees over the experimental period. Both beetle species showed a significant preference for *Calliphora* larvae over slugs. *P. madidus* showed a significant preference for earthworms over slugs. No preference was shown between earthworms or *Calliphora* larvae by *P. madidus* females or *N. brevicollis*, however, *P. madidus* males showed a significant preference for *Calliphora* larvae over worms. *P. madidus* showed no preference between slug eggs and aphids, *N. brevicollis* showed a significant preference for aphids over slug eggs.
- 4 The results from this study indicate that generalist beetles will often attack other prey in preferences to adult slugs. Slugs may not be preferred because of their mucus. Other prey items occur frequently in arable soils and generalist carabids may ignore slugs altogether and may only feed on them when slug density is high or other prey are unavailable.

4.2 Introduction

Previous studies have indicated that carabid beetles are important polyphagous predators in a number of ecosystems including agricultural crops (Luff, 1987; Lövei & Sunderland, 1996). There are several species of specialist carabid beetles, however, the majority are polyphagous feeding on any prey they encounter and are able to overcome (Hengeveld, 1980). The diet of these generalist species varies between individuals as well as temporally throughout the year (Hengeveld, 1980). Carabids can survive without food for weeks depending on their physiological state and water availability (Luff, 1987) although the weight of daily food intake is usually equal to body weight (Lövei & Sunderland, 1996). The majority of temperate carabid species are nocturnal and prey are located via olfactory and tactile cues (Luff, 1987).

Slugs attack a wide range of crops including wheat, oilseed rape and potatoes and are important agricultural pests in moist, temperate climates (Port & Port, 1986). Of the thirty three species found in the UK (South, 1992), *D. reticulatum* (Müller) is considered to be one of the most economically important (South, 1992). The role of potential natural enemies of slugs is being studied in another approach to management of these pests and, if effective, may lead to a reduction in molluscicide usage. Whilst some species of carabid beetle are specialist mollusc feeders these are relatively uncommon in arable crops (Ayre, 1995; Kromp, 1999). Several species of more common polyphagous carabids have been identified as mollusc predators using gut dissection and more recently serological techniques (Stephenson, 1965; Davies, 1953;

Luff, 1974; Sunderland, 1975; Tod 1973; Sunderland, 1988; Symondson & Liddell, 1993; Ayre, 1995; Symondson & Liddell, 1996a).

P. madidus and *N. brevicollis* are polyphagous predators which have been found to contain a wide variety of prey items in their guts including mollusc remains. *P. madidus* feeds on a wide range of insects including lepidopteran larvae, aphids and has also been found to feed on strawberries (Luff, 1973; 1974). *N. brevicollis* has been found to feed on prey items up to 4 mm in length including Collembola, small spiders, mites, Hemiptera, small Diptera, small earthworms and Coleoptera (Davies, 1953; Penny, 1966; Sunderland, 1975).

Successful biological control is achieved by the predator maintaining the number of pests below an economic threshold. Ideally these predators need to be (i) host-specific (ii) synchronous with the pest (iii) able to increase rapidly in numbers (iv) require few pests to complete their life-cycle (so the predator can remain in areas of low pest numbers) and (v) have a high searching efficiency (Murdoch *et al.*, 1985). Generalist beetle species are considered to be poor biological control agents as they are polyphagous, not synchronised with the pest and do not have a high rate of population expansion (Murdoch *et al.*, 1985). Generalists, however, unlike specialists can feed on a variety of prey items so can stay in the crop all year round and not only when the pest is present. This 'lying-in-wait' strategy may allow generalists to be effective control agents (Murdoch *et al.*, 1985).

Several authors have reported the significance of polyphagous carabid beetles as predators of cereal aphids (Sunderland, 1975; Sunderland & Vickerman, 1980; Sopp

& Wratten, 1986; Sunderland *et al.*, 1987; Chiverton, 1988). Some species, such as *Pterostichus melanarius* Illiger and *Harpalus rufipes* DeGeer have been shown to detect the primary constituent of the aphid alarm pheromone (E- β -farnesene) (Kielty *et al.*, 1996), and may thus seek out these pests as prey.

Slugs, however, may be less readily accepted as prey, partly because of their mucus production. Slug skin is relatively soft and permeable and slugs are therefore susceptible to water loss and mechanical and chemical damage. In order to reduce damage, allow movement and prevent desiccation mucus is secreted to act as a lubricant (South, 1992). This paper examines predation on the slug *D. reticulatum* by the generalist beetles *P. madidus* and *N. brevicollis* in the presence of alternative prey types (fly larvae and earthworms). Consumption of slug eggs in the presence of an alternative prey item (pea aphids) was also investigated.

4.3 Materials and methods

4.3.1 Experimental animals

4.3.1.1 Beetles

Adult *P. madidus* and *N. brevicollis* were collected from woodland and grassland habitats at Close House, Heddon on the Wall, Northumberland (Grid Reference NZ 1265) using dry pitfall traps (8 cm diameter x 10.5 cm depth). Traps were emptied three times per week. Prior to experimentation, beetles were maintained individually in 9 cm diameter petri dishes with a 9 cm disc of moist blotting paper in the base. Petri dishes with beetles were placed in batches in plastic bags, to maintain high humidity, and kept in a controlled environment room with a light:dark cycle of 16:8

and at a constant temperature of $12 \pm 2^\circ\text{C}$. Blotting paper discs were changed weekly and beetles were maintained by feeding with chopped blowfly larva/pupa (*Calliphora* sp.) provided *ad lib*. All beetles were held for a minimum of one week prior to experiments.

4.3.1.2 Slugs

Slugs of a single species, *D. reticulatum* were collected from beneath shelter traps (40 x 40 cm squares of hardboard) at Close House. Slugs were maintained in 20 x 20 x 12 cm tubs at $10 \pm 2^\circ\text{C}$ containing moist roll (Kimwipe) and fed a constant supply of Layers Mash (chicken feed). Slug eggs laid in the tubs were collected and also used in the experiments.

4.3.1.3 Earthworms

Earthworms were collected by digging in damp soil from gardens in Newcastle upon Tyne. All small earthworms were collected and maintained in 20 x 20 x 12 cm tubs containing moist soil.

4.3.1.4 *Calliphora* larva

Calliphora larvae were maintained in tubs (20 x 20 x 12 cm) in darkness at a constant temperature of 6°C .

4.3.1.5 Aphids

Pea aphids (*Acyrtosipon pisum* Harris) were collected from broad bean plants grown in a controlled environment room at a constant temperature of 20°C and an 18:6 light:dark cycle.

4.3.2 Experimental Protocol

4.3.2.1 No choice experiments

P. madidus and *N. brevicollis* individuals were presented with three different types of prey i.e. slug, earthworm or *Calliphora* larva, without alternative prey types. The different prey were of approximately the same size and weight (approximately 0.13g). Sandwich boxes (21.5 cm x 12 cm x 7.5 cm) were used as experimental arenas. Each box had a band of Fluon® (polytetrafluoroethylene) (Whitford Plastics, Cheshire, England) (2 cm wide) painted 5 cm from the base to prevent slugs crawling up the side of the box, out of the beetle's reach. The substrate was a 1 cm deep layer of moist Vermiculite (Dupre Vermiculite, Herts, England; Grade DSF 0.05-1.0 mm particle size). Beetles were presented with either a single *Calliphora* larva, worm or slug. Each beetle was only used once. Controls consisted of a single *Calliphora* larva, worm or slug without any beetle predator.

Beetles were also presented with slug eggs or pea aphids. Sandwich boxes (21.5 cm x 12 cm x 7.5 cm) were again used as the experimental arenas with a layer of very moist blotting paper on the base (to prevent eggs desiccating and eggs were easier to locate than if placed on Vermiculite). Five slug eggs (clumped together) or five dead aphids were given to each individual. Slug eggs and aphids of approximately the same weight were used in experiments (approximately 0.001g). Controls consisted of slug eggs or aphids without any beetle predator.

4.3.2.2 Choice trials

In choice trials *P. madidus* and *N. brevicollis* were presented with the three different types of prey (*Calliphora* larvae, worms and slugs) using the same arenas and conditions as described above. Each beetle was given a choice between two prey types i.e. one individual of each prey type was presented. The combinations of prey types presented were (i) slug and *Calliphora* larva (ii) slug and earthworm (iii) earthworm and *Calliphora* larva. Controls consisted of the pair-wise combinations of prey types without the presence of a beetle predator. For both choice and no-choice tests the beetles tested were *N. brevicollis*, female *P. madidus* and male *P. madidus*.

Beetles were also presented with a choice between slug eggs and pea aphids using sandwich box arenas and conditions as described for the no-choice tests. The numbers and sizes of prey were the same as for the no-choice. Controls consisted of the pair-wise combinations of prey types without the presence of a beetle predator.

All experiments were carried out in a light:dark cycle of 16:8 and at a constant temperature of $10 \pm 2^\circ\text{C}$. Experiments were run over three nights i.e. three beetle foraging periods. Numbers of prey eaten were recorded each day. No new prey were added over the course of the experiment. For each experimental combination there were ten replicates.

4.3.3 Analysis

The number (or preference) of prey consumed in each pair-wise combination was tested using χ^2 analysis using Yates' correction for continuity where appropriate.

4.4 Results

4.4.1 No choice experiments

The numbers of each prey type consumed in no choice trials are shown in Table 4.1. All five prey types were consumed to different degrees over the experimental period. *P. madidus* females consumed 100% of the *Calliphora* larvae presented, 80% of the worms, 10% of the slugs, 48% of the slug eggs and 50% of the aphids. *P. madidus* males consumed 100% of the *Calliphora* larvae presented, 80% of the worms, no slugs, 40% of the slug eggs and 52% of the aphids. *N. brevicollis* individuals consumed 80% of the *Calliphora* larvae, 30% of the worms, no slugs, 8% of the slug eggs and 44% of the aphids. No control *Calliphora* larvae, worms or slugs died over the experimental period. There was no sign of desiccation/damage to any of the control slug eggs.

4.4.2 Choice trials

Beetle preferences between slugs and *Calliphora* larvae over the experimental period are shown in Figure 4.1. *P. madidus* females, *P. madidus* males and *N. brevicollis* individuals all showed a significant preference for *Calliphora* larvae over slugs (*P. madidus* females: $\chi^2 = 8.1$, $P < 0.005$, $DF = 1$; *P. madidus* males: $\chi^2 = 8.1$, $P < 0.005$, $DF = 1$; *N. brevicollis*: $\chi^2 = 6.125$, $P < 0.025$, $DF = 1$

Beetle preferences between slugs and worms over the experimental period are shown in Figure 4.2. *P. madidus* females and *P. madidus* males showed a significant preference for worms over slugs (*P. madidus* females: $\chi^2 = 8.1$, $P < 0.005$, $DF = 1$; *P. madidus* males: $\chi^2 = 6.125$, $P < 0.025$, $DF = 1$). No significant preference was shown

for either slugs or worms by *N. brevicollis* ($\chi^2 = 2.25$, $P > 0.05$, $DF = 1$), however, only low numbers of prey were consumed over the experimental period.

Beetle preferences between *Calliphora* larvae and worms over the experimental period are shown in Figure 4.3. *P. madidus* females and *N. brevicollis* did not show any preference between the two prey types (*P. madidus* females $\chi^2 = 2.5$, $P > 0.05$, $DF = 1$; *N. brevicollis* $\chi^2 = 3.125$, $P > 0.05$, $DF = 1$). *P. madidus* males showed a significant preference for *Calliphora* larvae over worms (*P. madidus* males: $\chi^2 = 4.9$, $P < 0.05$, $DF = 1$).

The number of *A. pisum* adults and *D. reticulatum* eggs consumed over the experimental period is shown in Table 4.2. *P. madidus* females and males did not show any significant preference between the two prey types (*P. madidus* females $\chi^2 = 3.70$, $P > 0.05$, $DF = 1$; *P. madidus* males $\chi^2 = 2.82$, $P > 0.05$, $DF = 1$). *N. brevicollis* showed a significant preference for aphids over slug eggs (*N. brevicollis*: $\chi^2 = 13.88$, $P < 0.001$, $DF = 1$).

There was no difference in consumption rates in no-choice experiments compared to that in the choice experiments. Of the 390 prey items that were offered to *P. madidus* and *N. brevicollis* in no-choice trials 169 (i.e. 43.3%) were consumed. Of the 480 prey items that were offered to *P. madidus* and *N. brevicollis* in choice trials 182 (i.e. 37.9%) were consumed.

4.5 Discussion

All prey types were consumed to varying degrees over the experimental period. Only one live slug was consumed from all the experiments in both no-choice and choice trials. Both beetle species showed a significant preference for *Calliphora* larvae over slugs. *P. madidus* showed a significant preference for earthworms over slugs. No preference was shown between earthworms or *Calliphora* larvae by *P. madidus* females or *N. brevicollis*, however, *P. madidus* males showed a significant preference for *Calliphora* larvae over worms. All prey types were approximately the same weight therefore we assume that the choice of prey was not related to size.

Beetle preference for *Calliphora* larvae may have been enhanced as beetles were maintained on these prior to experiments. However, all beetles were collected one week prior to experiments and would only have been presented with one fly larva each before the experimental trials and this is unlikely to have affected prey choice.

Previous work has suggested that prey choice by polyphagous carabids may simply be a response to prey abundance. The efficiency of *P. melanarius* as a predator of codling moth larvae was influenced by the numbers of earthworms, scarabaeids and other large prey in orchard soil (Hagley *et al.*, 1982). Cornic (1973) & Hagley *et al.*, (1982) found that *P. melanarius* ate slugs on rainy days (slugs are more active in moist conditions). This indicates that *P. melanarius* changes its diet according to the availability of particular prey and may feed on slugs if they are abundant. However, the results of the present investigation suggest that there are more subtle interactions between polyphagous carabids and their prey and that prey choice is not solely affected by prey abundance. Sunderland & Vickerman, (1980) showed that the

percentage of carabid beetles which consumed aphids in the field was related to weather conditions as well as beetle sex and stage of sexual maturity and aphid density. *Pterostichus melanarius* and *N. brevicollis* were found to consume aphids even at very low prey densities.

In the present study, one factor which may influence the choice between *Calliphora* larvae, earthworms and slugs is the mucus covered integument. *Calliphora* larvae produce no mucus, earthworms an intermediate amount and slugs a relatively large amount. Live, healthy slugs are difficult animals for carabids to handle due to their defence mucus production (Pakarinen, 1994; Chapter 2). At the onset of an attack by a predator, mechanical stimulation or contact with a noxious substance *D. reticulatum* begin to produce a thick, white defence mucus secretion (Rollo & Wellington, 1979), and production can last up to three minutes in any twenty four hour period (Pakarinen, 1994). Mair & Port (1999; Chapter 3) found that *P. madidus* did consume slugs, but dead *D. reticulatum* were preferentially scavenged rather than attacking and killing live *D. reticulatum*. *P. madidus* could only overcome the defence mucus produced by small slugs (< 0.1g). The preference for dead prey may not be restricted to slugs, but may be common to other prey that are difficult to handle. In laboratory trials, five common North American carabids showed a preference for dead compared to live invertebrates (Best & Beegle, 1977).

In laboratory trials using video observations of beetles feeding upon slugs Ayre (1995), showed that *Pterostichus niger* foraged for a mean time of three hours before a kill and *Abax parallelepipedus* just less than two hours (no alternative prey were available). Mair (Chapter 2), again using video observations, found that *P. madidus*

foraged for up to 326 minutes before killing a healthy slug. Such long periods of time spent foraging before a kill, will increase the probability of the predator contacting other prey species in the field, which they may find more attractive (i.e. less difficult to overcome) and attack as an alternative to killing slugs.

Serological analysis of field caught beetles has found a number of species testing positive for slugs remains. Symondson & Liddell (1993), found that 89.5% of *A. parallelepipedus* and 42% of *P. madidus* tested positive. Ayre (1995) found a number of beetle species in Northumberland gave positive ELISA's and of the generalists *N. brevicollis* fed on slugs most frequently with 37.4% of beetles testing positive, 10.1% of the *P. madidus* tested were positive and 9.2% of the *P. melanarius*. Symondson *et al.*, (1996a), found that 84% of field caught *P. melanarius* tested positive whilst Bohan *et al.*, (2000) found eleven percent tested positive for slug tissue. The variable percentage slug consumption found in these studies indicates that these beetles have a variable diet and feed on a range of prey items and not mainly on slugs.

Some of the observed variation in the percentage of the carabid's diet comprised of slugs may be due to differences in the proportion of relatively small slugs that are easier to consume. Whilst some slugs show distinct population cycles, *D. reticulatum* populations contain individuals of all size classes at any time of year depending on weather conditions (Haynes *et al.*, 1996). However, there are likely to be peaks of juvenile (small) slug numbers in spring and summer following peaks of egg-laying by mature slugs. Overwintering *P. madidus* females become active in late spring, by summer there is a peak in numbers of newly emerged beetles of both sexes and by late summer the majority of active beetles are male. In the autumn female beetles are

active and eggs are laid from August to November (Luff, 1973). *P. madidus* are therefore active and can potentially consume small slugs in the autumn and in early spring. *N. brevicollis* adults have two activity peaks, one from May to July and the second peak from September to November (Penny, 1966) and this also coincides with the presence of small slugs. Thus, despite their variable food preferences, these carabid species may reduce the numbers of slugs in an area by feeding on particular size classes.

Slug eggs are a more suitable food item than juvenile or adult slugs as they have no defensive mucus covering. Large numbers of slug eggs were consumed in this study by *P. madidus* and there was no preference shown between aphids and slug eggs. - Slug eggs are present throughout the year, but with peaks in abundance in spring and autumn and this coincides with beetle activity. Therefore the consumption of eggs may be more important than consumption of juveniles and adults in reducing slug numbers. Further studies on the rates of egg consumption in the field need to be carried out.

4.6 Acknowledgements

This work was funded by the Perry Foundation and the University of Newcastle upon Tyne.

4.7 References

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Table 4.2

Cumulative numbers of slug eggs and aphids consumed over three nights (n = 10 in each class). Eggs and aphids were presented in groups of five in each trial. Data for night three show the total numbers of each prey type consumed. * = significant difference in numbers consumed.

	NIGHT 1		NIGHT 2		NIGHT 3	
	APHID	EGG	APHID	EGG	APHID	EGG
<i>P. madidus</i> FEMALE	17	7	19	8	19	8
<i>P. madidus</i> MALE	23	18	32	19	32	19
<i>N. brevicollis</i>	*19	2	*23	3	*23	3
CONTROL	0	0	0	0	0	0

Figure 4.1

Numbers of slugs and *Calliphora* larvae consumed by *P. madidus* males and females and *N. brevicollis* in choice trials over three nights (n=10 in each class). * = significant difference in numbers consumed.

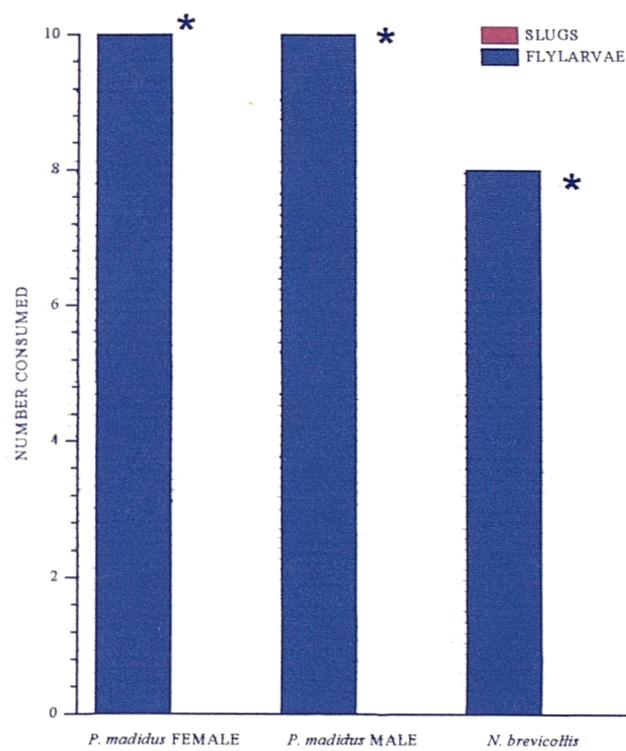


Figure 4.2

Numbers of slugs and worms consumed by *P.madidus* males and females and *N. brevicollis* in choice trials over three nights (n=10 in each class). * = significant difference in numbers consumed.

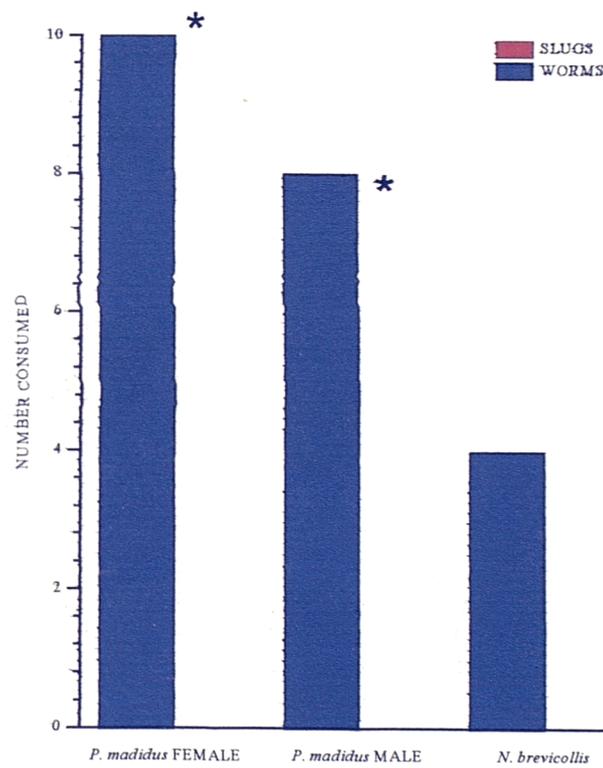
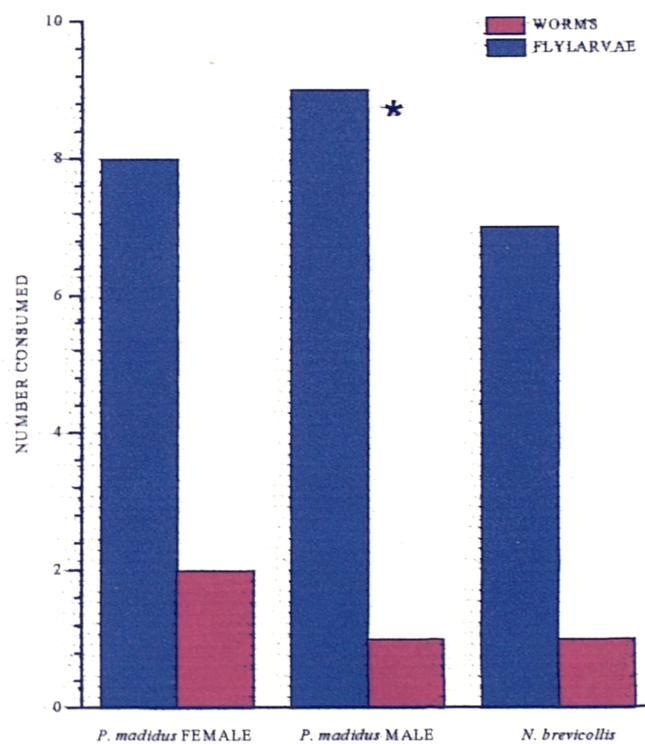


Figure 4.3

Numbers of *Calliphora* larvae and worms consumed by *P. madidus* males and females and *N. brevicollis* in choice trials over three nights (n = 10 in each class).

*** = significant difference in numbers consumed.**



5. The influence of mucus production by the slug *Deroceras reticulatum* on predation by *Pterostichus madidus* and *Nebria brevicollis* (Coleoptera: Carabidae).

5.1 Abstract

- 1 Slugs are important pests in many agricultural crops and potential biological control agents are being studied as an alternative to molluscicides. Carabid beetles may be able to reduce slug populations, but the defence mucus of slugs may deter some predator attacks.
- 2 Interactions between the carabids *Pterostichus madidus* (Fabricius) and *Nebria brevicollis* (Fabricius) with healthy and 'stressed' (unable to produce defence mucus) *Deroceras reticulatum* (Müller) were investigated in laboratory conditions.
- 3 Both beetle species consumed significantly more stressed slugs than controls. Defence mucus production by control slugs hampered beetle attacks. These generalist beetle species did not direct their attacks at vulnerable parts of the prey as equal numbers of contacts were made on the slug head, mantle and tail.
- 4 *Calliphora* sp. larvae are readily consumed by *P. madidus* and *N. brevicollis*. *Calliphora* larvae coated in slug defence mucus were less acceptable to both beetle species compared with control larvae.
- 5 Results indicate that these generalist beetle species are unable to overcome the defence mucus production of healthy slugs. Slugs sub-lethally poisoned

by molluscicides may be a more suitable prey item due a reduction in defence mucus production.

5.2 Introduction

Slugs attack a wide range of crops including wheat, oilseed rape and potatoes and are important agricultural pests in moist, temperate climates (Port & Port, 1986). Of the thirty three species found in the UK (South, 1992), *D. reticulatum* (Müller) is considered to be one of the most economically important (South, 1992).

The majority of slug control is achieved by treating crops with molluscicide baits (containing either metaldehyde or carbamates) and their success relies on slugs actively foraging for the bait and consuming a lethal dose (Kelly & Martin, 1989). Even when slugs do eat the bait, irritancy and lack of palatability caused by the active ingredient often prevents the consumption of a lethal dose and slugs often recover from temporary poisoning. Juvenile slugs may also be less well controlled than adults due to differences in activity or foraging patterns (Kelly & Martin, 1989). The role of potential natural enemies of slugs has been studied as another approach to management of these pests and, if effective, may lead to a reduction in molluscicide usage. However, relatively few invertebrates are specialist predators of slugs and one reason for this is the production of defence mucus by the slug.

Slug skin is relatively soft and permeable and slugs are therefore susceptible to water loss and mechanical and chemical damage. In order to reduce damage, allow

movement and prevent desiccation mucus is secreted to act as a lubricant (South, 1992). *D. reticulatum* has mucus glands near the surface of the skin and these can be divided into three types (i) pedal mucus cells found in the foot (ii) mantle mucus cells found in the mantle and dorsal surface (iii) peripodial cells along the foot margin (Triebkorn & Ebert, 1989). Mucus cells function in water regulation and ion exchange (Deyrup-Olsen *et al.*, 1989). At the onset of an attack by a predator, mechanical stimulation or contact with a noxious substance *D. reticulatum* begin to produce a thick, white defence mucus secretion from the mantle mucus cells (Rollo & Wellington, 1979). *D. reticulatum* can produce a constant flow of defence mucus for up to 3 minutes in any 24 hour period (Pakarinen, 1994).

Partly because of the defence mucus production, few invertebrates are specialist predators of slugs. Certain species of carabid beetle are specialist mollusc feeders (beetles from the genus *Cychrus*, *Scaphinotus* and *Carabus*), possessing powerful, elongated mandibles (Evans & Forsythe, 1985). These beetles prevent slugs producing large quantities of mucus by directing their attacks to kill/paralyse their prey quickly (Pakarinen, 1994). However, these carabids are either not commonly found in arable fields or not found at high enough densities to reduce slug populations. Better candidates for effective biological control of slugs in arable crops are polyphagous carabids which occur naturally in farmland (Port *et al.*, 2000). Several species of polyphagous carabid have been identified as mollusc predators. Laboratory studies have shown that these carabids attack slugs (Stephenson, 1965) and studies of field collected material used the presence of

mollusc remains in dissected beetle crops to confirm consumption (Davies, 1953; Luff, 1974; Sunderland, 1975). Serological techniques using precipitin tests have been used to determine feeding on molluscs by carabids in the field (Tod 1973; Sunderland, 1988) as well as more sensitive techniques, such as ELISA using polyclonal antisera (Symondson & Liddell, 1993; Ayre, 1995) or using monoclonal antibodies (Symondson & Liddell, 1996). Some of these serological studies have shown that a significant proportion of carabid beetles may consume slug tissue (Symondson *et al.*, 1996). None of these serological techniques can distinguish between true predation and scavenging, however, and Mair & Port (1999) have suggested that generalist beetle species will preferentially scavenge dead slugs (which do not produce mucus) rather than live slugs.

This study examined the effect of mucus production by *D. reticulatum* on slug survival and interactions with the generalist beetle predators *P. madidus* and *N. brevicollis*. An alternative prey (*Calliphora* larvae), known to be readily consumed by both beetle species, was coated in slug defence mucus and the reaction of *P. madidus* and *N. brevicollis* recorded.

5.3 Materials and methods

5.3.1 Experimental animals

5.3.1.1 Beetles

Adult *P. madidus* and *N. brevicollis* were collected from woodland and grassland habitats at Close House, Heddon on the Wall, Northumberland (Grid Reference

NZ 1265) using dry pitfall traps (8 cm diameter x 10.5 cm depth). Traps were emptied three times per week. Prior to experimentation, beetles were maintained individually in 9 cm diameter petri dishes with a disc of moist blotting paper in the base. Petri dishes with beetles were placed in batches in plastic bags to maintain high humidity and kept in a controlled environment room with a light : dark cycle of 16:8 and a temperature of $10 \pm 2^\circ\text{C}$. All experiments were carried out under the same conditions. Beetles were maintained by feeding with chopped blowfly larva/pupa (*Calliphora* sp.) provided *ad lib*. All beetles were held for a minimum of one week prior to experiments.

5.3.1.2 Slugs

Slugs of a single species, *D. reticulatum* were collected from beneath shelter traps (40 x 40 cm squares of hardboard) at Close House. Slugs were maintained in 20 x 20 x 12 cm tubs at $10 \pm 2^\circ\text{C}$ containing moist paper roll (Kimwipe) and fed a constant supply of Layers Mash (chicken food). The moist environment ensured that slugs for use in the experiments were fully hydrated.

5.3.2 Mucus removal

D. reticulatum individuals were weighed prior to mucus removal. Defence mucus production was encouraged by mechanical stimulation. *D. reticulatum* individuals were touched from head to tail with a pair of blunt forceps. Stimulation was carried out for three minutes at a constant rate of one stroke per second. After three minutes *D. reticulatum* individuals were no longer able to produce defence

mucus although the production of pedal mucus was not affected. Pakarinen (1984) termed this treatment 'stressing' and this term will also be used in this paper.

5.3.3 Survival after stressing

Stressed slugs i.e. unable to produce defence mucus, were placed individually in 9 cm diameter petri dishes with a layer of moist blotting paper. No carabids were present. Three size classes were used (i) small 0.01 - 0.1 g (ii) medium 0.12 - 0.2 g (iii) large >0.22 g (n = 10 in each class). Defence mucus production after 24h was recorded and classed as either (i) little/none (ii) moderate (iii) copious. Slug survival was also recorded. Defence mucus production and survival in control slugs (which had not been stressed) was recorded (n = 10 in each size class).

5.3.4 Susceptibility of stressed slugs to predation

P. madidus and *N. brevicollis* were presented with either a single stressed or control slug from the size classes described previously (Section 5.3.3; n = 10 in each class). Experiments were carried out in 9 cm diameter petri dishes with a layer of moist blotting paper on the base. Slug mortality was recorded after 24 h as was the amount of defence mucus produced in surviving slugs.

5.3.5 Choice trials

P. madidus and *N. brevicollis* individuals were presented with a choice between two slugs i.e. one stressed and one control (able to produce defence mucus). The two slugs were both from the same weight class. Experiments were carried out in sandwich boxes (21.5 x 12 x 7.5 cm) with a layer of moist blotting paper in the

base and a band of Fluon® (polytetrafluoroethylene) (Whitford Plastics, Cheshire, England) 3 cm wide on the walls to prevent slugs crawling onto the roof out of the beetle's reach. Slug weight before and after the experiment was recorded as was survival and defence mucus production after 24 h. There were ten replicates in each weight class.

Controls consisted of two slugs from the same weight class i.e. one stressed slug and one slug able to produce defence mucus with no carabid beetles present.

5.3.6 Video Studies

Behavioural interactions between beetles and slugs were studied using time-lapse video recordings over single nights. Experiments were carried out in a 15 x 38 x 7 cm plastic arena filled to a depth of 2 cm with soil. Any other organisms observed in the soil were removed and large clods of soil broken up. A refuge made from a square of cardboard (5 x 5 cm) was placed in the arena. Due to irregularity of the soil surface beetles were able to move under the refuge. A band of Fluon® 3 cm wide, was painted around the rim of the arena to prevent slugs and beetles escaping. Beetle and slug activity was recorded using a video camera (Baxall CD9242/IR sensitive to low light levels and infra-red illumination from an array of light emitting diodes) and a Panasonic AG-6040 time-lapse video recorder. A single beetle was placed in the arena with one stressed slug and one slug able to produce defence mucus. Medium weight class slugs (0.12-0.2g) were used as this was the class of control slugs (i.e. able to produce defence mucus) that were not likely to be consumed by either beetle species (Section 5.4.2). Slug weight was

recorded at the start and end of the experiment. Beetles were starved for 7 days prior to the start of the experiment. As *D. reticulatum* and the two carabid species are nocturnal foragers only night time behaviour was examined

In these experiments the active components of slug and beetle behaviour were examined and classified as follows:

- (i) number of contacts with slug
- (ii) time of attack
- (iii) target of attack i.e. head, mantle, tail
- (iv) number of attacks before consumption
- (v) time each slug spent active on soil surface

The amount of mucus exuded at the end of the experiment was assessed following mechanical stressing. Controls consisted of slugs only in the arena (to determine prey behaviour in the absence of a predator).

5.3.7 Maggots in Mucus

5.3.7.1 No-choice experiments

In order to determine whether defence mucus production was a major obstacle for generalist beetle species to overcome, an alternative prey type known to be readily consumed by both *P. madidus* and *N. brevicollis* i.e. live blowfly larvae (*Calliphora* sp.) were covered in slug defence mucus and presented to both beetle species. A number of *D. reticulatum* individuals (approximately 10) were placed in a small glass vial with a gauze lid and exposed to CO₂ for 30 s. This caused the

slugs to become anaesthetised and exude copious amounts of defence mucus. *Calliphora* larvae were then mixed with this defence mucus and immediately used in experiments. Experiments were carried out in 9 cm diameter petri dishes with a layer of moist blotting paper in the base. *P. madidus* and *N. brevicollis* were presented with either a single live *Calliphora* larva covered in mucus or a control live *Calliphora* larva (n = 10). *Calliphora* larvae were also killed (frozen) then covered in mucus. *P. madidus* and *N. brevicollis* were presented with a dead *Calliphora* larva covered in mucus or a control dead *Calliphora* larva (n = 10). Predation by beetles was recorded at 5, 15, 30 min after *Calliphora* larvae were presented. The number of *Calliphora* larvae consumed after 24 h was also recorded.

5.3.7.2 Choice trials

P. madidus and *N. brevicollis* individuals were presented with two dead *Calliphora* sp. larvae. One of the two larvae was coated in slug defence mucus as described previously (Section 5.3.7.1). Cream and red coloured larvae were used to identify which larva had been covered in mucus. Experiments were carried out in sandwich boxes (21.5 x 12 x 7.5 cm) with a layer of moist blotting paper in the base. There were ten replicates in each class (5 cream *Calliphora* larvae covered in mucus with red controls and 5 red *Calliphora* larvae covered in mucus with cream controls). Predation by beetles was recorded at 5, 15, 30 min and 24 h after larvae were presented. To determine whether or not beetles showed a preference for a certain colour of *Calliphora* larvae the experiment was repeated with neither larva being covered in mucus.

5.3.8 Statistical Methods

Differences in numbers of attacks by beetles, slugs surviving etc. were examined using χ^2 analysis with Yates' correction for continuity. Differences in beetle and slug activity were analysed (after confirming the data were normally distributed) using the Student's t-test.

5.4 Results

5.4.1 Survival after stressing

Stressed *D. reticulatum* individuals were unable to produce defence mucus 24 h after stressing (Table 5.1). The stressing process itself did not cause an increase in slug mortality as there was no difference between the survival of stressed or control slugs after 24 h (100% of stressed and control slugs surviving; $\chi^2 = 0.005$, $P > 0.05$, DF = 1).

5.4.2 Susceptibility of stressed slugs to predation

Stressed and control slugs were consumed by both beetle species over the experimental period. The numbers of stressed and control slugs consumed are shown in Table 5.2. *P. madidus* females consumed small stressed and control slugs to the same extent. The numbers of stressed medium and large slugs which were consumed was significantly greater than that of the controls (medium: $\chi^2 = 6.12$, $P < 0.05$, DF = 1; large: $\chi^2 = 3.96$, $P < 0.05$, DF = 1). *P. madidus* males consumed significantly more small stressed slugs than small control slugs ($\chi^2 = 3.96$, $P < 0.05$, DF = 1). There was no significant difference in the numbers of medium and large

stressed and control slugs consumed i.e. no medium stressed or control slugs were consumed and only one large control slug was consumed. *N. brevicollis* consumed very few slugs of any category and there was no significant difference between the numbers of stressed and control slugs consumed in all three size classes ($P > 0.05$ in all classes).

When all slug size classes were grouped together only *P. madidus* females showed a significant preference for stressed slugs over control slugs ($\chi^2 = 8.52$, $P < 0.05$, $DF = 1$). *P. madidus* males and *N. brevicollis* individuals showed no significant differences between stressed and control slugs, however, this may be due to the low numbers of slugs consumed by these beetles over the experimental period.

Of the slugs which were not consumed those that had been stressed still showed impaired mucus production after 24 h whilst mucus production was greater in those that had not been stressed (Table 5.3).

5.4.3 Choice trials

Slugs were consumed by both beetle species over the experimental period. Figure 5.1 shows the numbers in each slug class consumed by *P. madidus* females. No significant preference was shown between small stressed and control slugs ($\chi^2 = 2.28$, $P > 0.05$, $DF = 1$). There was a significant preference for medium sized stressed slugs against controls ($\chi^2 = 3.96$, $P < 0.05$, $DF = 1$) and a significant preference for large stressed slugs over control slugs ($\chi^2 = 4.16$, $P < 0.05$, $DF = 1$).

The numbers of slugs consumed by *P. madidus* males is shown in Figure 5.2. In the smallest slug size class males showed a significant preference for stressed slugs against controls ($\chi^2 = 3.96$, $P < 0.05$, $DF = 1$) There were no significant preferences shown for stressed or control medium and large slugs.

N. brevicollis did not show a significant preference for stressed or control slugs over the experimental period, with only two small stressed slugs consumed (Figure 5.3).

All surviving control slugs produced copious amounts of mucus at the end of the experiment. In the control group (i.e. no beetles present) no stressed slugs or those able to produce defence mucus died over the experimental period.

5.4.4 Video analysis

The results of behavioural interactions between beetles and slugs are shown in Table 5.4. *P. madidus* individuals contacted control slugs significantly more than stressed slugs over the experimental period (stressed total 126 , control total 222; $\chi^2 = 25.9$, $P < 0.01$, $DF = 1$). *P. madidus* showed similar numbers of contacts with the head, mantle and tail of both stressed and control slugs. Three stressed slugs were consumed and none of the control slugs. Two of the stressed slugs were killed on the first attack (one attack directed to the head after 30 min and one to the mantle after 1 min) and the third slug was killed on the fourth attack (attack directed to the tail after 182 min). Beetles spent a mean time (\pm SE) of 65.5 min \pm

6.6 feeding on the killed slugs. No slugs were totally consumed during the first bout of feeding. Stressed and control slugs spent a similar time inactive on the soil surface in the presence of *P. madidus* (stressed slugs mean inactivity $119.1 \text{ min} \pm 42.4$, control slugs mean inactivity $65.8 \text{ min} \pm 22.2$; $T = 1.11$, $P > 0.05$, $DF = 13$). Stressed and control slugs also spent a similar time under refuges (stressed slugs mean time under refuge $107.2 \text{ min} \pm 43.7$, control slugs mean time under refuge $212.8 \text{ min} \pm 38.9$; $T = 1.8$, $P > 0.05$, $DF = 17$).

The data for *P. madidus* males and females ($n = 5$ in each case) were examined separately. Both males and females contacted control slugs significantly more than stressed slugs (females $\chi^2 = 5.95$, $P < 0.05$, $DF = 1$; males $\chi^2 = 29.6$, $P < 0.001$, $DF = 1$). Contacts did not appear to be directed, as there was a similar number with the head, mantle and tail. *P. madidus* males did not kill any stressed or control slugs over the experimental period whilst *P. madidus* females killed three stressed slugs. Stressed slugs were inactive for significantly longer in the presence of male ($323.8 \text{ min} \pm 53.3$) than female ($114.6 \text{ min} \pm 67.7$) beetles ($T = 2.43$, $P < 0.05$, $DF = 9$). Control slugs spent significantly longer inactive on the surface in the presence of female ($114.8 \text{ min} \pm 26.9$) beetles than males ($16.8 \text{ min} \pm 16.8$) ($T = 3.09$, $P < 0.05$, $DF = 9$).

N. brevicollis individuals contacted control slugs significantly more than stressed slugs ($\chi^2 = 5.26$, $P < 0.05$, $DF = 1$). Similar numbers of contacts were directed to the head, mantle and tail of both stressed and control slugs. Five of the stressed slugs were consumed: three on the first contact with one attack directed to the

head (after 2 min) and two to the mantle (after 1 and 14 min). One slug was consumed on the second contact with an attack directed to the head (after 301 min) and one consumed after 17 contacts with an attack directed to the tail (after 25 min). Beetles spent a mean time of $71.7 \text{ min} \pm 14.7$ feeding on the dead slugs. No slugs were totally consumed during the first bout of feeding. No control slugs were consumed. When *N. brevicollis* were present stressed and control slugs spent a similar time inactive on the soil surface (stressed $304.9 \text{ min} \pm 36.3$, control $355.3 \text{ min} \pm 41.5$; $T = 0.42$, $P > 0.05$, $DF = 17$) and also a similar time under refuges (stressed $169.0 \text{ min} \pm 40.4$, control $156.9 \text{ min} \pm 52.8$; $T = 1.25$, $P > 0.05$, $DF = 16$).

P. madidus contacted stressed slugs significantly more often than *N. brevicollis* ($\chi^2 = 22.7$, $P < 0.001$, $DF = 1$). Both species contacted the head, mantle and tail to the same extent. *P. madidus* contacted control slugs significantly more often than *N. brevicollis* ($\chi^2 = 56.0$, $P < 0.001$, $DF = 1$), with more contacts being directed at the mantle.

5.4.5 *Calliphora* larvae covered in mucus

5.4.5.1 No-choice experiments

5.4.5.1.1 Live *Calliphora* larvae

The numbers of live control *Calliphora* larvae and mucus covered larvae consumed is shown in Figures 5.4.1 - 5.4.3. Both beetle species had attacked and consumed all control *Calliphora* larvae within 5min. The larvae covered in mucus were not consumed as rapidly, but as live larvae were able to move, the covering

of slug defence mucus was quickly wiped off onto the moist blotting paper and they were then attacked.

5.4.5.1.2 Dead *Calliphora* larvae

The numbers of dead control *Calliphora* larvae and mucus covered larvae consumed are shown in Figures 5.5.1 - 5.5.3. Fewer larvae covered in mucus were consumed during the course of the experiment.

5.4.5.2 Choice trials

Neither *P. madidus* nor *N. brevicollis* showed any preference for cream or red coloured *Calliphora* larvae as similar numbers of each colour were consumed (Table 5.5.1).

The numbers of control *Calliphora* larvae and mucus covered larvae consumed in choice trials are shown in Table 5.5.2. *P. madidus* females consumed more controls than mucus covered larvae. After 15 min there was a significant preference shown for controls ($\chi^2 = 4.0$, $P < 0.05$, $DF = 1$). After 24 h seven control *Calliphora* larvae had been consumed and only 2 mucus covered larvae consumed. *P. madidus* males also consumed more controls than mucus covered larvae. After 5 min there was a significant preference for controls ($\chi^2 = 4.1$, $P < 0.05$, $DF = 1$). There were no significant preferences shown by *N. brevicollis* although there were more controls consumed than mucus covered *Calliphora* larvae.

Contacts between beetles and *Calliphora* larvae were observed. Larvae covered in mucus were rejected on first contact by both beetle species. Mucus covered larvae were contacted with the antennae and palps which appeared to cause the mandibles to open automatically, but there was no attack on the larva. If beetle mouthparts did contact a mucus covered larva a period of mandible cleaning followed. The majority of further contacts with mucus covered larvae resulted in rejection by the beetles. The mucus deterred beetles over the 24h of the experiment.

5.5 Discussion

Within a few seconds of a beetle attack, slugs produce a thick defence mucus. The low numbers of mucus producing slugs consumed in these experiments suggests that the generalist beetle species *P. madidus* and *N. brevicollis* find it difficult to overcome the defence mucus secreted by *D. reticulatum*.

In both no choice and choice trials *P. madidus* females showed no significant preference between small stressed or control slugs. The numbers of medium and large stressed slugs consumed was significantly greater than that of the controls, suggesting that these beetles were unable to overcome the defence mucus produced by control medium and large slugs. *P. madidus* males showed a significant preference for small stressed slugs over controls (males are unable to overcome the defence mucus produced by even small control slugs), but no preference was shown in the larger size classes as few slugs were preyed upon. *N. brevicollis* did not show any preference for stressed or control slugs of any size

class again due to the low numbers of slugs consumed over the experimental period. Pakarinen (1994) also showed that the generalist beetle species *P. niger* preferred stressed *D. reticulatum* significantly over control individuals.

When *P. madidus* and *N. brevicollis* were presented with both a stressed and control slug, control slugs were contacted more than stressed slugs although none were killed. These generalist beetle species did not target their attacks at particular parts of the slug as an even number of attacks were directed to the head, mantle and tail. Generalists attack slugs by opening their mandibles and attempting to bite the slug. Contacts with control slugs lead to the beetle mouthparts being 'gummed up' with defence mucus and after each contact there followed a period of mandible cleaning. Beetles that are mollusc specialists (*Cychrus*, *Scaphinotus* and *Carabus*) have developed morphological and behavioural adaptations to overcome defence mucus production. *Cychrus caraboides* L., directs its attacks to the head destroying the cerebral ganglion either by poison or mechanical damage whilst *Carabus violaceus* L., causes paralysis by attacking the posterior of the mantle (Pakarinen, 1994). These beetles have powerful mandibles that can quickly penetrate the skin before the slug can initiate defence mucus production. Slugs of all sizes are consumed by specialists as the mandibles are used to 'stab' the slug instead of biting, therefore the width of the mandible gape is not limiting (Wheater, 1988; Pakarinen, 1994).

P. madidus and *N. brevicollis* readily consume *Calliphora* sp. larvae. In choice trials, both beetle species consumed more control larvae than larvae covered in

mucus (*P. madidus* significantly so). *Calliphora* larvae covered in mucus were rejected on first contact by both beetle species and contacts were followed by a period of mandible cleaning. Both generalist beetle species had difficulty overcoming the defence mucus coating and control larvae were preferentially consumed due to a reduced handling time.

P. madidus and *N. brevicollis* have been shown to be capable of consuming small (<0.01g) healthy *D. reticulatum* (Mair & Port, 1999). Beetles appear unable to overcome the defence mucus produced by larger slugs. Dead slugs were also scavenged in preference to 'healthy' slugs. As dead slugs no longer produce mucus they may be a more suitable prey item due to a reduced handling time (Mair & Port, 1999). Pakarinen (1994), also showed that mucus produced by *D. reticulatum* aided survival when individuals were attacked by generalist beetle species and that generalists are likely to avoid attacking slugs if alternative prey were available.

The production of defence mucus by slugs utilises valuable resources as within a few minutes large amounts of body fluids are lost (Deyrup-Olsen *et al.*, 1989). The slug body is composed of approximately 80% water and slugs must regulate their water content to within very narrow limits (South, 1992). In the field, several factors may impede defence mucus production increasing the likelihood of slugs being attacked by beetles. Molluscicides (containing either metaldehyde or carbamates) are commonly applied to crops to control slugs. On contacting or consuming these toxins, slugs produce mucus to dilute the toxin and to form a

protective barrier preventing the toxin contacting epithelial cells on the skin (Triebkorn & Ebert, 1989). After ingesting carbamates, *D. reticulatum* produce increased levels of chemically modified mucus (damage to the digestive tract is less than after ingestion of metaldehyde) whilst after ingesting metaldehyde there is an initial increase in digestive tract mucus production followed by total destruction of the mucus producing system preventing mucus production by the skin (Triebkorn & Ebert, 1989). Slugs sub-lethally poisoned by metaldehyde (i.e. no longer able to produce defence mucus) which may recover in humid conditions, would be suitable prey items for generalist beetle species. No lethal or sub-lethal effects have been observed after the ingestion of metaldehyde by carabid beetles (Büchs *et al.*, 1989; Kennedy, 1990).

P. madidus females consumed more slugs than males. Female *P. melanarius* which had consumed slugs in the field, were also found to have a greater crop weight than males and it was suggested that females may consume double the number of slugs compared to males (Symondson *et al.*, 1996). In laboratory trials both male and female *A. parallelepipedus* were shown to have similar kill rates (0.3 and 0.22 slugs/day respectively, slug weight 0.3-0.6g), but females ate significantly more than males (Symondson, 1989). Female beetles require additional food resources for egg production, with the numbers of eggs laid correlated with the amount of food ingested (van Dijk, 1994). *P. madidus* females are slightly larger than males and may be able to overcome slightly larger slugs.

Although males did not consume any slugs, stressed slugs were inactive for longer in the presence of male beetles. *D. reticulatum* individuals have been shown to detect the odour of the generalist beetle *Pterostichus melanarius* (Dodds *et al.*, 1997). Air containing beetle defensive pygidial secretions were shown to induce rapid signalling in the slug olfactory nerve. It was suggested that although slugs move relatively slowly compared to their predators there could be an advantage for slugs to be able to detect potential predators. The detection of generalist beetle species may allow slugs to flee or to prepare for production of defensive mucus secretions which usually requires up to four seconds after the onset of an attack (Dodds *et al.*, 1997). Mair (Chapter 3) suggested that *D. reticulatum* may also be able to detect semiochemicals from *P. madidus* and alter its behaviour to reduce the chances of an attack. This may be achieved by remaining under a refuge or by being still on the soil surface primed for defence mucus production. Due to an increased period of inactivity in the presence of male beetles the slugs will not be actively foraging on the crop and this in turn leads to a reduction in damage.

The results presented in this paper suggest that the mucus produced by slugs is an effective defence mechanism preventing generalist beetle species from consuming all but the smallest size class of slugs tested in these experiments. Slugs which are 'stressed' due to dry weather conditions or sub-lethal ingestion of molluscicides and which can no longer mount an effective defence mucus barrier are potentially suitable prey items for generalist beetle species. Although generalist beetle species may not be an efficient predator of large 'healthy' slugs (Mair & Port, 1999), they may be an important tool in integrated pest management.

5.6 Acknowledgements

This work was funded by the Perry Foundation and the University of Newcastle Upon Tyne.

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Table 5.1

Defence mucus production by stressed and control *Deroceras reticulatum* slugs 24 hours after stressing. Slug weight in the size classes was (i) small = 0.01-0.1g (ii) medium = 0.12-0.2g (iii) large = >0.22g (n=10)

	Copious	Moderate	Little/None
Stressed			
Small	0	1	9
Medium	0	1	9
Large	0	0	10
Control			
Small	10	0	0
Medium	9	1	0
Large	10	0	0

Table 5.2

No choice trials. Susceptibility of stressed and control *Deroceras reticulatum* to predation by the beetles *Pterostichus madidus* and *Nebria brevicollis*. Slug weight in the size classes was (i) small = 0.01-0.1g (ii) medium = 0.12-0.2g (iii) large = >0.22g

	Stressed	Control		
	Eaten/Not Eaten	Eaten/Not Eaten	χ^2	<i>P</i>
<i>P. madidus</i> Female				
Small	7/3	4/6	0.81	n.s
Medium	8/2	0/10	6.12	0.05
Large	4/6	0/10	3.96	0.05
Total	19/11	4/26	8.52	0.01
<i>P. madidus</i> Male				
Small	4/6	0/10	3.96	0.05
Medium	0/10	0/10	0.0	n.s
Large	0/10	1/9	0.04	n.s
Total	4/26	1/29	1.8	n.s.
<i>N. brevicollis</i>				
Small	3/7	1/9	0.25	n.s
Medium	3/7	0/10	0.52	n.s
Large	0/10	0/10	0.0	n.s
Total	6/24	1/29	3.57	n.s.

Table 5.3

No choice trials. Mucus production by surviving stressed and control *Deroceras reticulatum* after 24h in the presence of *Pterostichus madidus* and *Nebria brevicollis*. Slug weight in the size classes was (i) small = 0.01-0.1g (ii) medium = 0.12-0.2g (iii) large = >0.22g

	No. Surviving	Mucus Production		
		Little/None	Moderate	Copious
Stressed				
Small	16	12	4	0
Medium	19	11	8	0
Large	26	26	0	0
Control				
Small	25	0	0	25
Medium	30	0	1	29
Large	29	0	0	29

Table 5.4

Behavioural interactions between stressed and control *Deroceras reticulatum* and the carabids *Pterostichus madidus* (n = 10 i.e. 5 males and 5 females) and *Nebria brevicollis* (n=10).

	Total Number of Contacts	Percentage of Total Contacts to			Number Eaten
		Head	Mantle	Tail	
Stressed					
<i>P. madidus</i>	126	38.8	30.9	30.1	3
(Females)	63	38	34.9	26.9	3
(Males)	63	39.6	26.9	33.3	0
<i>N. brevicollis</i>	60	40	35	25	5
Control					
<i>P. madidus</i>	222	36	32.8	31	0
(Females)	80	37.5	40	22.5	0
(Males)	142	35.2	28.8	35.9	0
<i>N. brevicollis</i>	89	38.2	29.2	32.5	0

Table 5.5.1

Preferences for cream (C) and red (R) coloured *Calliphora* larvae by the carabids *Pterostichus madidus* and *Nebria brevicollis*. The numbers of each colour of larvae eaten after 5, 15, 30 min and 24 h is shown. There were no significant preferences shown for either colour of larva.

	5 min		15 min		30min		24h	
	R	C	R	C	R	C	R	C
<i>P. madidus</i> Females	3	4	4	4	5	4	5	4
<i>P. madidus</i> Males	2	3	3	4	4	4	4	4
<i>N. brevicollis</i>	2	3	2	3	2	3	2	3

Table 5.5.2

Preferences for control (C) and mucus covered (M) *Calliphora* larvae by the carabids *Pterostichus madidus* and *Nebria brevicollis*. The numbers of each type of larva eaten after 5, 15, 30min and 24h is shown. Significant differences between the numbers attacked are indicated by *.

	5 min		15 min		30min		24h	
	M	C	M	C	M	C	M	C
<i>P. madidus</i> Females	1	1	1	8*	2	8	2	8
<i>P. madidus</i> Males	0	6*	1	6	1	8*	1	9*
<i>N. brevicollis</i>	1	3	2	3	2	4	2	5

Figure 5.1

Numbers of stressed and control *Deroceras reticulatum* consumed in choice-trials by *Pterostichus madidus* females (* = $P < 0.05$).

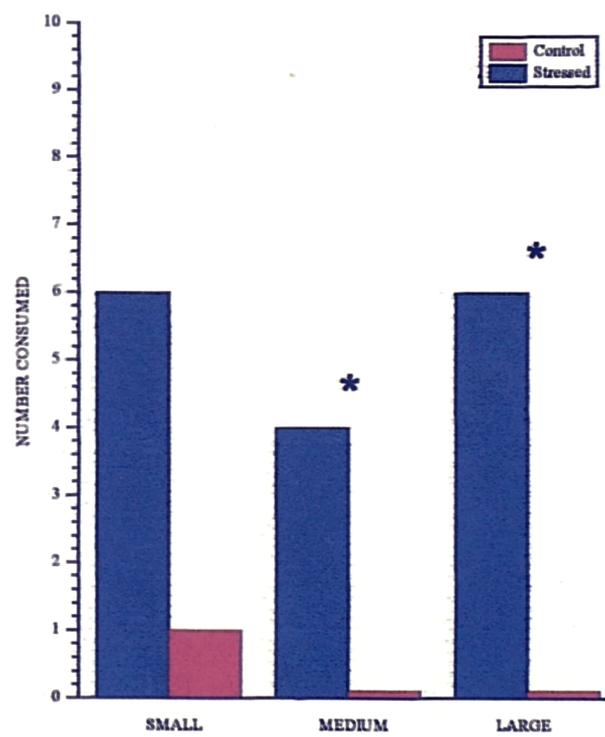


Figure 5.2

Numbers of stressed and control *Deroceras reticulatum* consumed in choice-trials by *Pterostichus madidus* males (* = $P < 0.05$).

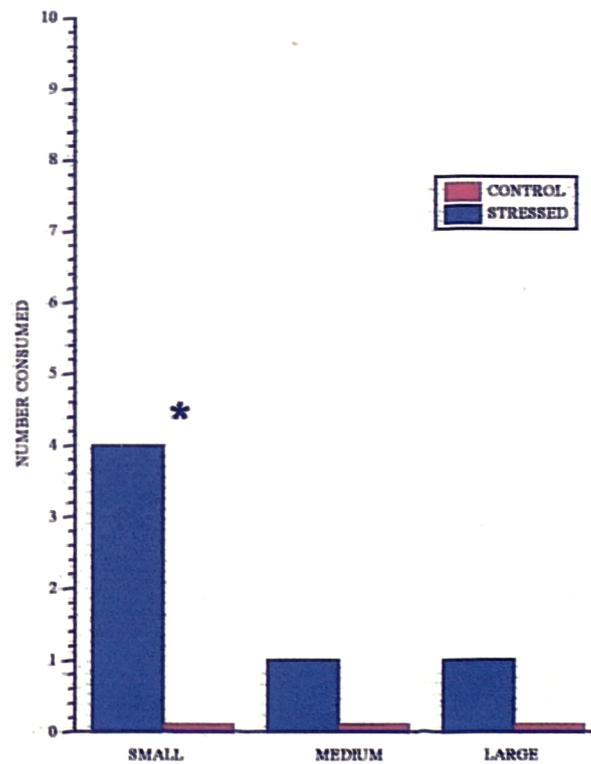


Figure 5.3

Numbers of stressed and control *Deroceras reticulatum* consumed in choice-trials by *Nebria brevicollis*.

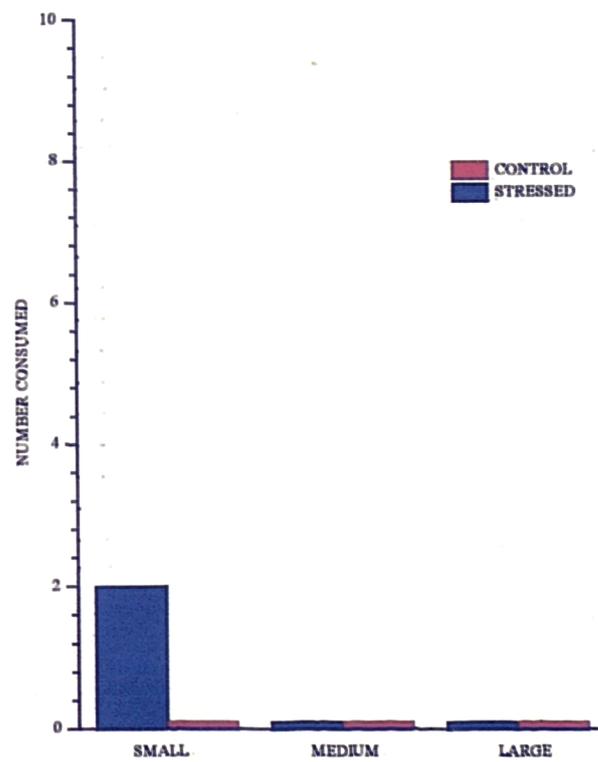


Figure 5.4.1

No-choice trials. Percentage consumption of live mucus covered *Calliphora* larvae and control larvae by *Pterostichus madidus* females. C = control, M = larva in mucus.

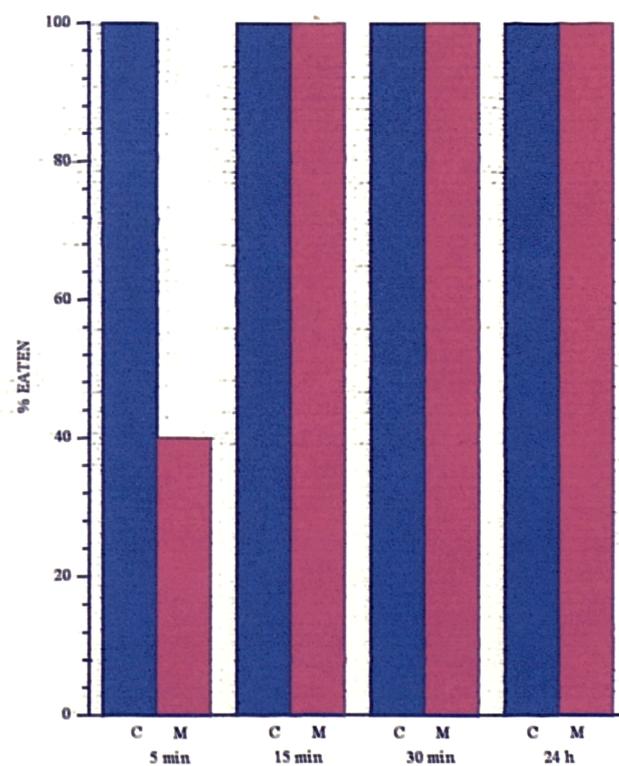


Figure 5.4.2

No-choice trials. Percentage consumption of live mucus covered *Calliphora* larvae and control larvae by *Pterostichus madidus* males. C = control, M = larva in mucus.

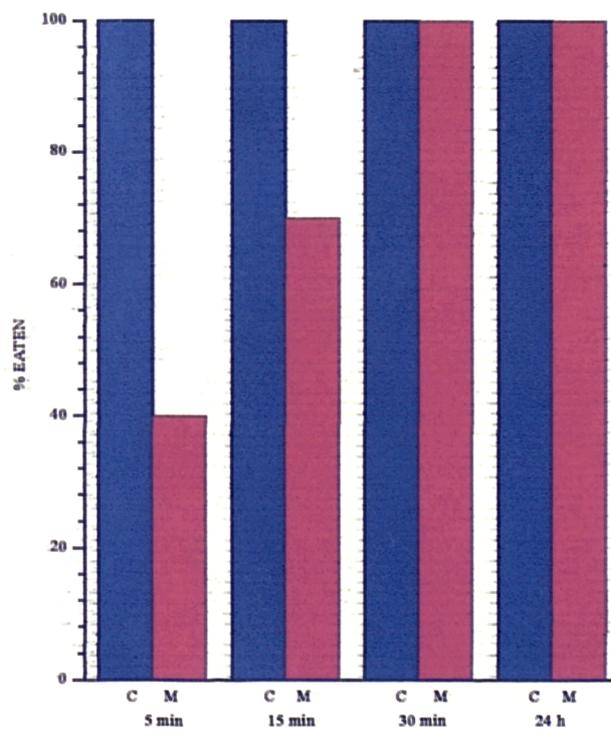


Figure 5.4.3

No-choice trials. Percentage consumption of live mucus covered *Calliphora* larvae and control larvae by *Nebria brevicollis*. C = control, M = larva in mucus.

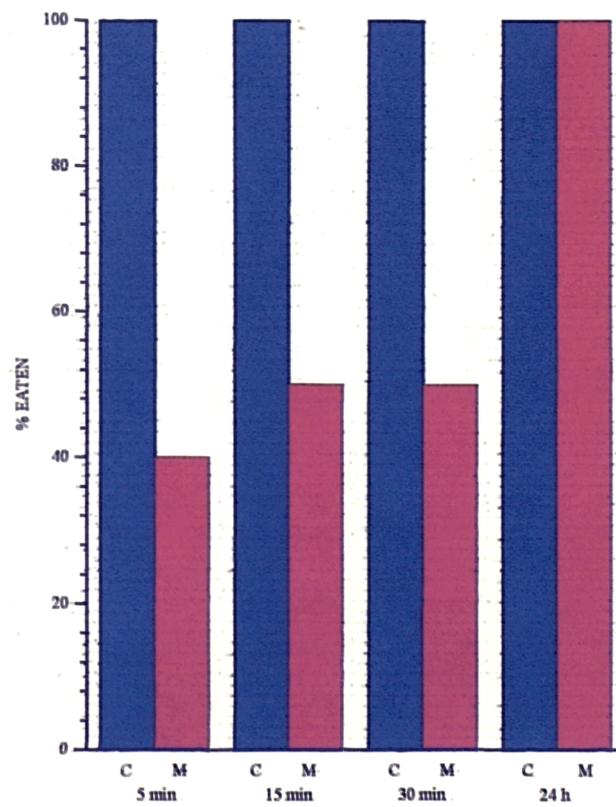


Figure 5.5.1

No-choice trials. Percentage consumption of dead mucus covered *Calliphora* larvae and control larvae by *Pterostichus madidus* females. C = control, M = larva in mucus.

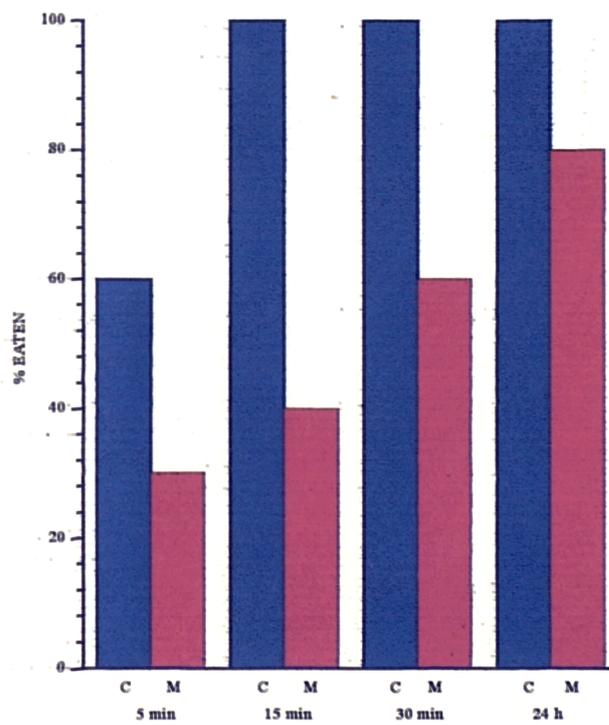


Figure 5.5.2

No-choice trials. Percentage consumption of dead mucus covered *Calliphora* larvae and control larvae by *Pterostichus madidus* males. C = control, M = larva in mucus.

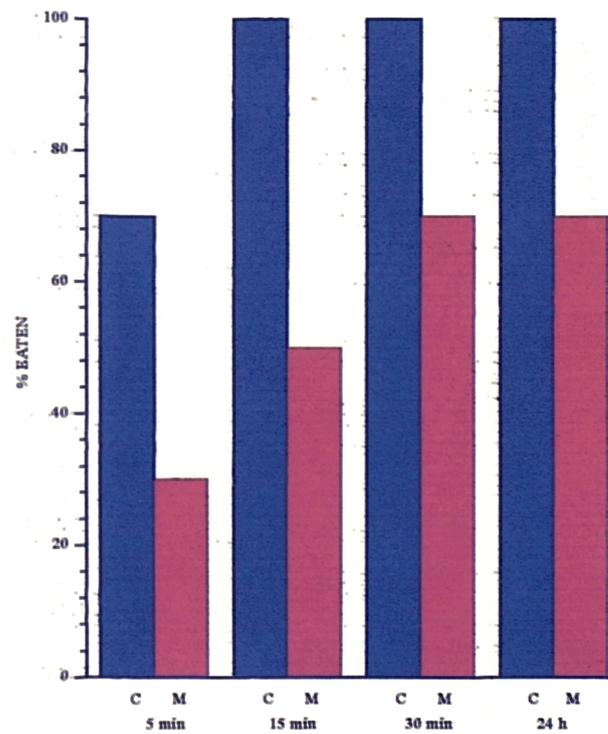
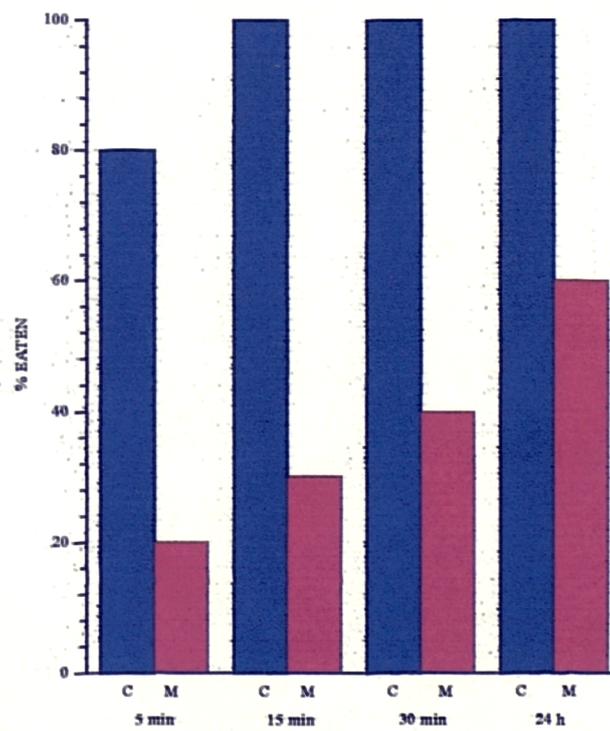


Figure 5.5.3

No-choice trials. Percentage consumption of dead mucus covered *Calliphora* larvae and control larvae by *Nebria brevicollis*. C = control, M = larva in mucus.



6. General Discussion

This project aimed to investigate the role of *Pterostichus madidus* (Fabricius) and *Nebria brevicollis* (Fabricius) as predators of the slug *Deroceras reticulatum* (Müller). These two generalist beetle species are commonly found in agricultural crops in Northern England (Ayre, 1995) and previous authors, using serological techniques have identified them as potential predators of slugs (Tod, 1973; Sunderland, 1988; Symondson & Liddell, 1993, 1996; Ayre 1995). These techniques however, do not distinguish between scavenging and true predation nor do they provide information on the size or other characteristics of the prey consumed. The behavioural studies carried out in this project aimed to address these questions.

Both *P. madidus* and *N. brevicollis* consumed *D. reticulatum* under laboratory conditions. *P. madidus* and *N. brevicollis* were only capable of killing small (<0.1g) healthy slugs. *D. reticulatum* populations contain individuals of all size classes at any time of year (Haynes *et al.*, 1996), however, there are likely to be peaks of juvenile (small) slug numbers in spring and summer following peaks of egg-laying by mature slugs. Overwintering *P. madidus* females become active in late spring, by summer there is a peak in numbers of newly emerged beetles of both sexes and by late summer the majority of active beetles are male. In the autumn female beetles are active and eggs are laid from August to November (Luff, 1973). *P. madidus* are therefore active and can potentially consume small slugs in the autumn and in early spring. *N. brevicollis* adults have two activity peaks, one from May to July and the second peak from September to November (Penny, 1966) and

this also coincides with the presence of small slugs. Thus, despite the fact they are generalists, these carabid species may reduce the numbers of slugs in an area by feeding on particular size classes (small slugs).

When presented with healthy, injured and dead slugs both *P. madidus* and *N. brevicollis* preferentially consumed dead slugs. Healthy and injured slugs were contacted more than dead slugs, however few contacts lead to a successful kill. Observations indicated that beetles attacking healthy and injured slugs were deterred by slug defensive mucus production, fouling the beetle mouthparts and forelegs following an attack. As dead slugs no longer produce mucus they may be more suitable prey items due to a reduced handling time. The results presented indicate that the relatively high proportion of positive serological results from field caught carabids (Ayre, 1995; Symondson & Liddell, 1996) may reflect a high scavenging rate or consumption of incapacitated slugs rather than actual predation on healthy slugs. Scavenging of dead slugs is possible throughout the year as slugs have a fairly constant mortality rate. In the field, several factors may impede defence mucus production increasing the likelihood of slugs being attacked by beetles. Slugs which have consumed a sub-lethal dose of metaldehyde (no longer able to produce defence mucus) (Triebkorn & Ebert, 1989), but which may recover in humid conditions, would be suitable prey items for generalist beetle species.

Behaviour studies, such as those carried out in this project, are required to aid interpretation of serological results. Bohan *et al.*, (2000) suggested that predation

by the generalist beetle *P. melanarius* on slugs was not random and that there was a trophic association between beetles and large slugs (> 0.025g). In June there were lots of large slugs and lots of beetles giving positive ELISA results for slug tissue whilst in July there were few large slugs, but still many slug positive beetles (11% of beetles tested positive over the two month sampling period). In certain areas of the field, slug density had been reduced by up to 50% and this was attributed to beetle predation. It was suggested that *P. melanarius* individuals contacted and consumed a large slug and remained in the vicinity following predation (accounting for the distribution of slugs and beetles in June). Before consuming a second slug there followed a period of satiation and searching time which was suggested to be approximately 23 days (11% of beetles tested positive with a detection period of 2.5 days), accounting for the beetle distribution not changing by July.

Behavioural studies, however, do not support the conclusions drawn by Bohan *et al.*, (2000). It is extremely unlikely that *P. melanarius* would be satiated for such a long period i.e. 23 days. Generalist carabids are known to be voracious feeders consuming at least their own body weight daily (Thiele, 1977; Kromp, 1999) and *P. melanarius* has been found to consume three times their body weight per day (Thiele, 1977) and also kill when satiated (Hagley *et al.*, 1982). In laboratory experiments specialist carabids have been found to consume 0.43 slugs/night (Ayre, 1995) with generalists species including *Abax parallelepipedus* consuming 0.3 slugs/day (Symondson, 1989). In certain areas of the field the slug population was reduced by 50% between months and, if we assume an initial density of 100

slugs/m² then almost 2 slugs must have been eaten each night. As 11% of trapped beetles tested positive, 4.4% fed on slugs each night (11% divided by the length of time slug remains can be detected i.e. 2.5 days) indicating that the required *P. melanarius* density would have been 45/m². This is an unrealistic density as *P. melanarius* densities in the field have been found to range from 0.25-11/m² (Thiele, 1977; Scheller, 1984; Ayre 1995; Kromp, 1999). Therefore it is unlikely that the reduction in slug numbers is due solely to predation by *P. melanarius*. As the distribution of *P. melanarius* did not change between months it is unlikely that beetles were detecting and aggregating in areas of high slug density as suggested. Thus the decline in the slug population observed by Bohan *et al.*, (2000) must have been due to other factors.

A large number of dead or incapacitated slugs are available following molluscicide applications and the consumption of these poisoned slugs could have an impact on beetle behaviour and survival. Slugs poisoned by metaldehyde are more likely to remain on the soil surface whilst those poisoned by methiocarb are more likely to move underground before dying (Howling & Port, 1989). Carabid beetles are unlikely to show any sub-lethal or lethal effects following the consumption of metaldehyde poisoned slugs (Büchs *et al.*, 1989; Kennedy, 1990), however, the consumption of methiocarb poisoned slugs has been shown to cause sub-lethal effects in species including *Pterostichus melanarius*, *N. brevicollis* and *Abax parallelepipedus* (Kennedy, 1990). These results indicate that in Integrated Pest Management programmes the use of metaldehyde pellets is recommended, as this chemical is unlikely to affect potential natural enemies such as Carabidae.

Neither *P. madidus* or *N. brevicollis* appeared to target their attacks to vulnerable parts of the slug as equal numbers of attacks were directed to the head, mantle and tail. Beetles that are mollusc specialists (*Cychrus*, *Scaphinotus* and *Carabus* species), are able to overcome slug defence mucus via behavioural and morphological adaptations. *Cychrus caraboides* L. and *Carabus violaceus* L. have powerful mandibles capable of penetrating slug skin before the slug can be producing defence mucus and 'stabbing' attacks are directed towards the head destroying the cerebral ganglion and towards the mantle causing paralysis (Pakarinen, 1994). Generalist beetle species attack slugs by opening their mandibles and attempting to bite. The width of the mandible gape has been found to limit prey size which these beetles can consume (Wheater, 1988; Pakarinen, 1994).

Due to the defence mucus production large slugs are difficult prey items for generalist beetles to overcome. Both *P. madidus* and *N. brevicollis* in choice trials consumed alternative prey types (earthworms and *Calliphora* larvae) in preference to slugs. In laboratory trials using video observation of beetles feeding upon slugs, Ayre (1995) showed that *Pterostichus niger* foraged for a mean time of 180 minutes before a kill and *Abax parallelepipedus* just less than 120 minutes (no alternative prey were available). This study has shown that *P. madidus* forages for up to 326 minutes before killing a healthy slug, despite making numerous contacts with the slug during that time. Such long periods of time spent foraging before a kill will increase the probability of the predator contacting alternative prey species

in the field which they may find more acceptable (i.e. less difficult to overcome) and attack as an alternative to killing slugs. In this study, slug eggs (with no defensive mucus covering) were a more suitable prey item than juvenile or adult slugs. Slug eggs are present throughout the year, with peaks in spring and autumn coinciding with beetle activity. Egg consumption by beetles may be more important in controlling slug numbers than the consumption of juveniles and adults.

P. madidus males consumed fewer slugs than females, however slug behaviour was altered in the presence of male beetles. Slugs that could no longer produce defence mucus were inactive for longer in the presence of male beetles. Beetles also caused slugs to become active by displacing them from refuges. This may cause slugs to become active during conditions they find unfavourable e.g. low moisture leading to desiccation of active slugs. These results indicate that *D. reticulatum* can detect the presence of *P. madidus* beetles via semiochemicals (as slug behaviour was altered both before and following contacts with beetles) and alter its behaviour to reduce the chances of an attack. Dodds *et al.*, (1997) found rapid signalling in the olfactory nerve of *D. reticulatum* when exposed to odour from the generalist beetle species *P. melanarius*. Although *P. madidus* males may not be efficient slug predators their presence may lead to a reduction in slug damage to crops as slugs will reduce their activity in the presence of beetles. Slugs of all sizes exhibited these behavioural changes. Even if slugs were too large to be attacked and killed, beetles would still be able to exert some control by altering slug foraging behaviour.

As carabids influence slug behaviour, increasing beetle numbers in crops could lead to a decrease in the duration of slug feeding and a resulting reduction in crop damage. Crop diversification (increasing weediness and crop heterogeneity), intercropping and the presence of crop boundaries such as conservation headlands and grassy field margins have been shown to enhance numbers of beetles in crops (Kromp, 1999). Frank (1998) has shown that wildflower strips sown to encourage beneficial arthropods are used as refuges by slugs, allowing them to move back into the adjacent crop (oilseed rape) causing increased damage. The disadvantage of sowing wildflower strips, however, may be offset due to the increased beetle numbers in these strips which may consume slugs or cause a change in their foraging behaviour. As an alternative to wildflower strips, Kirkland *et al.*, (1999) have shown that beetle numbers in crops can be increased by attracting carabids into crops using semiochemical dispensers. This increase in beetle numbers may increase the predator pressure on slugs (whether beetles are consuming slugs or altering their foraging behaviour).

Successful biological control is achieved by the predator maintaining the number of pests below an economic threshold. Ideally these predators need to be (i) host-specific (ii) synchronous with the pest (iii) able to increase rapidly in numbers (iv) require few pests to complete their life-cycle (so the predator can remain in areas of low pest numbers) and (v) have a high searching efficiency (Murdoch *et al.*, 1985). Generalist beetle species are considered to be poor biological control agents as they are polyphagous, not synchronised with the pest and do not have a high rate of population expansion (Murdoch *et al.*, 1985). This type of biological

control is successful in stable environments e.g. glasshouses. Agricultural land, however, is an unstable habitat due to annual disruption (e.g. tillage) and the equilibrium between pest and predator is broken. Generalists, however, unlike specialists can feed on a variety of prey items so can remain in the crop all year round and not only when the pest is present. When pest numbers begin to increase, generalist species are present in the crop and can immediately begin to consume the pest species, whilst in the case of specialists there is often a time-lag before sufficient numbers are present thus allowing pest numbers to increase. This 'lying-in-wait' strategy may allow generalists to be effective control agents (Murdoch *et al.*, 1985).

6.1 Future work

- Observations indicate that beetles that attack, but do not successfully kill slugs consume slug mucus in the process. Experiments to determine whether these beetles would give positive ELISA results need to be carried out. If these beetles tested positive this would lead to overestimates of the actual predation rate and would alter the interpretation of the high proportion of field caught beetles testing positive.
- Further work on slug olfactory responses should be carried out to determine which beetle species elicit olfactory signalling (and a resulting change in behaviour) in slugs. Semiochemicals emitted by these beetle species need to be investigated, as these could be important tools in IPM strategies.

- Mini-plot and field trials need to be carried out to assess plant damage caused by slugs when beetles are present (without actual predation on slugs) to determine if the presence of beetles alone can protect crops. If beetle presence alone is effective at reducing damage the best means of increasing beetle numbers in crops, without particular reference to their predatory potential, needs further investigation.
- Some immunological studies have been carried out on slug egg predation (Mendis, 1997), however, further field investigations are needed to determine the importance of slug eggs as food items for generalist beetle species.

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