The Influence of a Carabid Beetle Predator on the Behaviour and Dispersal of Slug Pests

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Abstract

It is becoming increasingly recognised that natural enemies in arable land, particularly polyphagous predators such as carabid beetles, may help to suppress populations of pests, and that appropriate management of such predators may lead to a satisfactory level of pest control. Previous work has shown that a polyphagous carabid predator, *Pterostichus melanarius*, is capable of affecting the spatial and temporal dynamics of slug pest populations. This project is an examination of how *P. melanarius* affects the survival, behaviour and dispersion of two important slug pests, *Deroceras reticulatum* and *Arion intermedius*, and how this information applies to spatial and temporal data previously obtained from the field.

Deroceras reticulatum, but not *Arion intermedius*, elicited a variety of anti-predator behaviour in the presence of substrates previously exposed to the predator. The movements of slugs in arenas, incorporating a zone containing paper upon which the predatory beetles had previously been maintained and a control zone, were recorded at intervals. Significantly more slugs of all the size classes tested accumulated on the control half of arenas after 24 hours, with small slugs being quickest to respond. Slugs avoided paper exposed to both male and female beetles. Slugs also avoided paper exposed to another predatory carabid, *Pterostichus madidus*, but not to *Harpalus affinis*, a phytophagous carabid. Slugs did not respond to paper that had been exposed to beetles and then stored for five days prior to the test. Changes in parameters of movement of slugs when in the presence of *P. melanarius* chemicals were detected using a video-tracking system. These changes are consistent with a kinesis that would enable slugs to rapidly escape from areas where beetles were recently present. Despite this no changes in the rate of dispersion of *D. reticulatum* juveniles was detected in mini-plots previously exposed to *P. melanarius*. However, adult *D. reticulatum* reduced feeding and egg-laying and increased refuge on soil previously exposed to *P. melanarius*. Chemicals on the exterior of *P. melanarius* were isolated and two compounds were found to reduce feeding by *D. reticulatum* on leaf discs compared to control discs. One of these chemicals is currently being analysed using mass spectroscopy and NMR to determine it's structure and identity. It was concluded that *D. reticulatum* have evolved behavioural responses to chemical cues from either this generalist carabid predator in particular, or carabid beetles generally, many species of which include molluscs in their diets.

During a video-tracking study of beetle movements, some evidence was found for the detection and response of beetles to slug mucus, and amputation work suggested that the palps may be important in slug detection. Previous feeding experience was found not to influence prey choice in *P. melanarius* during a food choice experiment, with beetles always selecting a mixed diet when offered. Analysis of spatial data showed that slug size, but not beetle sex, was important in the spatial relationship between the predator and the prey, and this agrees with results from the laboratory experiments of slug behaviour.

This work has added to the evidence provided by other studies that some generalist predators can have significant effects on the dynamics of pest populations and therefore may become useful biocontrol agents for pests if effectively managed. The implications of this work in the field of predator-prey ecology and for the efficacy of *P. melanarius* as a biological control agent of slugs are discussed.

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Abbreviations

A. Units

Units of length

m	metre
cm	centimetre (10^{-2} metres)
mm	millimetre (10^{-3} metres)

Units of time

s	second
min	minute
h	hour

Units of volume

ml	millilitre (10^{-3} litres)
µl	microlitre (10^{-6} litres)

Units of weight

g	gram
mg	milligram (10^{-3} gram)
ng	nanogram (10^{-9} gram)

Other units

°C	degrees centigrade (temperature)
°/cm	degrees per centimetre (meander)
°/s	degrees per second (angular velocity)
cm/s	centimetres per second (velocity)
cm day[-1]	centimetres per day (velocity)
cm^2	centimetre squared (area)
m^2	metre squared (area)
ha	hectare (area)

g m^{-2}	grams per metre squared
tsp	teaspoon
%	percent
I_a	index of aggregation
P_a	probability of aggregation
D	distance to regularity
□	cluster index
X	index of association
P_t	probability of association

B. Other abbreviations

2D	two-dimensional
3D	three-dimensional
ANOVA	analysis of variance
CE	controlled environment
CI	confidence interval
CV	canonical variate
CVA	canonical variates analysis
D	dark
df	degrees of freedom
DNA	deoxyribosenucleic acid
EAG	electroantennagram
ed	edition
ELISA	enzyme-linked immunosorbant assay
FLUON	polytetrafluoroethylene
L	light
LSD	least significant difference
M	million
ML	maximum likelihood
n	number of observations
NMR	nuclear magnetic resonance
p-value	probability value

PCR	polymerase chain reaction
PVC	polyvinyl chloride
REML	residual maximum likelihood
SADIE	spatial analysis by distance indices
se	standard error
TLC	thin layer chromatography
VCR	video cassette recorder

Chapter 1

Introduction

It is widely recognised that slugs are important pests of arable land and are a cause of great concern to farmers worldwide (Glen, 1989). Slugs are difficult to control because they are subterranean, only occasionally coming to the surface to feed. At any one time only a small proportion of the resident slug population is on the surface, therefore after control has been initiated, populations can recover and return to the surface when conditions are right.

Traditional methods of control involve the use of chemical molluscicides, which are effective but they are also broad-spectrum and may not provide a long-term solution to slug control. Research into alternative methods of control is advancing. Most success has centred on better understanding of the effects of farming practices and predation by biological control agents on slug populations.

Carabid beetles are polyphagous predators of arable land, consuming a wide range of invertebrate prey, including many pest species. Better understanding of their spatial and temporal dynamics, their relationships with prey species and habitat preferences has led to extensive research on the value of carabids as biological control agents for slugs. Work described in this thesis is part of ongoing research into the effects of a carabid beetle predator, *Pterostichus melanarius* (Illiger), on behaviour and dispersion of two common slug pests, *Deroceras reticulatum* (Müller) and *Arion intermedius* (Normand).

1.1. Slugs as pests

The most abundant and problematic species present in UK arable crops is the field slug, *D. reticulatum*. *D. reticulatum* are small slugs from the family Limacidae and although endemic to the Palearctic, have also been introduced to, and become pests in Australia, New Zealand and North America (South, 1992). *A. intermedius*, another important pest, is a small slug from the family Arionidae. Abundant in arable land, this species has also been introduced to continents across the world (South, 1992).

1.1.1. Damage to crops and yield losses

Although slugs also eat barley and oats, wheat is the preferred food of the main slug pest *D. reticulatum* (Duthoit, 1964). Barley and oats have a tough seed coating, which provides partial protection from mollusc feeding. Barley is also drilled earlier in the season when slugs are not so abundant (Glen and Moens, 2002).

It was estimated that between 1960 and 1970, 0.2 - 2.2 % of wheat was lost to slugs (Glen, 1989; South, 1992; Glen & Moens, 2002). Port & Port (1986) point out that more recent estimates are not available but farmers clearly perceive slug problems to have increased considerably since then because there has been a 67-fold increase in molluscicide use since the 1970s (Garthwaite & Thomas, 1996). Changes in agricultural practices may be contributing to greater slug numbers (see Section 1.1.2).

Slugs cause most damage to wheat during crop establishment. Slugs attack seeds by eating the embryo and endosperm, which destroys the seed and prevents growth. Slug feeding may also destroy seedling shoots, but seedlings can recover from partial grazing of emerging leaves (Barker, 1989; Glen, 1989; South, 1992; Glen & Moens, 2002).

1.1.2. Changing agriculture and the effects on slug populations

Slug populations are strongly influenced by agricultural practices. Cultivation of oilseed rape has increased since the 1970s. Large populations of slugs can build up in this crop and attack the following wheat crop (Glen *et al.*, 1996). Gould (1961) observed that wheat was at greater risk from slug damage if preceded by crops that give good cover (peas, leys, brassicas and cereals) than by crops giving poor cover (potatoes and sugar beet). This is attributed to cover and humidity of dense crops providing the slugs with protection from desiccation and predation. The soil cultivation that is associated with root crops may also help to destroy slugs.

Straw burning helps to reduce the numbers of slugs, probably through the removal of food and shelter (Glen *et al.*, 1984; Glen *et al.*, 1988) but it has been banned since 1993. Farmers now dispose of straw by baling and removing from the field, or chopping and spreading the straw for incorporating into the soil (Glen & Moens, 2002). Both methods encourage the growth of slug populations (Glen *et al.*, 1994; Glen, 2000).

1.1.3. Why is *Deroceras reticulatum* such an important slug pest?

Many aspects of the biology of this species have contributed to it becoming an important pest. *D. reticulatum* is a highly surface-active species (South, 1965), can remain active

during winter (South, 1989) and reproduce at temperatures as low as 5 °C (South, 1982). Eggs were laid by adult *D. reticulatum* when they were stored at 5 °C (+/-2 °C) (personal observation). South (1989) found fresh plant material in the guts of slugs taken from turf buried under snow.

Airey (1987) found that *D. reticulatum* can maintain a high level of activity even during food deprivation, suggesting that the metabolic costs for its activity are low compared with other slugs.

Growth of *D. reticulatum* is not adversely affected by dry conditions except in very hot spells. They simply shelter in a moist refuge (South, 1965) or move deeper into the soil (Glen & Moens, 2002). *D. reticulatum* egg hatching was reported by Barker (1991) to peak in May and early September and each adult may lay up to 500 eggs a year (South, 1992). South (1989) suggested that this slug would breed continuously throughout the year if conditions were right. *D. reticulatum* is an opportunistic species and it is has adapted to the dry, changing conditions experienced in arable land. The lack of competition with other molluscs has allowed it to flourish under such conditions (South, 1992).

1.1.4. Biology and ecology of *Deroceras reticulatum* and *Arion intermedius*

D. reticulatum is a medium sized slug (3.5 - 5 cm), generally creamy in colour with a mottled appearance and keel truncated at the tail (South, 1992). *A. intermedius* is a small slug (1 - 2 cm), generally light in colour with distinctive prominent tubercles over the surface of the body giving rise to the name 'hedgehog slug' (South, 1992). The optimal temperature for growth in both species is approximately 18 °C (Wareing & Bailey, 1985; South, 1992). In both species eggs are laid in batches in holes or gaps in the soil. *D. reticulatum* may lay up to 500 eggs in a year and on average lays 22 eggs in each batch (Carrick, 1938). Depending on environmental conditions, on average eggs of *D. reticulatum* hatch in 3 - 4 weeks and those of *A. intermedius* in approximately 3 weeks (South, 1992). At low winter temperatures eggs can take as long as three or four months in both species (South, 1992). *Arion intermedius* sp. has an annual lifecycle (South, 1989; Barker, 1991). Eggs are laid by mature slugs in late summer, hatch in winter, and juvenile slugs grow slowly until spring, when activity increases and slugs enter a stage or rapid growth culminating in mature adults in late summer (South, 1989; Barker, 1991). In dry years egg laying is delayed until late winter or early spring (South, 1992). *D. reticulatum* has two main breeding seasons a year and peaks of egg hatching occur in late spring and early autumn (Karlin & Naegele, 1958). Hunter & Symonds

(1970), Duval & Banville (1989) and Barker (1991) found in their studies that there are two overlapping sets of generations a year. Both species show a build up of numbers in the egg stage, followed by a rapid decline in numbers in the neonate stage and a more gradual decline as slugs reach maturity (South, 1989). Mortality is highest in the egg and neonate stages.

1.2. Methods of controlling slugs

Chemical control is currently the most effective and widely used method of slug control. In 1994 27 % of the total wheat area in the UK was treated with molluscicides (Glen & Moens, 2002). Damage to crops varies from year to year and is affected by a combination of factors such as weather, farming practices, predation and slug population dynamics. Without a reliable method of forecasting slug damage, much molluscicide use may not be economically justified by the loss of yield (Port & Port, 1986; Glen *et al.*, 1993). This highlights the importance of research into slug population dynamics and alternative methods of control that may be more cost effective and more environmentally acceptable.

There has been a considerable amount of research into the effects of farming practices on slug populations. Cultural methods of control are attractive prospects for farmers as they usually only involve small adjustments to current farming practices, and are relatively cost effective. Integrated control with the complementary use of chemicals and cultural techniques is also a possibility, and may be more effective than if one method is used alone.

Biological control of slugs would most likely have to be achieved through augmenting, conserving and enhancing the populations of natural enemies already present in the field. Unfortunately most predators of slugs are not in sufficiently high enough numbers to have a large impact on slug numbers (Port *et al.*, 2000). Recently, significant success has been achieved with the use of a parasitic nematode *Phasmarhabditis hermaphrodita* (Schneider) (Wilson *et al.*, 1994a, b; Hass *et al.*, 1999a, b; Glen *et al.*, 2000; Iglesias *et al.*, 2001) for the control of slugs within horticultural crops and gardens. Predation upon slugs by certain species of ground beetles is also being researched as they have the potential to suppress the growth of slug populations; this is discussed in detail in Section 2.3.

1.2.1. Chemical control

In 1996 it was reported that approximately 800,000 hectares of crops were treated with molluscicides annually in Great Britain at an estimated cost of £10 M (Garthwaite & Thomas, 1996). There are many problems associated with the development and use of chemicals to control slug pests in crops. The problems are discussed in detail in Henderson and Parker (1986), and summarised below:

- Obtaining and culturing test animals for the development of chemical control is very difficult for slugs: the lifecycle is long compared to insects, they do not survive well in culture and regular collection from the field would be a labour intensive process.
- The market for slug control has been judged to be too narrow for research and development of molluscicidal chemicals to be economical. New molluscicides are only likely to be detected if they have already shown insecticidal properties i.e. methiocarb. Novel molluscicides are likely to have undesirable broad-spectrum effects on other fauna in the field.
- Contact poisons are generally unsuitable for use against molluscs as the animals are protected by a layer of mucus. Poisons would therefore have to be highly water soluble to dissolve in the mucus layer – an undesirable characteristic if they are to survive periods of rain in the field.

An alternative to contact poisons is baited stomach poisons. Most current methods of chemical control rely on metaldehyde (first used in 1936) and methiocarb (originally developed as an insecticide, but eventually marketed for mollusc control in 1967). The poisons are delivered to the slug in attractive bait form, which are then eaten (Henderson & Parker, 1986). However, non-target animals may consume them, and the toxicity of the pellet has to be balanced against the repellent effect of the poison. Purvis & Bannon (1992) found that applications of methiocarb pellets severely depressed populations of winter-active carabids, known slug predators, although activity had usually recovered by next spring. Buchs *et al.* (1989) found that methiocarb caused 25 % mortality of the slug predator *P. melanarius*, although metaldehyde seemed to have no adverse effects on carabids tested (Glen & Wilson, 1995). Significant mortality of *P. melanarius* has also been caused when the beetles consumed slugs sub-lethally poisoned by methiocarb, but not metaldehyde (Langan *et al.*, 2004). Methiocarb baits, but not metaldehyde, may also affect earthworms. The earthworm *Lumbricus terrestris* L. experienced over 20% mortality when exposed to pellets in soil-filled funnels (Bieri *et al.*, 1989). However, no significant difference in mortality of *L.*

terrestris between methiocarb and untreated controls were detected when the recommended application rate was used in another lab test (Wellman & Heimbach, 1996). Incidents of poisonings to birds, badgers, hedgehogs, foxes and domestic animals such as dogs are also reported, though usually in connection with bad storage, spillage or abuse (Meredith, 2003). It must be noted though that poisonings of wild animals by pellet spreading on fields are likely to go mostly unnoticed by humans.

Slugs appear to be less capable of recovering from poisoning by methiocarb than metaldehyde based pellets. Howling (1991) found that slugs are not attracted to metaldehyde pellets, they find and consume them by chance, unlike methiocarb pellets, which are attractive from a distance. *D. reticulatum* will however preferentially feed on plant material as an alternative to methiocarb pellets when both are present (Airey, 1986). Despite laboratory findings, both poisons give similar levels of protection in the field.

Observed effects of poisoning by metaldehyde include immobilisation, writhing, excessive mucous production and inhibition of feeding (Mills *et al.*, 1989; South, 1992). Methiocarb pellets inhibit acetylcholinesterase activity. Symptoms include paralysis and loss of muscle tone. Both poisons have comparatively low toxicity and death may take several days to occur (South, 1992).

Some naturally occurring plant chemicals have been shown to have repellent and antifeedant effects on slugs, and show promise as potential wheat seed dressings (Dodds *et al.*, 1996; Powell & Bowen, 1996) or as slug repellents coated onto physical barriers (Ali *et al.*, 2003)

1.2.2. Cultural control

The effects of crop rotation and methods of straw deposition on the growth of slug numbers have already been discussed (see Section 2.1.2). A variety of other cultural methods of control are described in this section.

1.2.2.1. Intercropping

Increasing the diversity and density of crops, or allowing weed growth in fields may provide shelter and over-wintering sites for predators of crop pests, and provide alternative food for slugs (George *et al.*, 1995; Theunissen, 1995; Cook *et al.*, 1996; Chapman *et al.*, 1999; Frank & Barone, 1999). Cook *et al.* (1996) found that some weeds, *Taraxicum officinale* (Weber), *Capsella bursa-pastoris* (L.), *Trifolium repens* (L.) and *Chenopodium album* (L.), were more palatable to *D. reticulatum* than wheat, and they suggested that these weeds could provide

alternative food for slugs during wheat establishment. Frank and Barone (1999) found that intercropping oilseed rape with the weeds *Stellaria media* (L.) and *C. bursa-pastoris* provided protection against slug damage equal to that of metaldehyde pellets at low slug density but not high slug density. More successfully, George *et al.* (1995) showed that feeding damage to wheat caused by *D. reticulatum* was reduced by 50-60 % when grown in mixed culture with clover cv.Milkanova than in monoculture. Powell *et al.*, (1985) found that plots containing dense weed growth contained higher numbers of carabid and staphylinid larvae (adults of some species are predators of slugs), and provide beetles with better conditions for egg-laying. However, larger carabids showed reduced activity in dense vegetation, and predation may be hindered. Diverse, open vegetation is favoured by carabids and does not severely hinder activity. It has been suggested that providing weed strips may be more effective by providing oviposition sites and temporary shelter during the day than completely undersowing the entire crop (Armstrong & McKinlay, 1997).

1.2.2.2. Seedbed condition

Wheat seeds are better protected in fine seedbeds than rough cloddy seedbeds. Slugs cannot move easily through fine soil to find buried seeds due to the reduction in spaces between soil aggregates (Glen, 2000). Consolidation (i.e by rolling) can reduce slug access to seeds if it effectively breaks soil down into finer particles (Davis, 1989; Glen *et al.*, 1989).

1.2.2.3. Tillage

Direct drilling into stubble rather than non-inversion tillage, or ploughing results in larger numbers of slugs (Glen *et al.*, 1994; Glen, 2000) and seeds are more accessible to slugs if this method is used. Tillage and ploughing disturbs the soil and may expose slugs to predation and desiccation, as well as mechanical injury (Glen & Moens, 2002), but may also result in lower numbers of some beneficial invertebrates (Holland & Reynolds, 2003), including slug predators such as *P. melanarius* (Symondson *et al.*, 1996).

1.2.2.4. Seed depth

In coarse seedbeds, increased protection of seeds can be achieved by sowing deeper (Glen *et al.*, 1989). Seeds sown at 40 mm instead of at 20 mm provided protection equal to that given by methiocarb pellets broadcast over seeds sown at 20 mm (Glen *et al.*, 1990). In 1989, seeds sown at 50 mm, rather than 2.5 mm, gave a 9 % increase in the wheat yield that year, and no significant delay in seedling emergence was associated with deeper sown seeds (Glen *et al.*,

1994). It appears that deeper sown seeds have grown past the most vulnerable stage by the time they emerge from the soil (Glen, 2000).

1.2.2.5. Crop resistance

Cook *et al.* (1996) found no significant differences in palatability of different wheat cultivars. Research has revealed the possibility of growing transgenic crops that display resistance to slug feeding. The cysteine proteinase inhibitor E-64 inhibited the activity of an enzyme present in the digestive gland of *D. reticulatum* and caused a reduction in growth rates but not feeding rates (Walker *et al.*, 1998). Growth and survival of juvenile *D. reticulatum* was significantly reduced when fed on transgenic *Arabidopsis thaliana* (L.) tissue expressing the cysteine proteinase inhibitor OC-IΔ86 (Walker *et al.*, 1999). More recent studies have however shown that oilseed rape plants expressing the cysteine proteinase inhibitor OC-1 did not successfully reduce growth or feeding rates of juvenile *D. reticulatum* (Mulligan *et al.*, 2003). It might be possible to develop transgenic crops expressing cysteine proteinase inhibitors to provide partial protection from slug feeding but more research into this is required.

1.2.2.6. Sowing date

Cereals sown in autumn experience wetter conditions and are at increased risk of slug damage compared to spring sown cereals (Glen, 1989; Glen & Moens, 2002). Sowing slightly earlier in the autumn may ensure that seed-bed preparation is more effective in deterring attack (Glen & Wilson, 1995) and that seedlings have grown past the most vulnerable stage before slug numbers start to increase.

1.2.3. Biological control

Despite a vast array of invertebrate and vertebrate predators of molluscs, most do not have a large enough impact on mollusc populations to be considered for biological control. Most of these species are generalists, and molluscs only form a small part of their total diet. Many of them are also only present in the field at low densities so are unlikely to reduce slug numbers significantly. Species that have been considered for the biological control of slugs include the parasite *Microsporidium novocastriensis* (Jones and Selman) (Jones & Selman, 1985), the ciliate parasite *Tetrahymena rostrata* (Kahl) (Wilson *et al.*, 1998), the parasitic nematode *P. hermaphrodita*, and some species of predatory carabid beetles (see Section 1.3).

1.2.3.1. Microsporidium novocastriensis

Many individuals of *D. reticulatum* have been found to be infected with this parasite (Selman & Jones, 2004). Infected slugs have lower growth rates and reduced fecundity, as well as reduced feeding, but are rarely directly killed by the infection (Jones & Selman, 1985). A number of factors have made it unsuitable to be considered: it can only be mass-reared inside living *D. reticulatum*, high doses of spores are required to kill slugs and it is specific only to *D. reticulatum* therefore other slugs remain unaffected. This parasite is not being considered as a suitable biological control agent in the near future.

1.2.3.2. Tetrahymena rostrata

This ciliate protozoan is a highly virulent pathogen and effective at killing *D. reticulatum* in laboratory bioassays (Wilson *et al.*, 1998). The life span of the slugs was significantly reduced at 17 °C but not 10 °C. Lower temperatures inhibit the growth and reproduction of the protozoan, making it unsuitable as a biological control agent in the field.

1.2.3.3. Phasmarhabditis hermaphrodita

Phasmarhabditis hermaphrodita is a bacterial-feeding nematode, cultured by MicroBio Ltd for the successful control of pest slugs. Infective larvae enter the shell cavity of the host, reproduce, spread over the body of the slug and re-enter the soil to infect new slugs (Wilson *et al.*, 1993).

The nematodes are mass cultured. Dauer juveniles are separated from medium and mixed with clay to produce a formulation that can be mixed with water and sprayed onto the soil. Experiments in laboratory bioassays (Wilson *et al.*, 1994a; Glen *et al.*, 2000; Speiser *et al.*, 2001), mini-plots (Wilson *et al.*, 1994b; Hass *et al.*, 1999a) and field trials (Hass *et al.*, 1999b; Iglesias *et al.*, 2001) have proven the effectiveness of the nematode sprays in protecting crops from slug damage.

Mortality of slugs is dose dependent (Wilson *et al.*, 1995; Glen *et al.*, 2000). Slug feeding is inhibited shortly after infection and death occurs within a few days. Even at low doses, death may occur more slowly but feeding is still inhibited early on, resulting in protection of the crop from herbivory (Glen *et al.*, 2000). *P. hermaphrodita* can infect and kill *D. reticulatum* within a temperature range of 5 - 25 °C, similar to the range of temperatures experienced by slugs in the field (Port *et al.*, 2000). Despite being able to kill a wide range of pest molluscs, including some species of snails, non-target species such as earthworms are not affected (Wilson *et al.*, 2000; Grewal & Grewal, 2003). Numbers of non-

target species of snails living in field margins, which may be important for conservation purposes, were also not adversely effected by spraying of the nematode into nearby fields, or by confinement with infected soil under laboratory conditions (Wilson *et al.*, 2000). Most nematodes do not move more than 1 cm from the site of application and are therefore unlikely to spread from arable fields into vulnerable habitats in any case.

P. hermaphrodita is cultured in association with a strain of bacterium called *Moraxella osloensis* (Bovre and Henriksen). *M. osloensis* is pathogenic in the absence of nematodes when injected into the shell cavity of *D. reticulatum* (Tan & Grewal, 2001). Axenic nematodes were not pathogenic to slugs and it has therefore been hypothesised that *M. osloensis* causes the death of the slugs, and the nematodes are merely a vector for the bacterium.

Although research shows that the nematode is capable of controlling slugs in the field, widespread use of this method of control has not yet been achieved. It is constrained by the need to store the agent under refrigerated conditions and has a shorter shelf-life than molluscicidal pellets (Port *et al.*, 2000). However, nematode use may be suitable for organic farmers who cannot use chemical molluscicides, and for use on high-value horticultural crops.

1.2.3.4. Natural predators

Birds, mammals, amphibians, reptiles and even fish have been recorded feeding on slugs though none of the vertebrates would be suitable candidates for biological control (South, 1992; Allen, 2004).

A few species of spiders and harvestmen have been reported to feed on slugs and snails, both in captivity and in the field (Nyffeler & Symondson, 2001; Pollard & Jackson, 2004). Some terrestrial gastropods are themselves carnivorous, and many species, including *Deroceras panormitanum* (Lessona and Pollonera) and *Deroceras laeve* (Müller) are cannabalistic (Barker & Efford, 2004). A variety of other invertebrates including the flatworm *Geoplana septemlineata* (Hymen) (Winsor *et al.*, 2004), certain Diptera species (Barker *et al.*, 2004; Coupland & Barker, 2004) and staphylinid beetles (Symondson, 2004) also prey upon slugs though none are being considered for enhanced biological control (South, 1992).

A rich variety of carabid beetles and some of their larvae have been reported to feed on molluscs (Symondson, 2004). Some genera have become morphologically adapted for feeding on snails e.g *Cychrus* and *Scaphinotus* (Pakarinen, 1994a). The majority of mollusc

feeding carabid beetles are generalist species that consume a range of other invertebrate prey as well as plant material. Many are woodland species, which are not found in high densities in arable fields, or at least not enough to have a significant impact on mollusc populations (Port *et al.*, 2000).

1.2.3.5. Generalists as biological control agents

There has been extensive research on the effects that assemblages of natural enemies, such as carabid beetles, may have on pest populations. Much of this research has focussed on conserving farmland biodiversity, particularly techniques that preserve generalist predators (Powell *et al.*, 1985; Lys & Nentwig, 1992; Pickett & Bugg, 1998; Landis *et al.*, 2000; Symondson *et al.*, 2002b). There is little possibility of the use of carabids for classical biological control, however, it may be possible to increase the natural protection afforded by predatory carabids by enhancing the resident field populations. Slight modifications to farming practices have had a positive impact on carabid numbers in the field: the provision of overwintering sites and food by building beetle banks (Thomas *et al.*, 1991; Collins *et al.*, 2002; 2003), sowing weed-strips (Lys & Nentwig, 1992; 1994; Lys *et al.*, 1994; Frank, 1997) and sensitive management of field margins (Chiverton & Sotherton, 1991; Dennis & Fry, 1992; Hassall *et al.*, 1992; Thomas & Marshall, 1999). Ostman *et al.* (2001) showed that four polyphagous carabid beetles, including *P. melanarius*, were found in higher numbers in fields that had a high perimeter-to-area ratio, and on organic farms compared to conventional farms. Molluscicidal pellets and some broad-spectrum insecticides can be harmful to carabids, therefore applying these when the beetles are not present in the field in high numbers may also be beneficial (Gholson *et al.*, 1978; Purvis & Bannon, 1992).

The effect of increasing the resident predator population on prey numbers has been examined in a few studies. Menalled *et al.* (1999) showed that prey removal rates increased in plots where numbers of carabid beetles had been artificially enhanced. Populations of Cicadellidae, Thysanoptera and Aphididae were depressed in plots where numbers of carabids and wolf spiders were artificially increased compared to control plots (Lang *et al.*, 1999). Collins *et al.* (2002) found that predation of aphids decreased with increasing distance from an over-wintering strip (beetle bank). Winder (1990) reported fewer aphids returning to plants after falling when carabid densities in plots below were artificially increased. Finally, a study by Riechart & Bishop (1990) showed that by providing suitable habitat for spider predators in vegetable plots, pest numbers and consequently plant damage was reduced.

Generalists are more likely to aggregate to and prey upon a particular pest if that pest is the preferred prey item for that species or forms a major constituent of the total available prey (Symondson *et al.*, 2002b). Very little research has been carried out to understand the relationship between an individual generalist predator and its prey item over time. Symondson *et al.* (1996; 2002a) found that slugs formed a major part of the diet of *P. melanarius* and the crop mass of the beetles was positively related to the abundance of slugs in the soil. Slug numbers and beetle crop mass were related to changes in the beetle population between years. Similarly the predators had a significant effect on the population growth of the slugs between years: evidence of a coupled relationship between the two species with changes in the beetle population buffered by feeding on alternative prey (Symondson *et al.*, 2002a).

Specialist predators are highly effective at reducing prey numbers but will themselves decline when prey numbers are low, due to a lack of available food. Generalist predators may be able to sustain high populations by switching to an alternative food source whilst the population of the main prey item is low (Murdoch *et al.*, 1985; Boer, den 1986). Alternatively, large populations of generalists can build up in the crop before the emergence of pest species by feeding on other prey and plant material, thus ensuring that prey populations cannot reach high numbers before being brought under natural biological control (Settle *et al.*, 1996). Specialists have certain advantages over generalists however in that they may be specially adapted to overcome defence mechanisms of specific prey (den Boer, 1986); for example carabids specially adapted to feed on molluscs can kill them almost instantly but generalist carabids have difficulty in overcoming defensive mucus production (Pakarinen, 1994a; Section 1.3.1.).

Symondson *et al.* (2000) used an earthworm specific monoclonal antibody to detect earthworm protein in beetle guts and they found that 36 % of *P. melanarius* beetles in the fields contained earthworm remains. Foregut biomass was negatively related to the quantity of earthworm protein consumed, indicating that earthworms are only eaten when the total prey availability is low. When the activity-density of beetles was high, more earthworm proteins were found in beetle guts suggesting that high numbers of searching beetles caused a decline in total prey availability and encouraged feeding on alternative prey. Earthworms may therefore help to sustain beetle populations in the field when preferred pest prey numbers are low. Evidence that declining prey availability causes an increase in beetle activity density is supported by Chiverton (1984), who found that a reduction in prey

availability caused by applications in insecticides resulted in increased pitfall trap catches of *P. melanarius.*

1.3. Ecology and biology of *Pterostichus melanarius*

P. melanarius is a medium-large, black carabid beetle that is very common in arable land and eats a wide variety of prey types. Food found in the guts of this species include molluscs, aphids, collembola, nematodes, diptera, coleoptera, spiders, earthworms and plant material (Sunderland, 1975; Pollet & Desender, 1985; Ayre & Port, 1996; Symondson *et al.*, 2000). *P. melanarius* has been shown to be capable of eating three times its own body weight daily under laboratory conditions (Thiele, 1977). The preferred habitat type of this beetle is uncultivated open land (Hagley, 1996). Like other beetles that inhabit open-field habitats, this species is primarily active during the day (Kegel, 1990).

P. melanarius usually live for 1-3 years and overwinter in the soil as adults and larvae (Wallin, 1989; Hagley, 1996). Most adults emerge in June and July and larvae between June and August. Over-wintering adults breed earlier than newly-hatched adults as the females need time for egg-ripening (Basedow, 1994). The main period of activity and breeding occurs between July and August, with adults becoming inactive in September/October and either dying off or overwintering (Den Boer & Den Boer-Daanje, 1990; Hagley, 1996). Eggs are laid individually in the soil during the peak breeding season (Hagley, 1996). Larvae of *P. melanarius* are free-moving, carnivorous and undergo three stages before pupating (Lövei & Sunderland, 1996).

1.4. Carabid beetles and slugs

A number of polyphagous carabid beetles have been reported to feed on slugs (Symondson, 2004). Some carabid beetles are specialist mollusc feeders and have become morphologically adapted for feeding on them. Many carabids of the tribe Cychrini are snail specialists and have elongated and narrowed mouthparts, head and thorax and spoon-shaped palps for entering and extracting snails from their shells (Digweed, 1993). The mollusc specialists *Carabus violaceus* (L.) and *Cychrus caraboides* (L.) rapidly immobilise and kill slugs by

biting the head or mantle cavity, whereas generalist carabid beetles have not developed strategies for handling slugs (Pakarinen, 1994a; see Section 1.4.1).

There are many generalist carabid beetles that feed on molluscs, slugs in particular. *Pterostichus madidus* (Fabricius) is closely related to *P. melanarius* and is of a similar size, but is not such a voracious predator of slugs. These beetles have been reported feeding on slugs when motivated by hunger but in the laboratory will preferentially feed on alternative prey when present (Mair & Port, 2001). This is also true of *Nebria brevicollis* (Fabricius), which is a medium-sized carabid but is capable of attacking juvenile slugs (Ayre, 2001). Field captured specimens of *N. brevicollis* have been found with slug protein in their guts, tested using ELISA, plus a few other medium-sized carabids tested positive including *Harpalus rufipes* (Degeer) and several *Amara* sp (Ayre & Port, 1996). No small carabids are reported as slug feeders, they would not be sufficiently large enough to tackle such prey and overcome slug defensive mucus. Tod (1973) found 14 different carabid beetles that had fed on slugs and there was a highly significant correlation between the size of the carabid and the number of individuals in that species that had fed on slugs. A number of the larger carabid species and mollusc specialists have contained slug protein, but they are not in such high density as *P. melanarius*; these include *C. violaceus*, *Abax parallelepipedus* (Piller and Mitterpacher) and *C. caraboides* (Ayre & Port, 1996). The ability of *Pterostichus cupreus* (L.) to accept slugs and slug eggs was compared to *P. melanarius* in the presence and absence of alternative prey (Oberholzer & Frank, 2003). *P. melanarius* ate slugs and slug eggs in preference to alternative food such as crickets, aphids and dipteran larvae, but the presence of alternative prey reduced feeding on slugs and eggs by *P. cupreus*. This further suggests that although other carabids may eat slugs when other prey availability is low, slugs are a preferred prey item for *P. melanarius* even when other prey species are available.

1.4.1. Slug defences

Although slugs do not possess a protective shell, they have evolved many defences, which are effective against attack by generalist carabid species. Slugs release viscous mucus from dorsal glands when attacked, which can block the action of beetle mouthparts (Pakarinen, 1994a). *Arion* species, such as *A. intermedius,* react to attack by withdrawing the head under the mantle, forming the body into a tight hemisphere which is difficult to bite, followed by mucus production. Limacids, such as *D. reticulatum,* have soft delicate skin and cannot protect themselves by tightening the body form. They react to attack by immediately producing large quantities of viscous mucus, tail-wagging which may stun an attacker,

autotomization of the tail and fleeing (Pakarinen, 1994b). Autotomy was investigated in *D. reticulatum* by mechanical stimulation of the tail, and 50 % were found to autotomize the tail shortly before fleeing to find refuge (Pakarinen, 1994b). It was hypothesised that the purpose was to provide the attacker with enough food to keep them occupied so that the slug would have time to find cover.

Specialist predators such as *C. violaceus* and *C. caraboides* can kill slugs before large amounts of mucus can be produced (Pakarinen, 1994a). Larger carabids such as *P. melanarius* and *A. parallelepipedus* appear to be more successful at attacking slugs and overcoming their defences than smaller species (Ayre, 2001).

1.4.2. Predation studies

Studies on the prey preferences of beetles have mainly consisted of three different approaches: visual analysis of beetle gut contents, laboratory feeding studies and molecular techniques that identify prey remains in the beetle's guts.

Visual identification of beetle gut contents is of limited value when attempting to identify slug remains, as slugs are soft-bodied prey items. Identification can be achieved if the shell or radula is consumed, but this is not always the case (Digweed, 1993).

Laboratory studies can provide useful insights into feeding preferences of carabids, including distinguishing between size preferences of one particular prey item. Ayre (2001) tested the ability of 21 different carabid species to kill and consume 1 day old *D. reticulatum*. Smaller species of beetle were not able to kill slugs, whereas most of the medium and large sized beetles were capable of killing and consuming slugs. Successful predation of slugs varied with temperature but was different for the four beetles species tested, and reflected their annual activity periods in the field. Size of slugs will also have an impact on whether or not they can be attacked and consumed by beetles. McKemey *et al.*, (2000) found that *P. melanarius* would readily attack and consume *D. reticulatum* < 40 mg, but would only consume slugs > 40 mg after prolonged confinement together in Petri dishes. Larger slugs may be more difficult to attack because of the vast quantities of mucus produced. It is likely that consumption of larger individuals eventually occurred because mucus production was exhausted and/or beetles became increasingly motivated by hunger to kill. Results from the laboratory must be considered carefully as they may not represent conditions in the field. For example, although McKemey *et al.* (2000) found that smaller slugs were more readily consumed by beetles, larger individuals may be encountered more frequently in the field and thus are more likely to be preyed upon than smaller individuals (McKemey *et al.*, 2003).

A range of techniques have been developed for the molecular identification of prey remains in predator guts (reviewed in Symondson, 2002b). Paill *et al.* (2002) successfully used isoelectric focusing to detect esterases from the slug *Arion lusitanicus* (Mabille) in the crops of several predatory carabid beetles. Electrophoretic esterase banding patterns were compared between several species of slugs and the pattern produced by *A. luscitanicus* was distinguishable from species not closely related. The characteristic banding patterns could be detected in crops of carabids many hours after feeding. The benefits of using this technique are that it is species specific yet relatively cheap and quick to do. The unfortunate drawback of this technique is that it is impossible to separate banding patterns caused when several prey species have been consumed by a single predator (Walrant & Loreau, 1995).

Serological techniques have been used with great success in slug predation studies. The use of antisera to detect slug protein in carabid beetles was first reported by Tod (1973) and showed that at least 14 species of carabids tested had fed on mollusc tissue, and larger species were more likely to have fed on slugs. A general slug antibody was developed by Symondson & Liddell, (1993), which used polyclonal antisera. Prey antigen in the presence of beetle guts were tested for in enzyme-linked immunosorbant assays (ELISA). This antibody reacted strongly with a variety of molluscs tested and some earthworms, and has been used extensively in predation studies of *P. melanarius* (Symondson *et al.*, 1996; Bohan *et al.*, 2000a). Specific monoclonal antibodies have since been developed that can identify slug protein to species level. Antibodies currently available can specifically detect *D. reticulatum* slugs (Symondson & Liddell, 1996), *D. reticulatum* and *Arion ater* (L.) eggs (Mendis *et al.*, 1996), *Arion distinctus* (Mabille) and *Arion hortensis* (Ferussaci) (Symondson *et al.*, 1999), and *Tandonia budapestensis* (Hazay) (Symondson *et al.*, 1997) in gut remains of predators.

The time since a prey item was consumed is an important consideration when using serological techniques coupled with ELISA as proteins become degraded in predator guts and may become undetectable after a couple of days. Degradation of proteins is also faster under higher temperatures, therefore during warmer times of the year, the period of detection after prey is consumed becomes much shorter (Hagler & Naranjo, 1997). Another problem is that a predator may have consumed several of the same prey items but ELISA cannot tell us how many a predator will have consumed in the last few days. The problem of false positives caused by secondary predation was examined in an aphid-spider-carabid system by Harwood *et al.* (2001) and was found to be extremely unlikely. ELISA cannot distinguish between scavenging and predation, nor can it provide any information on the size of prey consumed,

only laboratory studies can test this. Mair & Port (1999) did laboratory studies on predation of dead, injured and live slugs by *P. madidus* and *N. brevicollis*. They found that dead slugs were preferentially consumed in favour of injured or live slugs, probably due to the lack of mucus production and the reduction in handling time/energy expenditure of feeding on dead prey. They suggest that many studies involving the use of ELISA to quantify predation may be giving false positive results due to beetle scavenging, and question the effectiveness of carabids to become biological control agents of slugs. The theory that beetles will preferentially prey on sick or dead slugs is further supported by Langan *et al.* (2001) who found that slugs poisoned by metaldehyde were eaten in preference to healthy slugs. It was however pointed out that positive identification of slug protein in beetle guts by the use of monoclonal antibodies was likely to indicate predation, not scavenging, since the occurrence of dead and dying slugs is uncommon in the absence of molluscicides.

There have been recent developments in techniques that allow identification of prey DNA in predator guts. These techniques may be more suitable at identifying the range of prey which is present in a predator gut at any one time, which would be costly to do with monoclonal antibodies (Zaidi *et al.*, 1999; Symondson, 2002b). Although DNA rapidly degrades in a predator gut, a PCR amplification step allows the detection of very small amounts of prey DNA to be identified. Recently, primers have been designed to amplify mitochondrial DNA from *D. reticulatum* and *A. hortensis* agg. for use in predation studies with *P. melanarius* (Dodds *et al.*, 2003).

1.4.3. Use of beetles to protect crops from slug damage: results from simulated field trials

Attempts have been made to test the ability of carabids to control slugs under simulated field conditions. Effective control of *D. reticulatum* in mini-plots of lettuces was achieved using the carabid beetle *A. parallelepipedus* (Symondson, 1989). Control and partial control of *D. reticulatum* in sward boxes sown with grass and clover was achieved using *A. parallelepipedus* and *P. madidus*, respectively (Asteraki, 1993). Control by *A. parallelepipedus* was as successful as an application of methiocarb.

Control of *D. reticulatum* in lettuces grown in a polythene tunnel was also achieved by introducing *A. parallelepipedus* (Symondson, 1993b). The beetles were able to prey successfully upon slugs found on the soil surface and on young lettuce plants, but not when the plants had matured. The slugs successfully avoided predation by the ground beetles by

climbing up into the plants out of reach of attack. Timing of control with the beetles is therefore an important factor if effective control is to be achieved.

Larvae of *P. melanarius* also eat slugs and were shown to reduce numbers of *A. intermedius* and *D. reticulatum* in mini-plots of winter wheat (Thomas, 2002).

1.4.4. Effect of *Pterostichus melanarius* on slug distribution and survival in the field

Although many species of carabids have been proven to be important predators of slugs and effective control of slugs can be achieved in simulated field conditions, it is important also that the relationship between the predator and prey is studied in the field. Effective biological control agents must be capable of searching out prey in the field and aggregating in areas of high prey density (Port *et al.*, 2000).

The main slug pests *D. reticulatum* and *A. intermedius* share similar patterns of parametric intensity but have different spatial arrangements (Bohan *et al.*, 2000b). Sampling the slug populations throughout the year showed that the variance to mean relationship was common for both species, and that the variance for a given mean was lower in the summer months (June and July). Aggregations of juveniles of both species were present at a spatial scale of 0.25 m in March but both populations had become spatially random at this scale by June. Since the slugs within the aggregations in March were observed to be very small for both species, it was postulated that slugs having recently emerged from egg batches caused the aggregations observed. Both species have a habit of laying eggs in batches between autumn and early spring, hatching in late spring (South, 1965). The dispersion of juveniles away from egg batches could therefore have caused the reduction in the variance and the proportion of zero counts in the summer. At spatial scales of 4 m and above *D. reticulatum* became randomly distributed where as *A. intermedius* resolved to a patch. The patch persisted for at least a year but conversely the spatial arrangement of *D. reticulatum* changed every month.

Bohan *et al.* (2000b) suggested that predation by *P. melanarius* could also result in a reduction in the variance to mean ratio for slug populations in the summer. Bohan *et al.* (2000a) found that the distributions of slugs and *P. melanarius* were dynamically associated with each other. In June, areas containing high numbers of slugs also contained many beetles, whilst in July areas containing many carabids contained fewer slugs. This was hypothesised to be caused by the effects of predation on slug population growth in areas of high slug density. Density-dependent predation of slugs would cause a reduction in population growth in areas of high slug density but would allow an increase in the slug population in areas of

low slug density, which would contain fewer carabids. The effect of predation would therefore even out the distribution of slugs in the field and lead to a reduction in the proportion of zero counts. There is also the possibility that slugs in areas of high beetle density could detect the presence of the predators and move away to areas of lower beetle density. This could also account for a reduction in the variance of the two slug populations.

Symondson *et al.* (1996), with the use of monoclonal antibodies specific to slug protein, found that 85 % of *P. melanarius* captured in a field of oilseed rape in 1992 contained slug haemolymph. Significantly higher numbers of beetles were found in plots containing the greatest amount of slug biomass and these beetles also contained the highest quantities and concentrations of slug protein. The results suggest that *P. melanarius* was capable of aggregating in areas of high slug biomass and has potential to become a biological control agent for slugs.

In 1997, 11 % of beetles were found to have ingested slug protein, and the distribution of these beetles in June was significantly associated with the distribution of slugs > 25 mg (Bohan *et al.*, 2000a). Growth of the larger slug classes between June and July declined as the local numbers of beetles increased. It was therefore concluded that beetles were aggregating to areas of high slug biomass and that predation on slugs was not opportunistic but direct and dynamic.

This opinion has been refuted by Mair *et al.* (2001), who suggest that considering only 11 % of beetles tested positive for consumption of slug protein, beetles are not in high enough densities to have caused the reduction in the growth of the slug population. They also suggest that the static distribution of the beetles over the summer is not due to beetle satiation and that if beetles truly were aggregating to areas of high slug density then their distributions should also have been associated in July. Bohan *et al.* (2001) however, further argue that Mair *et al.* (2001) have assumed beetles testing positive for slug protein have only ingested one slug, whereas in fact, a beetle testing positive may contain protein from several slugs. 11 % of beetles testing positive is also an underestimate as the threshold for a positive reading is set high to prevent cross-reactions with earthworms and the creation of false positives. Beetle density could therefore have been high enough to account for the reduction in growth of the slug population. The static distribution of the beetles over time is harder to explain but Bohan *et al.* (2001) suggest that as *P. melanarius* were sampled a week before the slugs, it is possible that this could account for the time delay in changes in *P. melanarius* distribution.

The static distribution of *P. melanarius* has also been demonstrated in work by Thomas *et al.* (1998; 2001). They used mark-recapture techniques, spatial analysis by

distance indices (SADIE) and calculations of displacement over time to show that the distribution of *P. melanarius* is aggregated in patches and that the spatial pattern of activity-density was stable over considerable amounts of time. They hypothesised that the beetle movements were lower than expected due to aggregation in patches of high prey density, preferred microclimatic conditions or preferred oviposition sites. They also found that the activity density of beetles was much higher in August than July or June. If activity density is low earlier in the summer then this might account for the static distribution of the beetles found by Bohan *et al.* (2000a).

1.5. Summary and project outlay

Slugs are economically important agricultural and horticultural pests. The traditional method of controlling slugs is to use chemical molluscicides but recent concern over their non-target effects has encouraged research into alternative methods of control. Recent research into the effects of farming methods and native predators on slug populations heralds the possibility of integrated control management for slugs that does not solely rely on the use of chemicals.

Carabid beetles are important polyphagous predators of arable land that have been the subject of extensive ecological research. Carabid beetles are traditionally viewed as generalist and opportunistic predators that feed on a wide range of prey types, depending on the local prey availability. Recent evidence shows that carabids have more complex relationships with their prey than this and orientate towards areas where specific prey are aggregated (Bohan *et al.*, 2000a; Winder *et al.*, 2001, 2004). One of the most abundant carabids of arable land, *P. melanarius*, has been found aggregated in areas of high slug density (Symondson *et al.*, 1996) and has been shown to effect the spatial and temporal dynamics of slugs (Bohan *et al.*, 2000a; Symondson *et al.*, 2002a).

Despite evidence suggesting that *P. melanarius* is an important predator of slugs, there is still some debate over just how much influence this carabid has on the population dynamics of slugs (Mair *et al.*, 2001). Further investigation into the dynamics of this predator-prey relationship is required before management of this carabid could be recommended as part of an integrated control program for slugs. In particular, questions are raised by the work of Bohan *et al.* (2000a) as to how *P. melanarius* affects the survival, behaviour and dispersion of two abundant arable slug species: *D. reticulatum* and *A.*

intermedius. Work by Bohan *et al.* (2000b) suggests that although *A. intermedius* and *D. reticulatum* share common patterns of parametric intensity, they display contrasting patterns of spatial arrangement. Whether this is due to differences in life-history parameters between the two species, and/or is influenced by carabid beetle predation was investigated. Several hypotheses were created for testing in this project:

1. The presence of *P. melanarius* causes increased dispersion of juvenile and adult slugs.
2. *A. intermedius* and *D. reticulatum* differ in parameters of dispersion and/or their behaviour towards *P. melanarius*.
3. Beetles respond to and aggregate in areas of higher slug density.

Chapter 2

Spatial relationships between *Pterostichus melanarius* and slugs

2.1. Introduction

The distributions of slugs and the predator *P. melanarius* have been shown to be spatially associated (Bohan *et al.*, 2000a). Populations were sampled across a 16 m grid in a uniform field of winter wheat in the summer of 1997. In June, areas containing high numbers of slugs also contained many beetles, whilst in July areas containing many beetles contained fewer slugs; evidence of density-dependent predation on slugs by *P. melanarius*. Association was significant only for the total slug distribution and not when species of slugs were analysed separately. 11 % of the beetles sampled tested positive for the ingestion of slug protein following ELISA testing and the spatial distribution of these slug-positive beetles was spatially associated with slugs > 25 mg.

Work presented in this chapter takes the analysis published in Bohan *et al.* (2000a) further with the aim of providing information about spatial relationships with slugs of different weights, and whether relationships with slugs were different for male and female *P. melanarius*.

The spatial distribution of beetles that had fed on slugs was associated only with slugs > 25 mg and hence it was concluded that beetles were preying preferentially upon large slugs (Bohan *et al.*, 2000a). Bohan *et al.* (2000a) separated slugs into two classes (< 25 mg and > 25 mg) but *D. reticulatum* can range in size from less than 1 mg to over 1000 mg. The large slug category that they used therefore included a much wider range of slug sizes than the smaller slug size category. This makes it difficult to identify which size range is optimal for *P. melanarius*. Laboratory feeding studies indicate that when given a choice of a range of slug sizes, *P. melanarius* selects the smallest slugs < 40 mg and only eats larger slugs after prolonged confinement together in Petri dishes (McKemey *et al.*, 2001). Larger slugs are able to produce more mucus and hence attack success is lower on large slugs than on smaller slugs (Pakarinen, 1994a). In mini-plot field studies, smaller slugs were not preferentially preyed upon and beetles killed and ate a whole range of slug sizes (McKemey *et al.*, 2003). In complex field environments it is likely to be easier for smaller slugs to find refuge from beetle predation, leading to predation rates no greater than those on larger size classes. Since

large slugs > 40 mg are better able to defend themselves and slugs < 25 mg would be difficult to find in the field, it was predicted that an intermediate size class of slugs would be preferentially preyed upon in the field. In this chapter, slugs > 25 mg were broken down into two further categories: 25 - 50 mg and > 50 mg. The null hypothesis that there is no difference in predation between weight classes was tested.

When the data from Bohan *et al.* (2000a) were originally analysed, beetles were not separated into males and females although this information was recorded. Male and female *P. melanarius* have been shown to have different levels of activity-density in the field. Thomas *et al.* (1998) achieved greater capture rates of males in pitfall traps in June and July, but in August more females were trapped than males. Ratios of females to males change according to level of nutrition. This could lead to different spatial patterns and spatial relationships with the slug distributions. Using the data the null hypothesis was tested that there was no significant difference in the spatial patterns of male and female *P. melanarius,* and no difference in the spatial associations with the slug distributions in June and July.

The final part of this chapter links the beetle foregut weights with slug numbers in the soil. Previous work by Symondson *et al.* (2002a) found that the foregut weights of the beetles were positively related to the abundance of slugs in the soil and that foregut weight was positively correlated with beetle population growth over five years. Beetles therefore consumed more prey where there were higher numbers of slugs, and slug feeding influenced beetle reproductive success and hence beetle numbers in the next year. In this chapter spatial analyses were performed on data collected by Bohan *et al.* (2000a), to look for any spatial association between beetle foregut weight and slug numbers in samples. The null hypothesis that there was no spatial association between slug numbers and beetle foregut weight was tested. In order to make a trophic link between slug numbers and beetle feeding, foregut as a proportion of the beetles' total body weight was compared between beetles that had tested positive and those that had tested negative for consumption of slug protein. The null hypothesis that foregut as a proportion of beetle body weight was not significantly different between beetles that tested positive or negative for slug protein was tested.

2.2. Methods

Full details of the study site, method of sampling slugs and beetles and ELISA testing of beetles are given in Bohan *et al.* (2000a).

2.2.1. Study site and sampling grid

The original study was carried out in Field 54 at Long Ashton Research Station in 1997. The data analysed in this chapter were collected from the field in mid-June and mid-July when the crop was winter wheat. Data was collected from a 5 × 6 array, 16 m scale sampling grid. The sample points were fixed for *P. melanarius*, but the grid used for sampling of slugs was offset by 2.5 m due to the destructive nature of soil sampling. On each date, the position of the slug grid was also offset by 1.5 m from the previous sample date. For analyses the slug and beetle grids were considered to overlay one another with sampling occurring at the same points.

2.2.2. Sampling for *Pterostichus melanarius*

Beetles were sampled on the 16 June and 14 July 1997, using paired pitfall traps placed 0.5 m on either side of each sample point. Data were pooled for each sample point. Traps were emptied every day for three days and beetles were taken from the field to be stored at -20 °C for later gut content analysis by ELISA. Beetles were sexed and weighed before further analysis.

2.2.3. Sampling for slugs

Slug sampling was carried out one week after beetle sampling in June and July. A 25 cm × 25 cm × 10 cm (depth) sample of soil was collected from each sample point using a template and undercutting with a spade. Soil samples were each placed into a plastic tub and flooded in troughs over a period of 8-10 days (see Section 4.2.1. Chapter 4). Slugs were collected each day and then identified and weighed.

2.2.4. Testing beetle guts for slug protein using ELISA

Quantification of slug predation was achieved using ELISA to detect the remains of slug protein antigens in the beetle foreguts. Beetles were removed from the freezer and allowed a short time to defrost before dissecting out the foreguts. Each foregut was weighed and processed for ELISA (see Bohan *et al.*, 2000b; Symondson & Liddell, 1993) using the general slug monoclonal antibody DrW-2d11 (Symondson & Liddell, 1995). Guts that contained a protein concentration greater than 21 ng were considered to be slug-positive. This level was set artificially high to prevent false positive readings that might be obtained by the antibodies cross-reacting with earthworm protein (Symondson & Liddell, 1993). Beetles with guts containing a protein concentration of less than 4.1 ng were classed as slug-

negative. Any beetles with protein concentrations between the range of 4.1 ng and 21 ng were classed as inconclusive, as they may or may not have eaten slugs.

2.2.5. Spatial and statistical analysis

SADIE (Perry, 1995; Perry, 1998) was used to measure spatial pattern and the association between the slug and beetle data. SADIE is used to analyse ecological data in the form of spatially referenced counts. This technique is especially useful in that it takes into account the spatial location of sample units and identifies areas in the sample site that contribute to spatial pattern. Spatial pattern is the degree to which the count data are non-randomly distributed across an area, and is measured by an index of aggregation (I_a). Regions within a study area contain counts that are either randomly distributed or have local clusters of counts that have similar densities. Clusters are termed patches if counts within them are high-density or gaps if counts within them are low-density. SADIE estimates I_a by finding the minimum *distance to regularity* (D) for individuals in the sample area. This is the minimum distance that individuals would have to move between sampling points until there is an equal number at each sample point. The greater the D, the more aggregated the spatial pattern. I_a is based on a series of randomizations, in which counts are randomly moved to new sample points and the distance to regularity is estimated. In this study, 1170 simulations were run for each analysis, using a constant random seed. I_a is then obtained by dividing the average value of D obtained from the random permutations (the value expected when the counts are randomly distributed among the sampling points), by the observed value of D. An index with a value well above unity indicates that the spatial pattern is aggregated whereas an index with a value well below unity indicates spatial regularity. Finally, a probability of aggregation (P_a) is calculated, which is the proportion of D values from the random permutations that are equal to or larger than the observed value of D. When the value of P_a is less than or equal to 0.025, the count data are significantly aggregated.

From the analysis of spatial pattern, each sampling point in each data set has a cluster index, υ, which is a measure of the point's contribution to local spatial pattern. To visualize clusters, cluster indices υ, were contoured to produce a red-blue map (using Surfer version 6, Golden Software, Inc®) (Perry *et al.*, 1999). Red areas indicate patches ($\upsilon > 1.5$) and blue areas indicate gaps ($\upsilon < -1.5$). The red-blue maps are overlaid with a classed post map, which indicates the value of υ at each individual sampling point.

SADIE techniques were also used to analyse the degree to which two data sets were spatially associated (Perry & Dixon, 2002), estimated as an index of association, X. Spatial

association occurs when the patches and gaps for one population coincide with the patches and gaps of a second population respectively. Spatial dissociation occurs when patches for one population coincide with the gaps from the second population and vice-versa. Association is measured by the correlation between the local cluster indices, υ, of the two data sets and is expressed as X (the index of association). A component value is given to each sample point which indicates the degree to which the cluster indices from the two data sets 'agree'. The index of association is calculated by summing the component values from all the sampling points. The significance of X is estimated by comparing it to values of X estimated from random permutations of the cluster indices amongst the sample points for both data sets. This produces a probability of association (P_t), the proportion of X values from the random permutations that are equal to or larger than the observed value of X. A value of P_t less than or equal to 0.025 indicates significant spatial association. A value of P_t greater than or equal to 0.975 indicates significant spatial dissociation.

For each analysis of spatial association, measures of local association at each sample point were contoured to produce a plum-green plot (Winder *et al.*, 2001) (using Surfer version 6, Golden Software, Inc®).

2.2.6. Spatial and trophic associations between beetles and slugs of different sizes

The slug data were broken down into three size classes: small (< 25 mg), intermediate (25 - 50 mg) and large (> 50 mg). SADIE was used to analyse the spatial patterns of slugs from the different size classes and the degree to which the slug-positive beetle data (beetles with > 21 ng slug protein in foregut) were spatially associated with each class.

2.2.7. Male and female beetle distributions and spatial associations with slugs

SADIE analysis was used to analyse the spatial patterns of male and female beetles in June and July. SADIE was then also used to analyse the degree to which the male and female beetles were spatially associated with slugs.

2.2.8. Spatial association between beetle foregut weights and slug abundance

Although originally designed for count data, non-parametric SADIE approaches can be used to analyse continuous data (Kelvin Conrad, pers. comm.), provided the data are transformed into integers. The foregut weight data were all multiplied by one thousand to remove the decimal places. The transformed data were plotted as histograms to check for normality. To analyse the spatial pattern of the beetle foregut weights, the mean transformed weight at each

sample point was calculated for the June and July data. Beetles were then separated into each sex and the average male and female foregut transformed weights at each sample point were calculated. Non-parametric SADIE was then used to examine the spatial pattern of male and female beetle foregut weight data, followed by analysing the spatial associations between total slug numbers and male and female beetle foregut weights.

2.2.9. Trophic association between beetle foregut biomass and slugs

Only data in which beetles definitely tested positive or negative for consumption of slug protein were included in this analysis. For each beetle sampled in June, July, August and September, the foregut weight was calculated as a proportion of the beetles' total body weight. The distribution of the proportion data was skewed and resembled most closely that of a log-normal distribution. This distribution was seen as the most appropriate to use for such biological data pertaining to weights, where it is often found that the median and the mean do not coincide. Using the GenStat (GenStat 7th ed. - VSN International Ltd) FITDISTRIBUTION procedure, distributions were fitted to the data. This procedure firstly creates a frequency distribution for the data. To do this it estimates the number of groups to use, including the upper and lower tail groups, as the square root of the number of observations and then calculates the class limits so that the number of observations falling into each class is as equal as possible, i.e. the groups will be of unequal width. The method of Maximum Likelihood (ML) is then used to obtain estimates of the mean and variance for the specified distribution given the frequency observations. The deviance, distributed as chi-squared, thus produced for the fit of the distribution to the data allows a statistical assessment of the quality of fit. It is then possible to assess the improvement in fit provided by the factors Slug (eaten or not eaten), Date (June – September) and Sex (male or female) by fitting multiple distributions within the same data set according to these factors and their combinations. REsidual Maximum Likelihood (REML) was used to analyse the unbalanced data set and assess the significance of sources of variability in the data, using the regression principle of forward selection (Patterson & Thompson, 1971; Genstat committee, 1993).

2.3. Results

2.3.1. Slug weights

233 and 307 slugs were collected from the samples in the 16 m grid in June and July respectively. Most of these slugs consisted of *A. intermedius* (27.8 %) and *D. reticulatum* (69.1 %). Slugs were divided into three weight classes, small (< 25 mg), intermediate (25 - 50 mg) and large (> 50 mg) for the purpose of this analysis. In June and July, slugs from the small and large size classes were mostly *D. reticulatum* whilst the intermediate size class contained proportionately more *A. intermedius* (Figure 2.1).

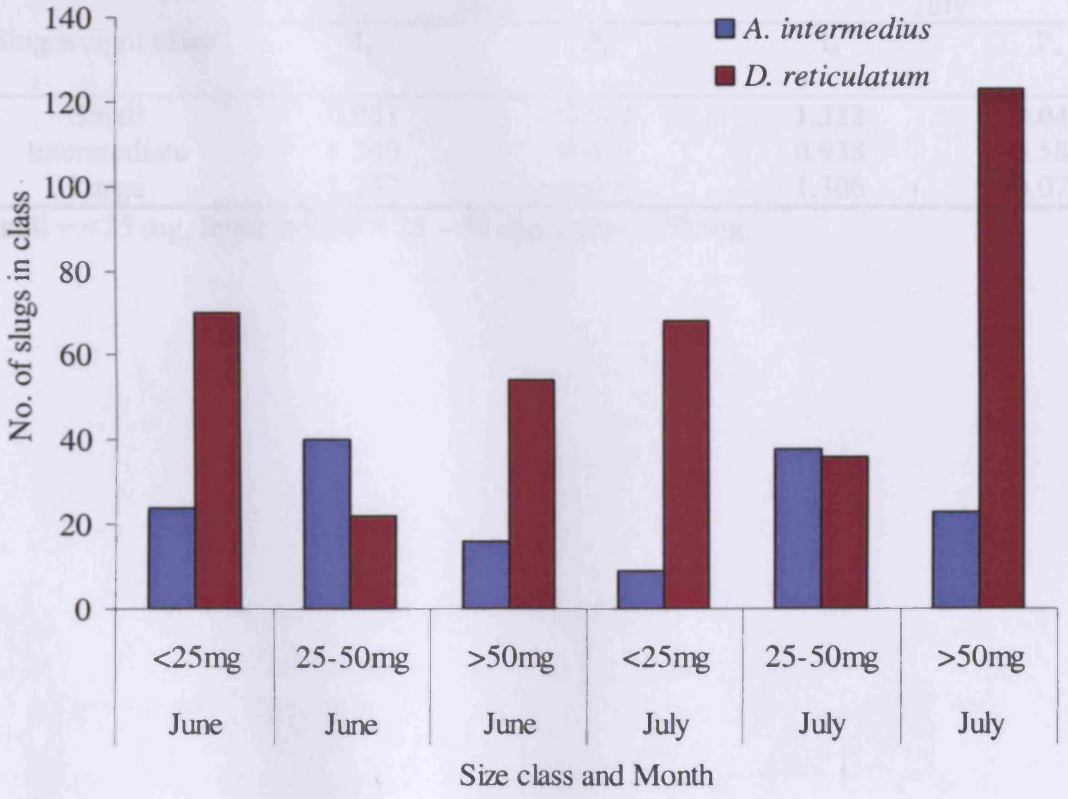

Figure 2.1. Slug weight frequency distribution. Number of *Arion intermedius* and *Deroceras reticulatum* from each size class sampled in June and July.

2.3.2. Spatial and trophic associations between beetles and slugs of different sizes

In June, small slugs were randomly distributed (Table 2.1, Figure 2.2.a). Intermediate and large slugs were also randomly distributed with a tendency towards aggregation in the west of the field (Table 2.1, Figure 2.2.b and 2.2.c). In July, small slugs were now aggregated,

with a large cluster in the south-eastern corner of the field (Table 2.1, Figure 2.2.d). Intermediate slugs were now randomly distributed but still with many slugs occurring along the western border (Table 2.1, Figure 2.2.e). Large slugs were randomly distributed with a tendency towards aggregation, particularly in the north of the field (Table 2.1, Figure 2.2.f). Although all slugs were clustered along the western border in June, slugs from different size classes occurred in different parts of the field in July. The distributions had been modified, possibly through dispersal, and predation by *P. melanarius*.

Table 2.1. Statistics for spatial distribution of small, intermediate and large slugs. Statistics are presented for the months of June and July.

Slug weight class[a]	June		July	
	I_a	P_a	I_a	P_a
Small	0.951	0.562	**1.322**	**0.041**
Intermediate	1.340	0.055	0.938	0.581
Large	1.257	0.092	1.306	0.071

[a]Small = <25 mg, Intermediate = 25 – 50 mg, Large = 50 mg.

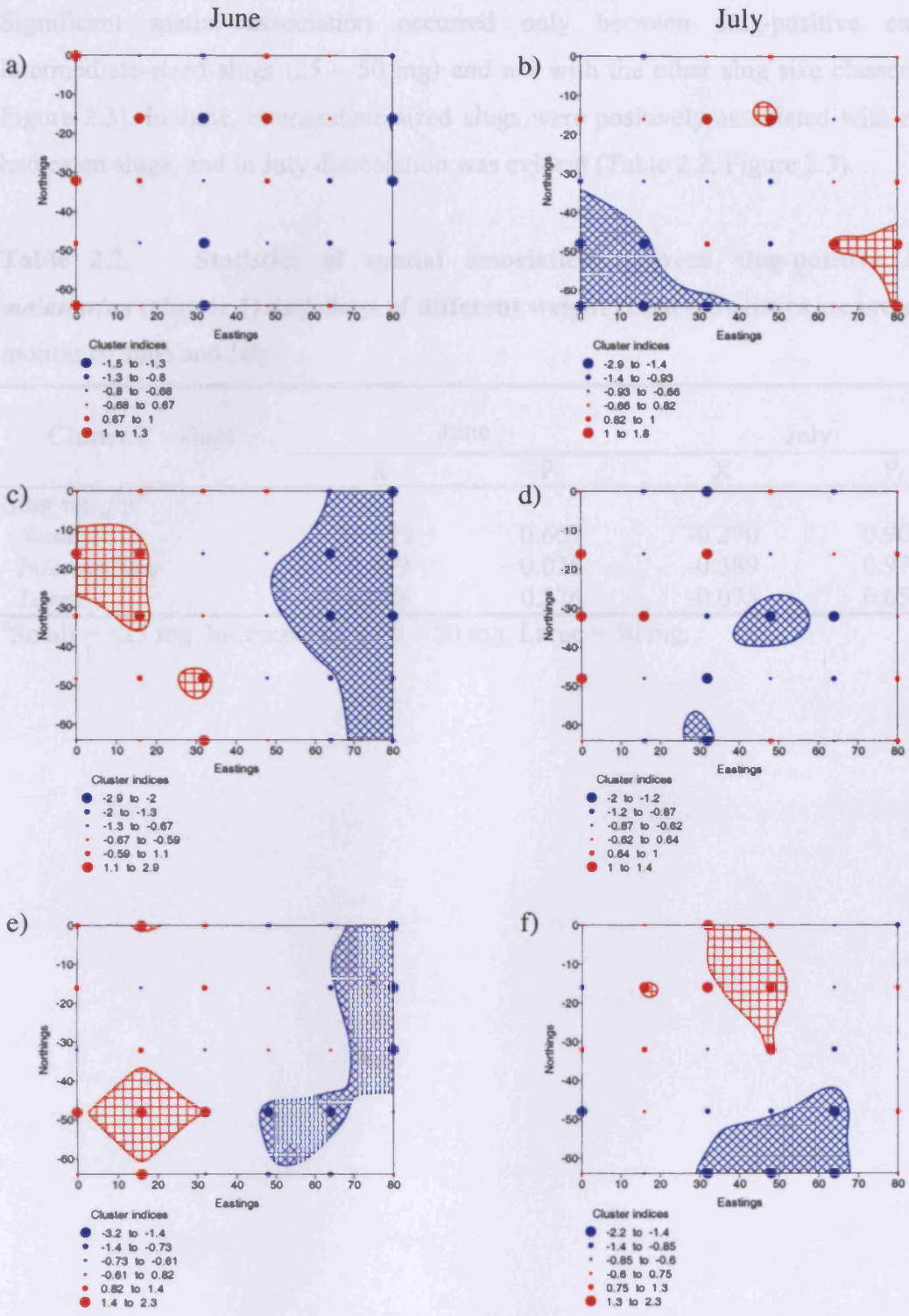

Figure 2.2. Red-blue contour plots of spatial distributions. Distribution patterns of small slugs in June (a) and July (b), intermediate slugs in June (c) and July (d) and large slugs in June (e) and July (f). Plots are overlaid with classed post maps of cluster indices. Shading represents areas where cluster indices (υ) either exceed 1.5 (red) or are below –1.5 (blue).

Significant spatial association occurred only between slug-positive carabids and intermediate-sized slugs (25 – 50 mg) and not with the other slug size classes (Table 2.2, Figure 2.3). In June, intermediate-sized slugs were positively associated with carabids that had eaten slugs, and in July dissociation was evident (Table 2.2, Figure 2.3).

Table 2.2. Statistics of spatial association between slug-positive *Pterostichus melanarius* (cluster 1) and slugs of different weight classes. Statistics are presented for the months of June and July.

Cluster 2 - slugs	June		July	
	X	P_t	X	P_t
Slug weight[a]				
Small	-0.059	0.607	-0.270	0.900
Intermediate	0.409	0.028	-0.389	0.971
Large	0.068	0.376	-0.075	0.654

[a]Small = <25 mg, Intermediate = 25 – 50 mg, Large = 50 mg.

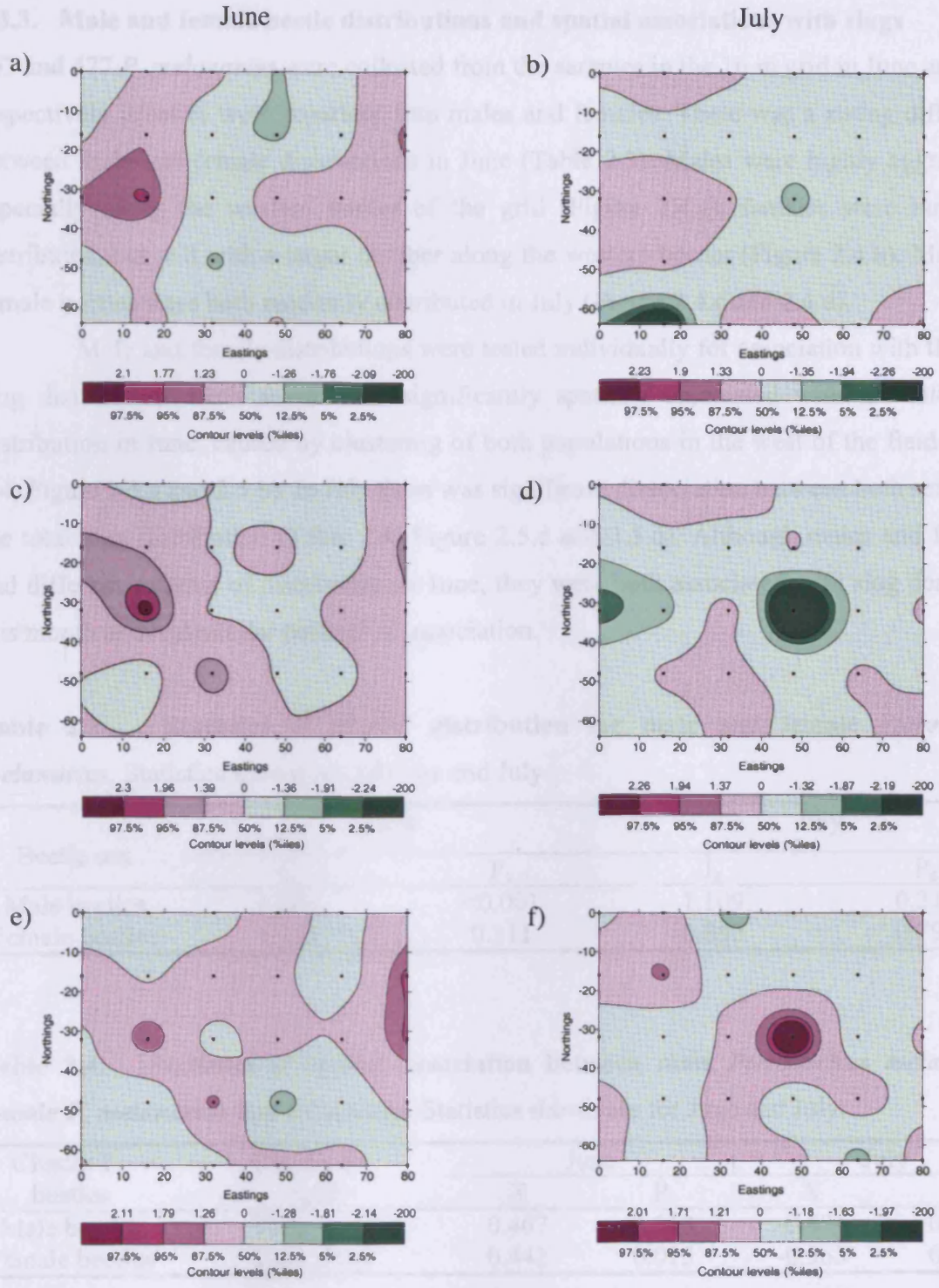

Figure 2.3. Plum-green contour plots showing spatial associations. Spatial association between slug positive *Pterostichus melanarius* and small slugs in June (a) and July (b), intermediate slugs in June (c) and July (d) and large slugs in June (e) and July (f) on a 64 m by 80 m grid in a field of winter wheat. Darkest colour represents areas where interpolated values of indices exceed 97.5 % confidence limits for association (plum) or dissociation (green). Posted symbols represent sample points.

2.3.3. Male and female beetle distributions and spatial associations with slugs

263 and 477 *P. melanarius* were collected from the samples in the 16 m grid in June and July respectively. Beetles were separated into males and females. There was a strong difference between male and female distributions in June (Table 2.3). Males were highly aggregated, especially along the western border of the grid (Figure 2.4.a); females were randomly distributed, but still with a larger number along the western border (Figure 2.4.b). Male and female beetles were both randomly distributed in July (Figure 2.4.c and 2.4.d).

Male and female distributions were tested individually for association with the total slug distribution. Both sexes were significantly spatially associated with the total slug distribution in June, caused by clustering of both populations in the west of the field (Table 2.4, Figure 2.5.a and 2.5.b). In July there was significant dissociation between both sexes and the total slug distribution (Table 2.4, Figure 2.5.c and 2.5.d). Although males and females had different patterns of distribution in June, they were both associated with slug density in this month and had similar patterns of association.

Table 2.3. Statistics of spatial distribution for male and female *Pterostichus melanarius*. Statistics shown are for June and July.

Beetle sex	June		July	
	I_a	P_a	I_a	P_a
Male beetles	1.890	<0.001	1.109	0.211
Female beetles	1.055	0.311	0.862	0.797

Table 2.4. Statistics of spatial association between male *Pterostichus melanarius*, female *P. melanarius* and total slugs. Statistics shown are for June and July.

Cluster 1 - beetles	Cluster 2 – slugs	June		July	
		X	P_t	X	P_t
Male beetles	Total slugs	0.467	0.010	-0.431	0.971
Female beetles	Total slugs	0.442	0.015	-0.363	0.967

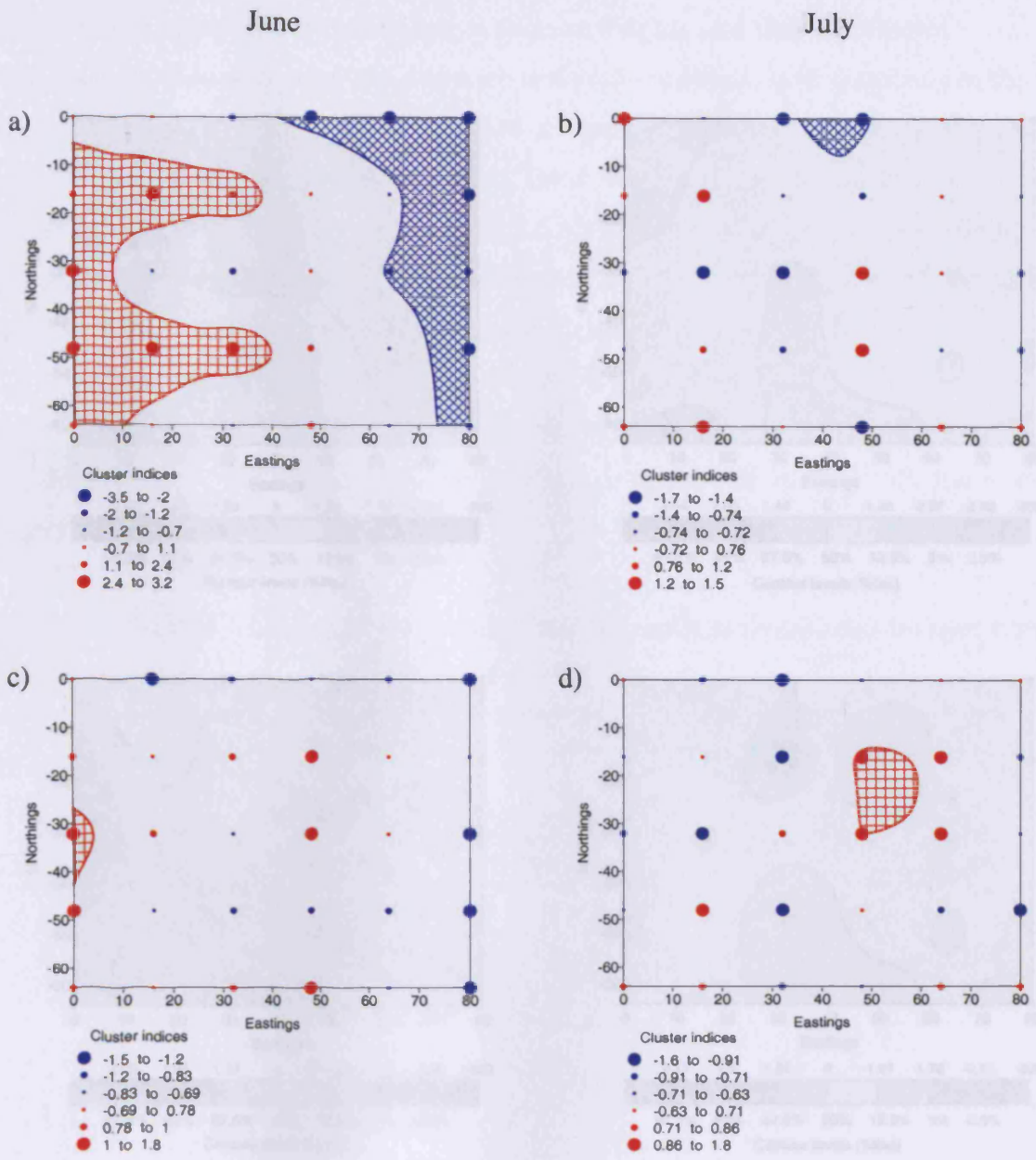

Figure 2.4. Red-blue contour plots of spatial distributions. Distribution patterns of male *Pterostichus melanarius* in June (a) and July (b), and female *P. melanarius* in June (c) and July (d) on a 64 m by 80 m grid in a field of winter wheat. Plots are overlaid with classed post maps of cluster indices. Shading represents areas where cluster indices (υ) either exceed 1.5 (red) or are below –1.5 (blue).

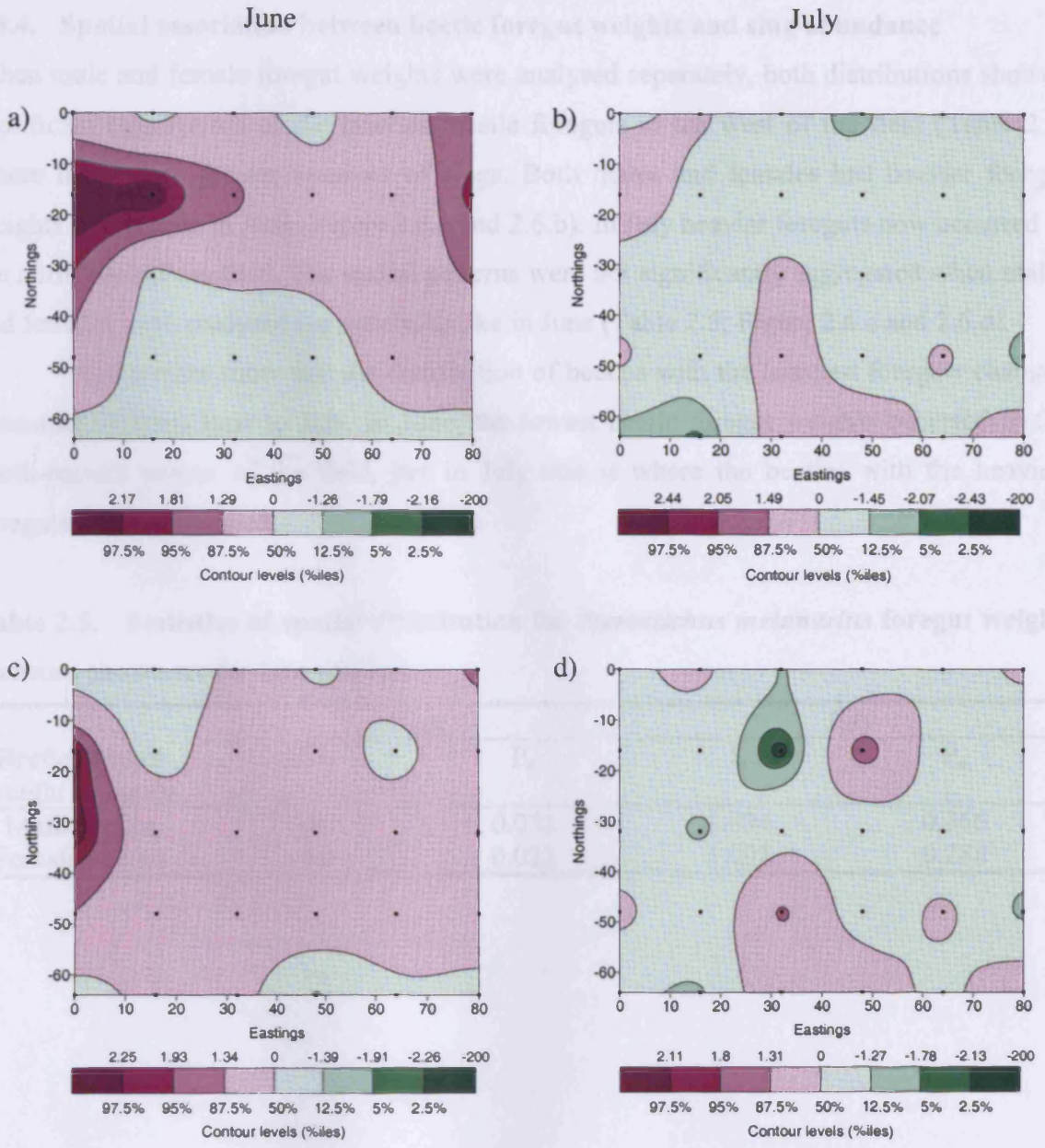

Figure 2.5. Plum-green contour plots showing spatial associations. Spatial association between male *Pterostichus melanarius* and slugs in June (a) and July (b) and association between female *P. melanarius* and slugs in June (c) and July (d) on a 64 m by 80 m grid in a field of winter wheat. Darkest colour represents areas where interpolated values of indices exceed 97.5 % confidence limits for association (plum) or dissociation (green). Posted symbols represent sample points.

2.3.4. Spatial association between beetle foregut weights and slug abundance

When male and female foregut weights were analysed separately, both distributions showed significant aggregation of the heaviest beetle foreguts in the west of the field (Table 2.5) where there were greater numbers of slugs. Both males and females had heavier foregut weights in this area in June (Figure 2.6.a and 2.6.b). In July heavier foreguts now occurred in the north-east of the field. The spatial patterns were not significantly aggregated when males and females were analysed separately, unlike in June (Table 2.5; Figure 2.6.c and 2.6.d).

The results show that the distribution of beetles with the heaviest foreguts changed dramatically from June to July. In June, the lowest beetle foregut weights occurred in the north-eastern corner of the field, but in July this is where the beetles with the heaviest foreguts were aggregated.

Table 2.5. Statistics of spatial distribution for *Pterostichus melanarius* foregut weight. Statistics shown are for June and July

	June		July	
Beetle foregut weight category	I_a	P_a	I_a	P_a
Male beetles	1.422	0.032	1.034	0.366
Female beetles	1.401	0.022	1.092	0.288

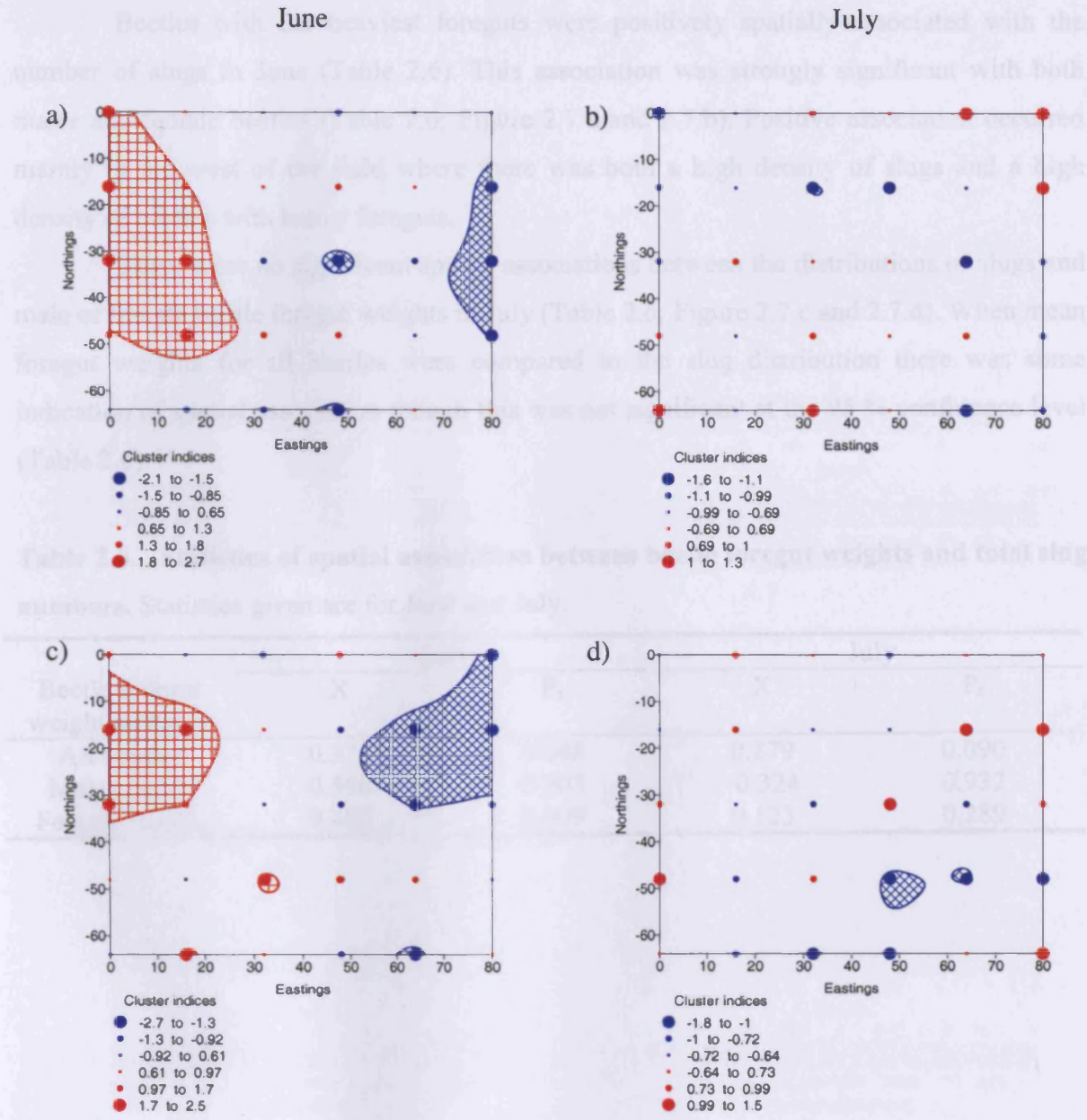

Figure 2.6. Red-blue contour plots of spatial distributions. Distribution patterns of male *Pterostichus melanarius* foregut weight in June (a) and July (b), and female *P. melanarius* foregut weight in June (c) and July (d) on a 64 m by 80 m grid in a field of winter wheat. Plots are overlaid with classed post maps of cluster indices. Shading represents areas where cluster indices (υ) either exceed 1.5 (red) or are below –1.5 (blue). Red areas indicate clustering of heavier beetle foreguts.

Beetles with the heaviest foreguts were positively spatially associated with the number of slugs in June (Table 2.6). This association was strongly significant with both males and female beetles (Table 2.6; Figure 2.7.a and 2.7.b). Positive association occurred mainly in the west of the field where there was both a high density of slugs and a high density of beetles with heavy foreguts.

There were no significant spatial associations between the distributions of slugs and male or female beetle foregut weights in July (Table 2.6; Figure 2.7.c and 2.7.d). When mean foregut weights for all beetles were compared to the slug distribution there was some indication of spatial association though this was not significant at the 95 % confidence level (Table 2.6).

Table 2.6. Statistics of spatial association between beetle foregut weights and total slug numbers. Statistics given are for June and July.

Beetle foregut weight category	June		July	
	X	P_t	X	P_t
All beetles	0.375	0.048	0.279	0.090
Male beetles	0.560	0.003	-0.324	0.932
Female beetles	0.482	0.009	0.123	0.289

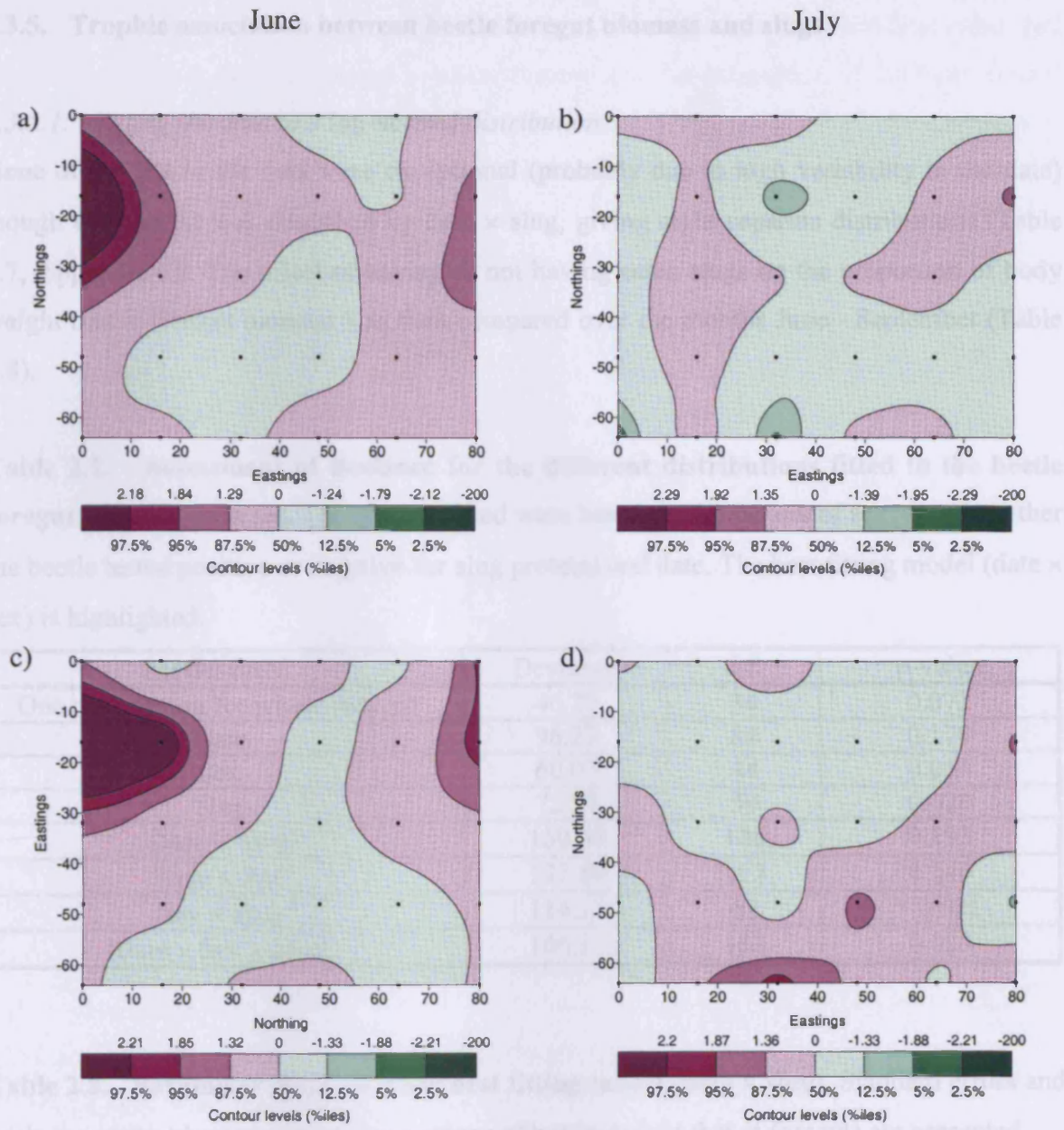

Figure 2.7. Plum-green contour plots showing spatial associations. Spatial association between male *Pterostichus melanarius* foregut weights and slugs in June (a) and July (b), and association between female *P. melanarius* foregut weights and slugs in June (c) and July (d) on a 64 m by 80 m grid in a field of winter wheat. Darkest colour represents areas where interpolated values of indices exceed 97.5 % confidence limits for association (plum) or dissociation (green). Posted symbols represent sample points.

2.3.5. Trophic association between beetle foregut biomass and slugs

2.3.5.1. Fitting the data to a log-normal distribution

None of the fits to the data were exceptional (probably due to high variability in the data) though the best fit was described by date × slug, giving eight separate distributions (Table 2.7, Appendix C). The effect of having or not having eaten slugs on the proportion of body weight that is foregut biomass was then compared over the months June - September (Table 2.8).

Table 2.7. Assessment of deviance for the different distributions fitted to the beetle foregut biomass data set. The models fitted were based on the factors of sex, slug (whether the beetle tested positive or negative for slug protein) and date. The best fitting model (date × sex) is highlighted.

Model fitted	Deviance	d.f.	p-value
One distribution for whole data set	46.70	34	0.072
Date	96.27	84	0.170
Sex	60.07	44	0.054
Slug	72.57	50	0.020
Date × Slug	**150.08**	**136**	**0.193**
Date × Sex	127.80	112	0.146
Sex × Slug	114.17	68	<0.001
Date × Sex × Slug	166.17	132	0.024

Table 2.8. Parameter details for the best fitting model (date × slug). Standard errors and back-transformed means (mean proportions of beetle weight that is foregut) are presented.

Date	Slugs eaten?	Mean	Standard error of mean	Variance	Back-transformed mean (g)
June	Negative	-2.872	0.028	0.565	0.062
	Positive	-2.680	0.051	0.658	0.069
July	Negative	-3.427	0.028	0.544	0.033
	Positive	-3.370	0.029	0.549	0.034
August	Negative	-3.781	0.135	0.687	0.023
	Positive	-3.647	0.118	0.737	0.026
September	Negative	-3.387	0.119	0.476	0.034
	Positive	-3.131	0.204	0.611	0.044

The proportion of beetle weight that is foregut is highest in June and September and lowest in July and August. Foregut biomass formed a higher proportion of the body weight of beetles that had tested positive than those that had tested negative for having eaten slugs in every month, though this difference was more prominent in June and September (Figure 2.8, 2.9, 2.10 and 2.11).

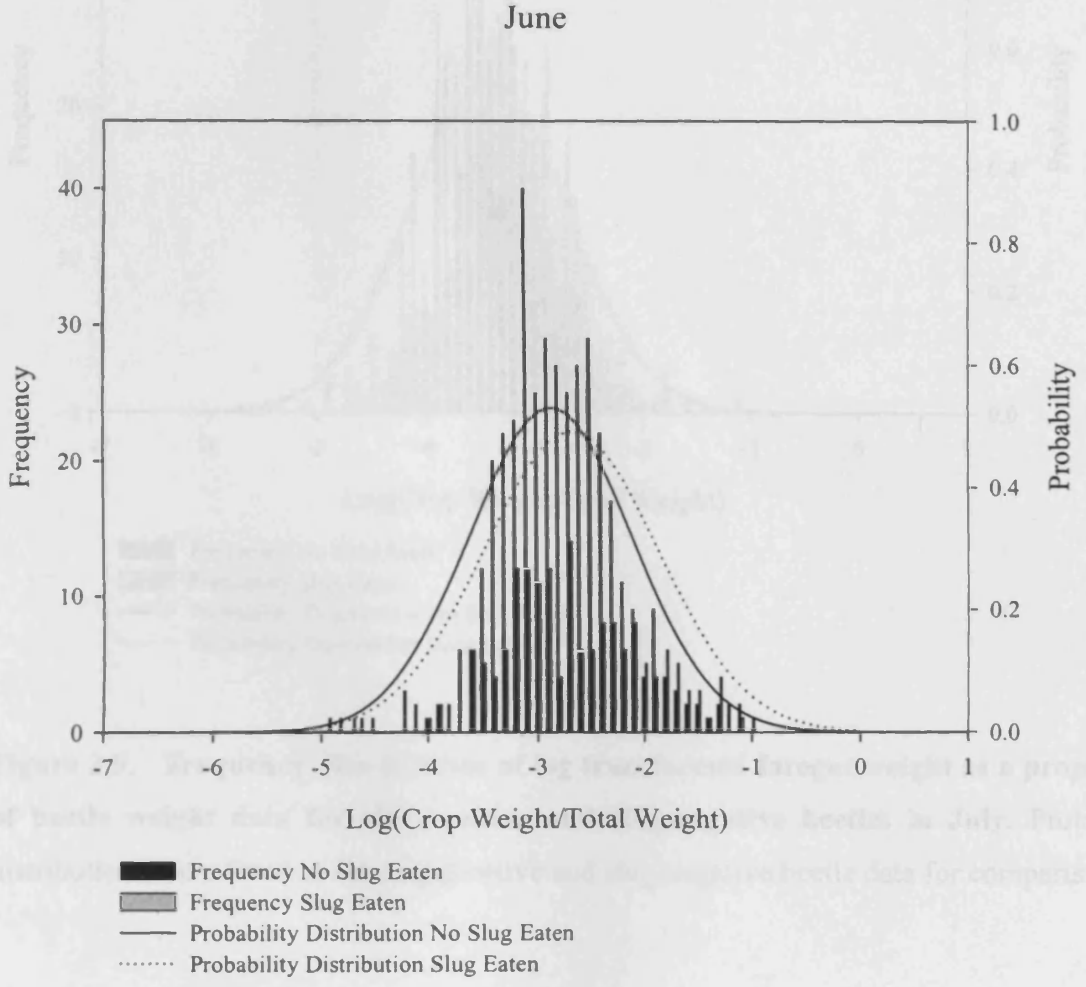

Figure 2.8. Frequency distributions of log transformed foregut weight as a proportion of beetle weight data for slug-positive and slug-negative beetles in June. Probability distributions were fitted to the slug-positive and slug-negative beetle data for comparison.

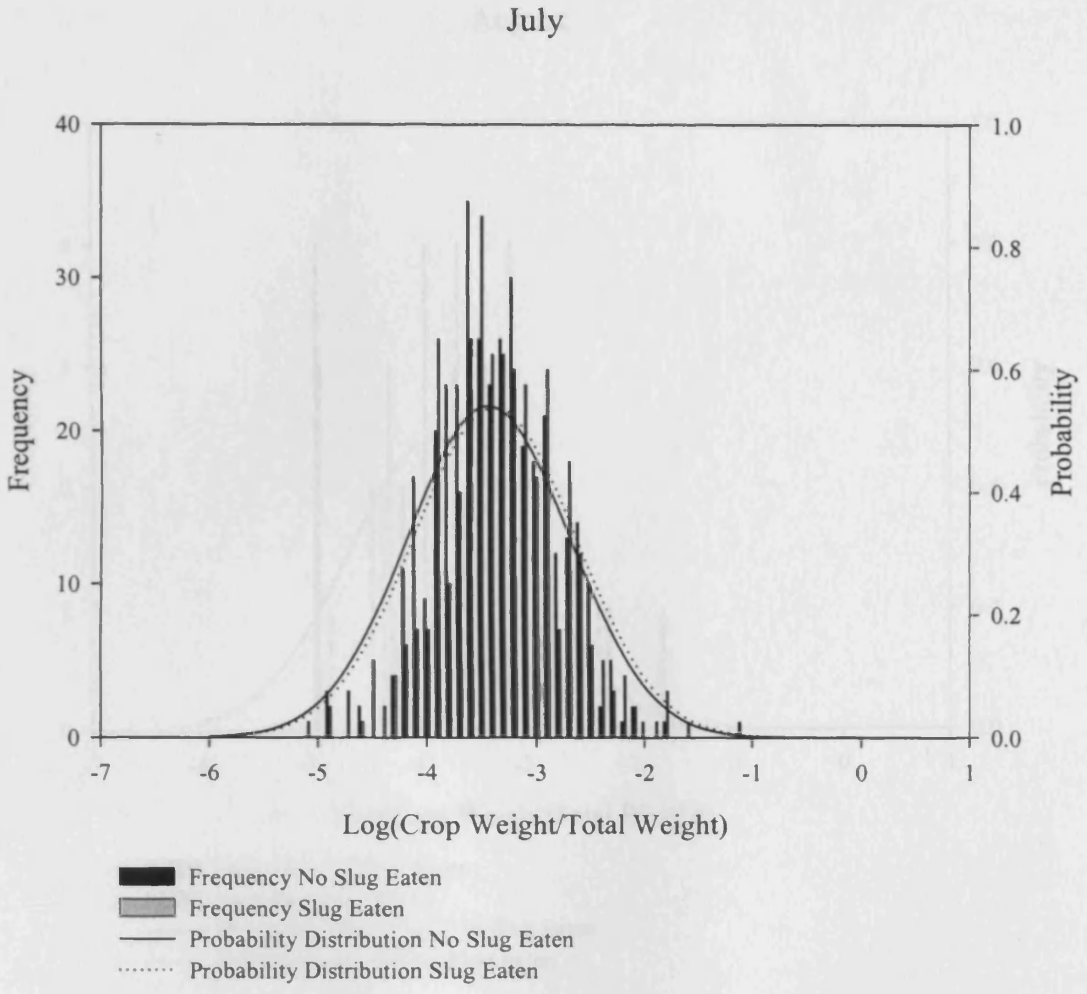

Figure 2.9. Frequency distributions of log transformed foregut weight as a proportion of beetle weight data for slug-positive and slug-negative beetles in July. Probability distributions were fitted to the slug-positive and slug-negative beetle data for comparison.

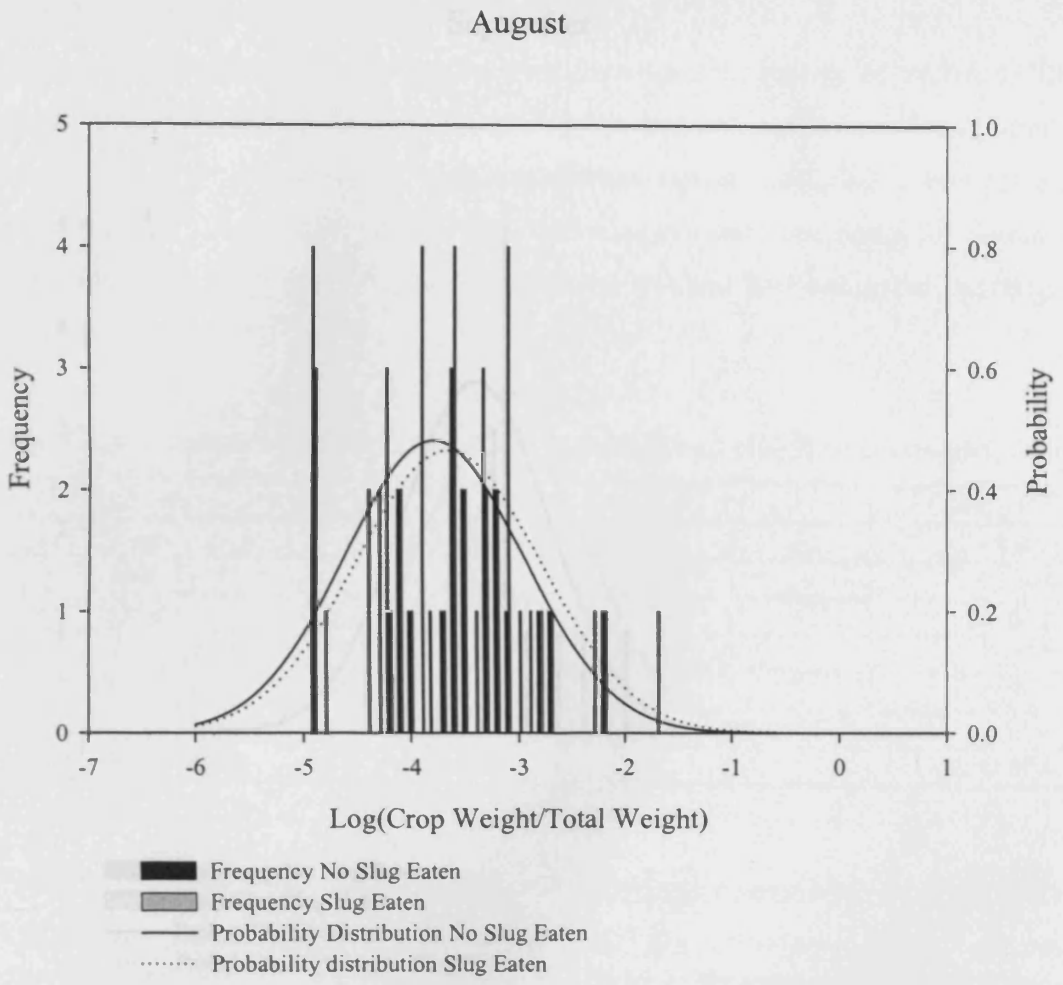

Figure 2.10. Frequency distributions of log transformed foregut weight as a proportion of beetle weight data for slug-positive and slug-negative beetles in August. Probability distributions were fitted to the slug-positive and slug-negative beetle data for comparison.

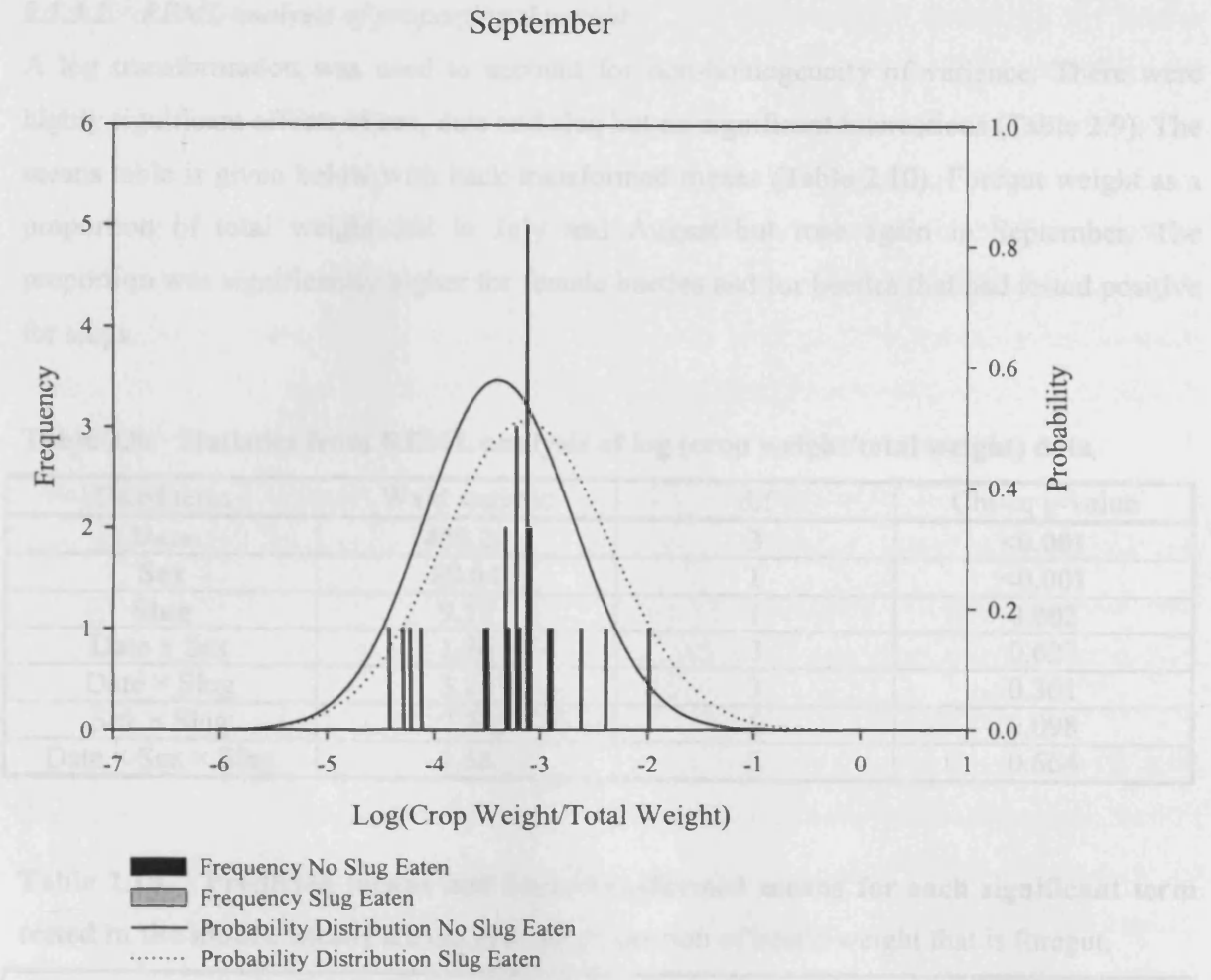

Figure 2.11. Frequency distributions of log transformed foregut weight as a proportion of beetle weight data for slug-positive and slug-negative beetles in September. Probability distributions were fitted to the slug-positive and slug-negative beetle data for comparison.

2.3.5.2. REML analysis of proportional weight

A log transformation was used to account for non-homogeneity of variance. There were highly significant effects of sex, date and slug but no significant interactions (Table 2.9). The means table is given below with back-transformed means (Table 2.10). Foregut weight as a proportion of total weight fell in July and August but rose again in September. The proportion was significantly higher for female beetles and for beetles that had tested positive for slugs.

Table 2.9. Statistics from REML analysis of log (crop weight/total weight) data

Fixed term	Wald statistic	d.f	Chi-sq p-value
Date	**403.28**	**3**	**<0.001**
Sex	**80.04**	**1**	**<0.001**
Slug	**9.17**	**1**	**0.002**
Date × Sex	1.74	3	0.627
Date × Slug	3.21	3	0.361
Sex × Slug	2.74	1	0.098
Date × Sex × Slug	1.58	3	0.664

Table 2.10. Predicted means and back-transformed means for each significant term tested in the model. Means are the average proportion of beetle weight that is foregut.

Factors		Mean	Back-transformed mean
Date	June	-2.785	0.062
	July	-3.413	0.033
	August	-3.620	0.027
	September	-3.337	0.036
Sex	Male	-3.430	0.032
	Female	-3.147	0.043
Slug eaten?	Negative	-3.388	0.032
	Positive	-3.189	0.041

2.4 Discussion

Beetles that had consumed slugs were significantly spatially associated only with intermediate-sized slugs in June and dissociated from these slugs in July. Therefore, in June, beetles may have been aggregating to areas where numbers of slugs were generally high (west of the field) but feeding preferentially on slugs 25 - 50 mg. Most of the intermediate

sized-slugs trapped in June were *A. intermedius*, but this could be caused by the beetles preferentially consuming intermediate-sized *D. reticulatum* before slug sampling and therefore there were fewer intermediate-sized *D. reticulatum* collected than *A. intermedius*.

The results from spatial analysis of the slug-positive carabids and slugs of different weights agree with Bohan *et al.*, (2000a), because beetles were associated with slugs greater than 25 mg. Further analysis has shown however that there is no relationship with the slugs > 50 mg. Slugs greater than 50 mg would be able to produce more mucus when attacked (Pakarinen, 1994a) and flee faster than small slugs, therefore would be less successfully preyed upon by beetles. Laboratory experiments show that smaller slugs are more readily taken by beetles than larger slugs (McKemey *et al.*, 2001; Oberholzer & Frank, 2003) but the results from this study and from semi-field work (McKemey *et al.*, 2003) do not support this for the situation in the field. Although the smallest slugs are more vulnerable if found, they are also less likely to be found in the first place. When neonate slugs were collected from mini-plots (Section 4.3.2. Chapter 4) two and three days after hatching, only 33 and 13 % of the recovered slugs were found on the soil surface respectively, and most of these were found under leaves, stones and straw. Larger slugs would be more easily detected on the soil surface than slugs < 25 mg, and therefore predation may be acting mainly on slugs in the region 25 – 50 mg. Experiments in other chapters take slug size into account as this appears to be an important factor in the prey choice of the beetles.

The distribution patterns for male and female beetles in June were very different, probably reflecting differences in activity density. Males are more active than females, especially earlier in the summer, which sometimes results in a higher probability of them falling into pitfall traps (Thomas *et al.*, 1998). Despite the apparently different patterns of spatial distribution, both sexes had similar patterns of spatial association with slugs in June and July. There appears to be no difference in the spatial relationship between male beetles and slugs and female beetles and slugs.

Female beetle foreguts were heavier on average than those of males in both months. Pollet & Desender (1985) also found that female foregut weights were heavier and fuller than males, and that female's guts contained remains from larger prey species. Previous studies have also shown that females' foreguts contain a greater percentage of solid food remains than males' (Sunderland, 1975; Chiverton, 1984). Female *P. melanarius* are larger than males and therefore may be able to tackle larger prey items. By analysing the proportion of the beetles' weight that is foregut biomass it was possible to discover that female beetles

had a higher proportion than males. Possibly females have to eat more food relative to their body size in order to gain enough energy for egg production.

Beetles with the heaviest foreguts were spatially associated with slug abundance in June (but not July); and in all months (but most noticeably in June) the foregut as a proportion of the total body weight was higher in beetles that had recently fed on slugs than those that had not. Beetles were also better fed in June as the proportion of the total body weight that was foregut biomass was approximately twice that of any other month. This supports the findings of Symondson *et al.* (2002a) who found that the gut weight of *P. melanarius* was positively related to slug numbers in the soil and that foregut mass was positively correlated with beetle population growth over five years. The difference in foregut biomass as a proportion of total body weight between slug-positive and slug-negative beetles tended to change over the course of the summer months, with there being larger differences in June and September than in July and August. In July and August there was little difference in the proportions between slug-positive and slug-negative beetles, indicating that beetles not feeding on slugs must have been eating other prey. It is therefore likely that in these months there were changes in prey availability, possibly fewer available slugs (due to beetle predation and it being hotter and drier) or other prey were now more available than they had been in June. This may explain why beetles were spatially associated with slugs in June but not in July. Possibly beetles reduced their feeding on slugs because slugs became less available and/or there was a greater diversity of other prey to choose from in these months and so they switched to feeding on alternative prey. Slugs are an important prey item for *P. melanarius* but it seems that they may have been more important in June and September than in July and August.

Chapter 3

Slug anti-predator behaviour - laboratory studies

3.1. Introduction

The detection and avoidance of predators is important because predation has severe and immediate consequences on fitness (Dicke & Grostal, 2001). Visual and mechanical detection of predators can be extremely risky but chemical detection may allow the prey to avoid areas of high predation risk without ever coming close to or into contact with the predator. Predators produce a variety of chemicals, such as for communicating with conspecifics or from excreta and faeces, which prey could use to avoid the predator. For organisms such as slugs, chemical cues may be important in anti-predator behaviour because visual input is limited and escape driven dispersion is a slow process relative to the speed of their predators. Although there are extensive reports in the literature about marine gastropod anti-predator behaviour (reviewed by Kats & Dill, 1998), usually mediated by chemicals diffusing in the water, it is not yet known how a terrestrial gastropod would detect and behave toward predators. Work described in this chapter tests the response of slugs to chemical cues from *P. melanarius* and other carabid beetles, the results are then related back to the spatial dynamics of the predation interaction in the field (Chapter 2).

The co-evolution in the responses of predators and their prey is an exciting area of research which has practical benefits for the development of biological control. *P. melanarius* is one of the most abundant carabids in arable land and an important predator of slugs (Symondson *et al.*, 1996, 2002a; Bohan *et al.*, 2000a). The detection of slugs by *P. melanarius* and the attraction of these beetles to slugs have been investigated under laboratory conditions using classical behaviour and electrophysiological approaches (McKemey *et al.*, 2004). Surprisingly little work has been done on predator avoidance behaviour by terrestrial molluscs. One might expect generalist predators, such as *P. melanarius,* to exert insufficient selection pressure on a single prey class (molluscs) for anti-predator responses to evolve in their prey. Evolution of behaviour might be expected if the prey is the preferred food for that species or forms a major constituent of the total available prey (Symondson *et al.*, 2002a). *P. melanarius* can affect the temporal and spatial dynamics of both aphids (Winder *et al.*, 2001, 2004) and slugs (Symondson *et al.*, 1996, 2002a; Bohan

et al., 2000a; Chapter 2). For example, changes in the spatial dynamics of the slug and beetle populations in the summer of 1997 were thought to depend on both attraction of *P. melanarius* to locales of high slug abundance and their predation of those slugs. Using a superset of the slug data presented in Bohan *et al.* (2000a), Bohan *et al.* (2000b) examined the parametric and spatial distributions of slugs at various spatial scales. The observed variance to mean ratio for slug numbers was found to decrease in the summer, when *P. melanarius* were present. It appeared that the presence of beetles, during June and July, significantly reduced the numbers of sample points that returned zero slug counts. Although it is conceivable that local slug reproduction, particularly within low abundance locales and predation within high abundance locales could reduce the variance of the slug samples, Bohan *et al.* (2000b) argued that these effects might stem from slug movements influenced by the presence of carabids. In essence the slugs might be adopting anti-predation avoidance behaviours such as seeking refuge or moving to areas of lower beetle density.

Avoidance/escape is the most commonly reported type of anti-predator behaviour (Kats & Dill 1998). Escape usually involves an increase in speed or overall activity to leave a high risk environment. For non-volatile chemical cues only the intensity of the stimulation can be measured and there is no directional information. Changes in kinesis behaviour are therefore the most likely response to this type of chemical stimulus (Fraenkal & Gunn 1940). Kinesis is defined as movement that is proportional to the intensity and is independent of the spatial properties of the stimulus (Gunn *et al.*, 1937). Responses usually take the form of a change in velocity (orthokinesis) or a change in the rate of turning (klinokinesis).

The experiments described in this chapter test the null hypothesis that slugs do not respond to residual carabid beetle chemicals, focussing mainly upon responses by *D. reticulatum* to *P. melanarius*. A classical choice experiment was set up to investigate avoidance by *A. intermedius* of paper that had been exposed to *P. melanarius*. Further experiments measured the effects of slug size, beetle sex, beetle density, chemical persistence (longevity) and slug age on avoidance behaviour by *D. reticulatum*. To measure responses of *D. reticulatum* to the beetle chemicals a video tracking program was used to measure quantitatively velocity and turning rates so that kinesis could be recorded accurately. The effect of beetle exposed soil on *D. reticulatum* feeding, sheltering and egg laying was examined in a further laboratory experiment. Finally a series of choice experiments were set-up to test the responses of *D. reticulatum* to paper exposed to three other medium/large carabid beetles present in the field: *P. madidus*, *P. cupreus* and *Harpalus affinis* (Schrank). *P. madidus* and *P. cupreus* are in the same genus as *P. melanarius*, are both abundant in

arable fields and have been shown to prey on slugs (Tod, 1973; Ayre & Port, 1996; Oberholzer & Frank, 2003). *P. cupreus* is in a different sub-genus to *P. melanarius* and *P. madidus*, and so is not as closely related. *H. affinis* is also abundant in arable fields but is phytophagous and does not eat slugs. The purpose of these choice tests was to find out if the response to *P. melanarius* was species specific or if the active chemicals are produced by other carabids present in the field, including those that do not eat slugs (see also Appendix A - Armsworth *et al.*, 2005).

3.2. Methods

Slugs and carabid beetles were collected from grassland and wheat fields around Rothamsted Research station, Harpenden, U.K. Slugs were collected using baited cover traps and beetles were collected using dry pitfall traps.

A. intermedius and *D. reticulatum* were stored in groups of 20 in boxes similar to the test arenas, lined with moistened cotton wool. The slugs were fed on Chinese cabbage and maintained in a dark incubator at 10 °C (± 2 °C) for up to a week prior to each experiment. This low temperature inhibits the spread of disease among individuals and reduces the mortality rate of stored slugs (personal observation), whilst a higher temperature of 15 °C was selected for running the choice experiments because this is closer to the optimum level for growth and activity (South, 1992).

Dry pitfall traps were used to collect live *P. melanarius* during July and August. The beetles were sexed, stored separately in groups of 20 and placed in Perspex boxes similar to the test arenas, containing 5 cm of moist peat. They were fed on Hilife Complete Moist Menus dog food. The boxes were stored in the dark at 10 °C (± 2 °C) for up to a month before the bioassays, and up to three months before the slug video-tracking experiment. Live *P. madidus*, *P. cupreus* and *H. affinis* were collected from dry pitfall traps in April and May the following year. Again the beetles were sexed and stored under the same conditions as *P. melanarius*.

3.2.1. Choice test bioassays

One experiment was done to test the responses of *A. intermedius* to *P. melanarius* residual chemicals and five to test the responses of *D. reticulatum* on paper previously exposed to *P.*

melanarius within arenas. A seventh experiment was designed to test the responses of *D. reticulatum* to paper previously exposed to *P. madidus*, *P. cupreus* and *H. affinis*. In all of the choice tests except choice test six (juvenile *D. reticulatum* bioassay) arenas were constructed from clear Perspex boxes (26 × 14 cm and 9 cm high). Each arena wall was painted with FLUON (polytetrafluoroethylene – Whitford Plastics) to ensure all slug movements were confined to the arena floor (Symondson, 1993a). The bases of the arenas were lined with a thin layer of cotton wool moistened with tap water. Beetle exposed and control paper could then be laid over the cotton wool and be kept uniformly moist throughout the experiments. Beetle exposed papers were created by covering the bases of similar Perspex boxes with two sheets of moistened filter paper (14 × 13 cm) placed side-by-side. A specified number of beetles were then introduced to these boxes, the lids closed, and the beetles allowed to walk over the paper for a treatment-dependent time period in a dark incubator at 15 °C (± 2 °C). After this period the beetles were removed and, for testing, the sheets were randomly assigned to one half of a test arena. The other half of each arena was then covered with a control of plain moistened filter paper that had not been exposed to beetles. For the *D. reticulatum* tests, six slugs were added to each arena, three randomly assigned to each piece of paper, and the lids closed. For the *A. intermedius* test, due to a scarcity of slugs, only two slugs were added to each arena, one on each piece of paper. For all tests, the arenas were numbered and stored in a dark incubator at 15 °C (± 2 °C). Half of the arenas were arranged with the control paper on the right and half with the control paper on the left. The numbers of slugs on the beetle exposed and control paper were then recorded at 1, 6 and 24 h. This basic design was used for choice experiments 1 - 5 and 7.

In choice test six, arenas were constructed from Petri dishes (9 cm diameter). The sides and undersides of the lids were painted with FLUON to prevent juvenile slugs leaving the arena floor. The bases of the arenas were lined with a thin layer of tap-water moistened cotton wool. Beetle exposed papers were created by covering the bases of similar Petri dishes with a moistened circular filter paper sheet (9 cm diameter) cut in half down the middle. A single male *P. melanarius* was added to each dish, the lids closed, and the dishes were then stored in a dark incubator at 15 °C (± 2 °C) for 24 h. After this period, the beetles were removed. Each sheet of beetle exposed paper was randomly assigned to one half of a test arena. The other half of each arena was then covered with a control of plain moistened filter paper that had not been exposed to beetles. Six juvenile slugs were added to each arena, three randomly assigned to each piece of paper, and the lids closed. The arenas were numbered and stored in a dark incubator at 15 °C (± 2 °C). Half of the arenas were arranged with the control

paper on the right and half with the control paper on the left. The number of slugs on the beetle exposed and control paper was then recorded at 1, 6 and 24 h.

3.2.1.1. Choice test one - Arion intermedius bioassay

Eight arenas were set up for this test. Three male and three female *P. melanarius* were stored in each box containing test paper (beetle exposed) for 24 h before the experiment. Sixteen adult *A. intermedius* were collected (> 200 mg) and two slugs were randomly assigned to each arena, one in the centre of each piece of paper.

3.2.1.2. Choice test two - slug size bioassay

Three male and three female *P. melanarius* were stored in each box containing test paper (beetle exposed) for 24 h before the experiment. Sixty *D. reticulatum* were selected, 20 from each of three size categories: small (30 - 100 mg), intermediate (100 - 200 mg) and large (200 - 500 mg). The small class represents pre-sexual slugs, the intermediate class represents slugs reaching sexual maturity and the large class represents sexually mature adults. Ten replicate arenas were set-up and each contained two slugs from each size category. One slug of each size group was placed in the centre of control paper and beetle exposed paper.

3.2.1.3. Choice test three - beetle sex bioassay

To test the effect of beetle sex on *D. reticulatum* behaviour, 16 arenas were setup with eight replicates for each beetle sex. Pairs of test papers (beetle exposed) were stored with either six female beetles or six male beetles for 24 h before the choice experiment. Slugs collected for this experiment weighed 200-800 mg and six slugs were randomly assigned to each arena, three in the centre of each piece of paper.

3.2.1.4. Choice test four - beetle density bioassay

This experiment was designed to test whether *D. reticulatum* were sensitive to the density of beetles and the length of time that paper had been exposed to the beetles. In this experiment a lower density of beetles was stored on the test paper (beetle exposed) compared to the previous two bioassays, and the paper was exposed for only 1 h instead of 24 h. Sixteen arenas were set-up for this bioassay, with eight replicates for each beetle sex. Pairs of test papers (beetle exposed) were stored with either two female or two male beetles for 1 h before the start of the bioassay. Slugs collected for this bioassay weighed 200-800 mg and six slugs, randomly selected, were placed in each arena, three in the centre of each piece of paper.

3.2.1.5. Choice test five - chemical persistence bioassay

Pairs of test papers (beetle exposed) were stored with either six female or six male beetles for 24 h. Beetles were then removed and the exposed papers were stored for a further five days before being placed in the arenas for the start of the choice experiment. *D. reticulatum* collected for this experiment weighed 200-800 mg and six slugs, randomly selected, were placed in each arena, three in the centre of each piece of paper. There were eight replicate arenas for each beetle sex.

3.2.1.6. Choice test six - juvenile slug bioassay

Nine replicate Petri dish arenas were set-up for this choice test. One male *P. melanarius* was stored with each pair of test papers (beetle exposed) in Petri dishes for 24 h before the experiment. Juvenile *D. reticulatum* were between 5 days - 2 months old, and all weighed < 20 mg. Six slugs were randomly selected and placed in each arena, three on each piece of paper.

3.2.1.7. Choice test seven - Pterostichus madidus, Pterostichus cupreus and Harpalus affinis bioassay

Ten replicate arenas were created for each of the beetles species tested. For each species, three male and three female beetles were stored with pairs of test papers (beetle exposed) for 24 h before the experiment. *D. reticulatum* collected for this experiment weighed 200-800 mg and six slugs, randomly selected, were placed in each arena, three in the centre of each piece of paper.

3.2.1.8. Statistical analysis of choice tests

For analysis the proportion of slugs on the control side, at each time interval and for each choice test was modelled using generalized linear models assuming a binomial distribution (McCullagh & Nelder, 1989). A constant term was fitted for the mean proportion of slugs on the control side. The standard error for this constant term was then used to test whether the mean proportion was greater than 0.5 using a one-tailed t-test. To check whether slugs were orienting to a particular side (left or right) regardless of treatment, a factor for arena orientation was tested in each model. In choice experiment two there were three size groups per arena, so the effect of arena was included as a blocking factor. In choice experiment three and four, beetle sex was fitted as a factor to see if there was a significant effect. Over-dispersion (see, for example, Collett, 1991) of the data, given that expected for a proposed

binomial distribution, was accounted for where necessary by setting the dispersion parameter to the residual mean deviance rather than assuming unity in the calculation of standard errors. In these analyses, given equal binomial totals, the result of this is to scale the standard errors of estimated parameters (proportions) by the square root of the residual mean deviance. All analyses were run using the GenStat (2003) (GenStat 7th ed. - VSN International Ltd.) statistical package.

3.2.2. Video tracking of slug movements

Pairs of arenas were filmed simultaneously over a video tracking period of 12 h. The beetle exposed papers had been stored in the dark with either six male or six female beetles for 24 h at 15 °C (± 2 °C) before each recording. The arenas were arranged on a bench in a controlled environment room (18 ± 1°C) under a video camera so that one arena had the control paper on the left and the other arena had the control paper on the right (Figure 3.1). The room was illuminated with a red strip light with an additional desk lamp fitted with a red bulb mounted directly overhead to provide uniform lighting over the arena floors. A monochrome camera was suspended overhead and this was connected to a VCR set in time lapse mode to record 12 h of footage on 30 mins of a 3 h tape when played back in normal mode.

One slug (400 - 800 mg) was transferred using a paintbrush and placed directly in the centre of each arena, along the divide between the two pieces of paper. Ten recordings were made (of pairs of arenas), each of 12 h duration, five for male beetles and five for females, giving ten replicates for each sex of beetle. Each slug and each piece of control and beetle exposed paper was only used once and arenas were washed thoroughly after each use.

The video data were analysed using a video tracking system, Noldus™ EthoVision Pro (Noldus Information Technology) (Noldus *et al.*, 2002) to calculate parameters for the movement and activity of the slugs. For each recording both the two test arenas and "zones" for the control and beetle exposed papers, within each arena, were defined on the computer screen. A frame grabber digitised the video image, converting each frame into a grid of pixels. The slug pixels were identified against the white contrasting background. The digitised images were sampled by the program at a rate of five per time-lapse second (this is equivalent to a real time sampling rate of once every 4.8 s over the 12 h period of filming). In each sample image the co-ordinate of the slug was recorded and each position was then marked on the screen with lines connecting successive points to produce the track to be analysed.

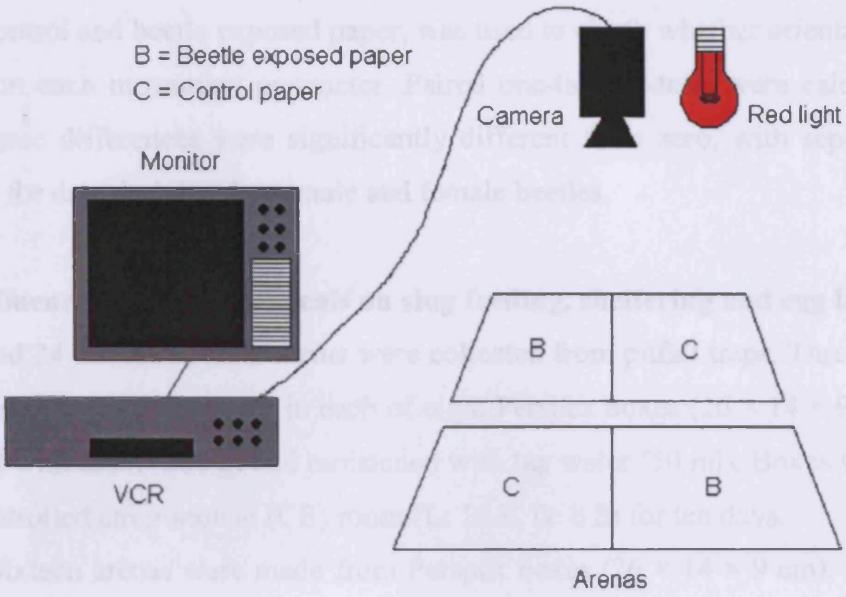

Figure 3.1. Experimental design for *Deroceras reticulatum* video tracking study. Video footage was recorded of slugs moving over beetle exposed and control zones within arenas. Two arenas were filmed simultaneously.

The movement parameters selected for analysis were the time spent in the zone, distance moved, mean velocity, mean absolute angular velocity, mean absolute meander and time spent moving. By nesting the data by zones it was possible to compare the values of each parameter on control and beetle exposed paper. Time spent in zone was calculated as the total time (s) that a slug spent in each zone. Distance moved was the total length of the tracks (cm) within each zone. Mean velocity was the average speed of slugs in each zone (cm/s). Mean angular velocity (°/s) was the average change in direction of the slug relative to time in each zone. Mean meander was the average change in direction of slugs in each zone relative to the distance moved (°/cm). The time spent moving was the total time spent moving in each zone and included any time when the slug moved more than 0.2 cm between image samples. This threshold was set to avoid slight movements caused by noise or body wobble being interpreted by the programme as movement. The time spent moving by slugs in each zone was then divided by the time spent in each zone to get the proportion of time in each zone that was spent moving. Any parameters involving time were recalculated to incorporate the time lapse factor.

One-way analysis of variance (ANOVA), applied to the data for the differences between control and beetle exposed paper, was used to check whether orientation was having an effect on each movement parameter. Paired one-tailed t-tests were calculated to assess whether these differences were significantly different from zero, with separate tests being performed for data deriving from male and female beetles.

3.2.3. Influence of beetle chemicals on slug feeding, sheltering and egg laying

24 male and 24 female *P. melanarius* were collected from pitfall traps. Three male and three female beetles were each stored in each of eight Perspex boxes (26 × 14 × 9 cm). The boxes were filled with loam (200 g) and moistened with tap water (50 ml). Boxes were stored at 18 °C in a controlled environment (CE) room (L: 16 h, D: 8 h) for ten days.

Sixteen arenas were made from Perspex boxes (26 × 14 × 9 cm). Soil from one of the beetle boxes (with beetles removed) was poured in to each of eight arenas and levelled. To the remaining eight arenas (control) fresh loam (200 g), moistened with tap water (50 g) was added. Twenty Chinese cabbage leaf discs (diameter 11 mm) were added to the centre of each arena in a five by seven open rectangle (Figure 3.2). Four slugs were then added to the centre of each arena and the lids replaced. The arenas were stored at 10 °C in a CE room (L: 16 h, D: 8 h) for seven days. The number of leaf discs eaten or partially eaten was recorded every day and all discs were replaced with fresh ones. The number of slugs on the soil or on the arena sides/lid on each day was recorded, as well as any deaths that had occurred. At the end of the trial the slugs were removed and the numbers of eggs and batches that had been laid in each arena were recorded.

For analysis, mortality of slugs in arenas had to be taken into account. The number of leaf discs eaten or partially eaten per live slug on each day was calculated for each arena. The percentage of live slugs in each arena on each day that were on or in contact with the soil was also calculated. Since repeated measures were used (by sampling the same arenas on consecutive days) the data were analysed with split-plot in time ANOVAs. For analysis of egg laying the number of eggs laid per live slug per day in each arena was calculated. A two-tailed t-test was used to compare the means of the eggs per slug per day values between control and beetle exposed arenas. An F-test was used to check for homogeneity of variance between the two sets of data.

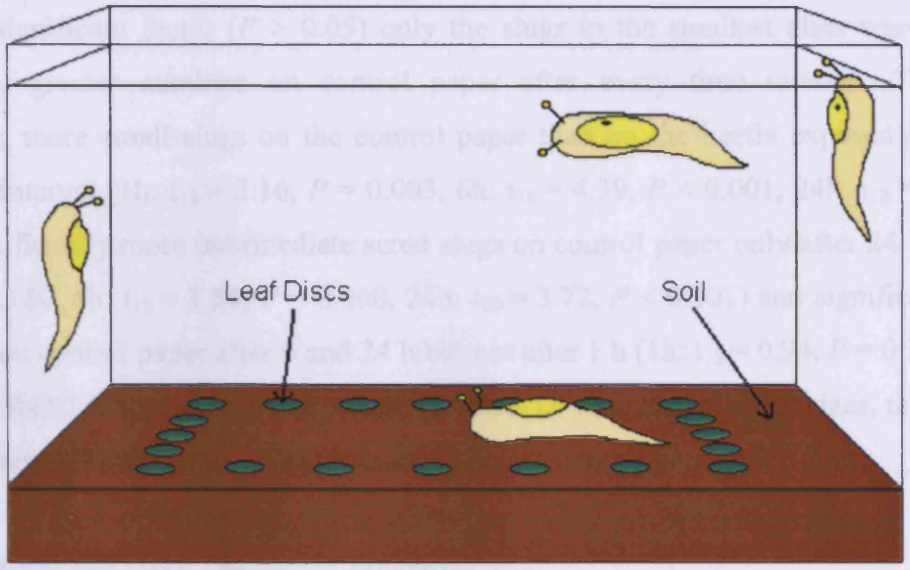

Figure 3.2. Arena design for testing the effects of *Pterostichus melanarius* exposed soil on feeding, sheltering and egg laying behaviour of *Deroceras reticulatum*.

3.3. Results

3.3.1. Choice test bioassays

The proportional data for the effect of arena orientation from each choice experiment after any interval were non-significant for all experiments ($P > 0.05$).

3.3.1.1. Choice test one - Arion intermedius bioassay

There was no significant difference in the number of slugs on the control or the beetle exposed paper after 1, 6 or 24 h (1h: $t_7 = 0.00$, $P = 0.500$; 6h: $t_7 = 0.86$, $P = 0.209$; 24h: $t_7 = 0.36$, $P = 0.365$).

3.3.1.2. Choice test two - slug size bioassay

When arena was fitted as a factor in the model it was significant after 1 and 6 h (Chi-square test: $X^2_9 = 19.74$, $P = 0.020$ and $X^2_9 = 17.80$, $P = 0.038$ respectively) and non-significant after 24 h (Chi-square test: $X^2_9 = 14.59$, $P = 0.103$). Arena was therefore maintained as a blocking factor in each model. When fitting this model to the data there was evidence of over

dispersion after 1 and 6 h, so the standard errors were adjusted accordingly. Although size was not a significant factor ($P > 0.05$) only the slugs in the smallest class were found in significantly greater numbers on control paper after every time interval. There were significantly more small slugs on the control paper than on the beetle exposed paper after every time interval (1h: $t_{18} = 3.16$, $P = 0.003$, 6h: $t_{18} = 4.39$, $P < 0.001$, 24h: $t_{18} = 3.72$, $P < 0.001$), significantly more intermediate sized slugs on control paper only after 24 h (1h: $t_{18} = 0.94$, $P = 0.180$, 6h: $t_{18} = 1.56$, $P = 0.068$, 24h: $t_{18} = 3.72$, $P < 0.001$) and significantly more large slugs on control paper after 6 and 24 h but not after 1 h (1h: $t_{18} = 0.94$, $P = 0.180$, 6h: $t_{18} = 2.72$, $P = 0.007$, 24h: $t_{18} = 2.94$, $P = 0.004$). Thus, *D. reticulatum* of all sizes, moved away from the beetle exposed paper and accumulated on the control paper.

3.3.1.3. Choice test three - beetle sex bioassay

When beetle sex was fitted as a factor to each model it proved to be non-significant at each time interval ($P > 0.368$). The model fit showed that there were significantly more slugs on the control paper than on the beetle exposed paper at each time interval (1h: $t_{15} = 3.92$, $P < 0.001$, 6h: $t_{15} = 4.30$, $P < 0.001$, 24h: $t_{15} = 2.35$, $P = 0.016$). Both male and female beetle exposed paper was avoided by slugs, and the effect persisted for at least 24 h.

3.3.1.4. Choice test four - beetle density bioassay

The effect of beetle sex was non-significant at each time interval ($P > 0.255$). There was no significant difference in the number of slugs on the control or the beetle exposed paper after 1 h ($t_{15} = 1.44$, $P = 0.085$) but significantly more slugs on the control paper after 6 h ($t_{15} = 2.09$, $P = 0.027$) and 24 h ($t_{15} = 3.66$, $P = 0.001$).

3.3.1.5. Choice test five - chemical persistence bioassay

As in the previous experiment, the effect of beetle sex was non-significant at each time interval ($P > 0.213$). There was no significant difference in the number of slugs on the control or the beetle exposed paper after 1, 6 or 24 h (1h: $t_{15} = 0.96$, $P = 0.176$, 6h: $t_{15} = 0.69$, $P = 0.252$, 24h: $t_{15} = 1.23$, $P = 0.119$). Thus any chemical cues left on the paper had faded after 5 days so that slugs no longer detected or responded to them.

3.3.1.6. Choice test six - juvenile slug bioassay

At each time period there were more slugs on the control paper than on the beetle exposed paper but because of high variability (due to the effect of arena) none of these differences

were significant (1h: $t_{15} = 0.28$, $P = 0.393$, 6h: $t_{15} = 0.31$, $P = 0.383$, 24h: $t_{15} = 0.90$, $P = 0.198$).

3.3.1.7. Choice test seven - Pterostichus madidus, Pterostichus cupreus and Harpalus affinis bioassay

There were significantly more slugs on test paper exposed to *P. cupreus* than on control paper after 1 h ($t_9 = -2.143$, $P = 0.030$) but no significant difference in the number of slugs on each paper after 6 h ($t_9 = -0.673$, $P = 0.259$) and 24 h ($t_9 = 0.1904$, $P = 0.425$). Slugs did not avoid paper exposed to *P. cupreus*.

There was no significant differences in the number of slugs on control paper and paper exposed to *H. affinis* after 1, 6 or 24 h (1h: $t_9 = 0.710$, $P = 0.248$, 6h: $t_9 = 1.073$, $P = 0.156$, 24h: $t_9 = 1.195$, $P = 0.131$). Slugs did not avoid paper exposed to *H. affinis*.

Unlike *H. affinis* and *P. cupreus*, paper exposed to *P. madidus* was avoided by the slugs. Significantly more slugs were counted on control paper than paper exposed to *P. madidus* at every time interval (1h: $t_9 = 1.933$, $P = 0.043$, 6h: $t_9 = 3.480$, $P = 0.003$, 24h: $t_9 = 2.435$, $P = 0.018$).

3.3.2. Video tracking of slug movements

On visual examination of the all the slug tracks (see example in Figure 3.3) there appeared to be little or no difference in the distance moved by slugs on either control or beetle exposed paper, however video tracking analyses indicated that there were significant differences in slug movement parameters in the presence of male beetle exposed paper, but not female (Figures 3.4 and 3.5). Arena orientation had no effect on any of the movement parameters ($P > 0.05$)

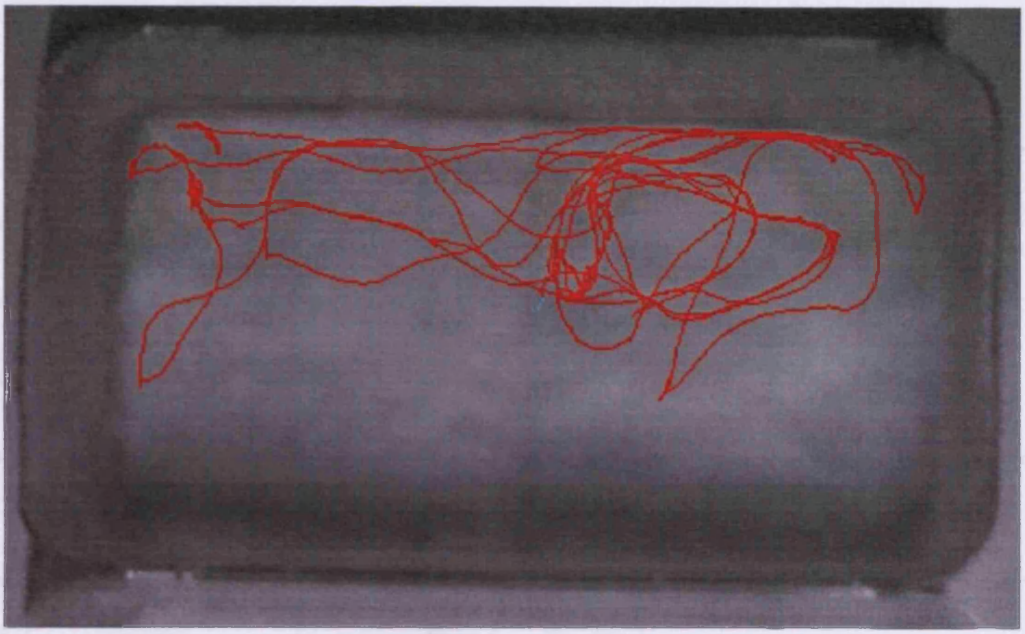

Figure 3.3. Example of a *Deroceras reticulatum* track in an arena. Control paper is on the right and paper exposed to male *Pterostichus melanarius* is on the left. The track is highlighted in red and the starting point of the slug is marked by a blue portion of the track.

Slugs spent significantly more time on control paper than on male beetle exposed paper (*t* test: $t_9 = 2.20$, $P = 0.027$) but the difference was not significant when test paper was exposed to female beetles (*t* test: $t_9 = 0.47$, $P = 0.326$) (Figure 3.4.a and 3.4.b). There was no significant difference between the distance moved by slugs on control and beetle exposed paper when paper had been exposed to either males (*t* test: $t_9 = 0.10$, $P = 0.462$) or females (*t* test: $t_9 = 0.11$, $P = 0.542$) (figure 3.4.c and 3.4.d). Slugs moved significantly faster on male beetle exposed paper than on control paper (*t* test: $t_9 = 3.34$, $P = 0.004$) but there was no difference in velocity on control paper and female beetle exposed paper (*t* test: $t_9 = 0.56$, $P = 0.295$) (Figure 3.4.e and 3.4.f). The proportion of time that slugs spent moving was significantly greater on male beetle exposed paper than on control paper (*t* test: $t_9 = 2.08$, $P = 0.034$) but this difference was not significant for slugs on control and female beetle exposed paper (*t* test: $t_9 = 0.76$, $P = 0.233$) (Figure 3.5.a and 3.5.b). Angular velocity of slugs was significantly greater on control paper than on male beetle exposed paper (*t* test: $t_9 = 2.73$, $P = 0.012$) but no differences in this parameter were detected when test paper had been exposed to female beetles (*t* test: $t_9 = 0.67$, $P = 0.260$) (Figure 3.5.c and 3.5.d). Finally, slugs meandered significantly more on control paper than on male beetle exposed paper (*t* test: $t_9 = 2.23$, $P = 0.026$) but there was no difference in meander on control and female beetle exposed paper (*t* test: $t_9 = 0.65$, $P = 0.265$) (Figure 3.5.e and 3.5.f).

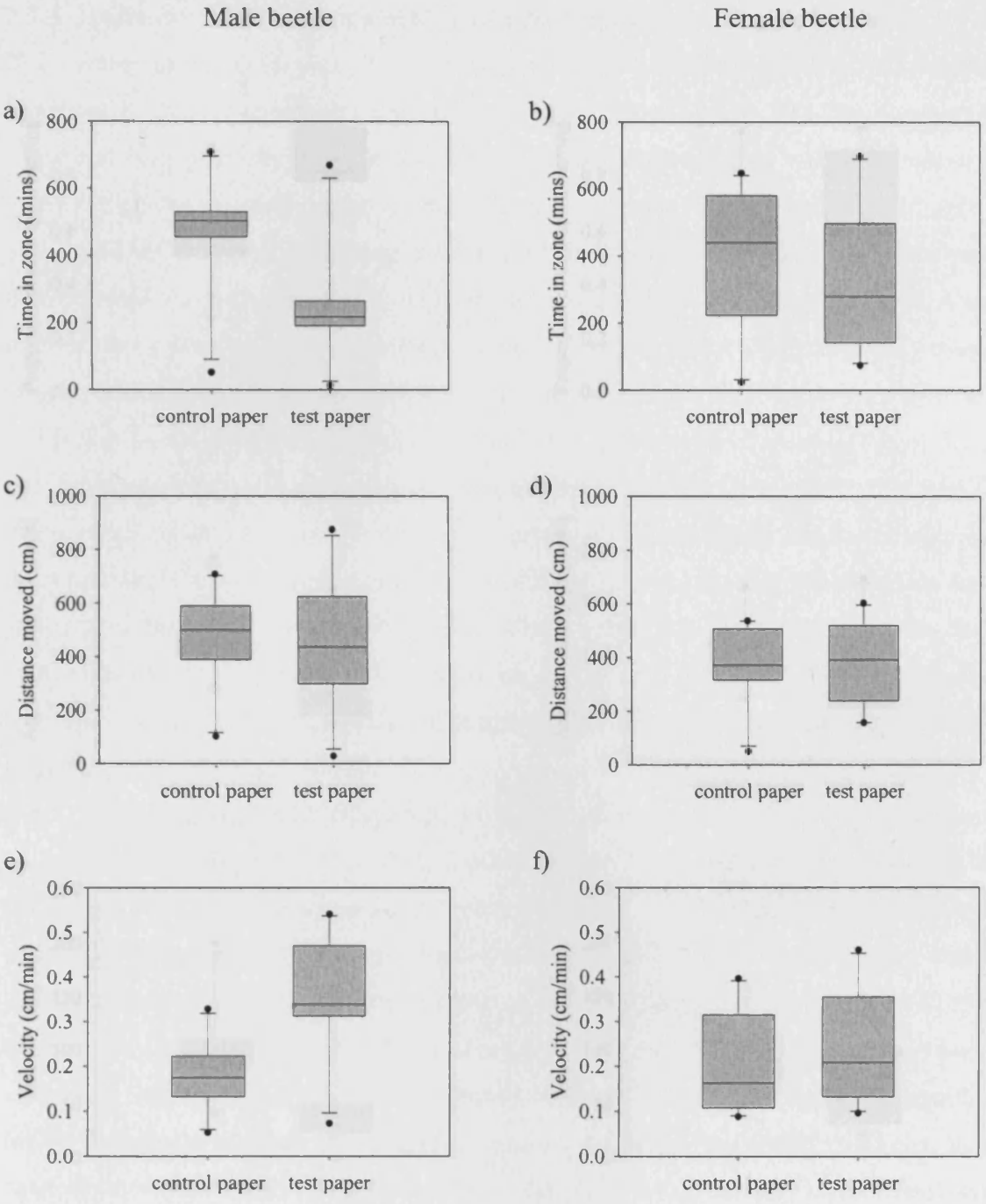

Figure 3.4. Boxplots for slug movement parameters. Time in zone (a, b), distance moved (c, d) and mean velocity (e, f) for slugs in arenas with male beetle exposed paper and control paper; and for female beetle exposed paper and control paper respectively. The median is indicated by the horizontal bar, the 1st and 3rd quartile by the box 1.5 times the interquartile range is represented by the bars and the outliers are represented by closed circles.

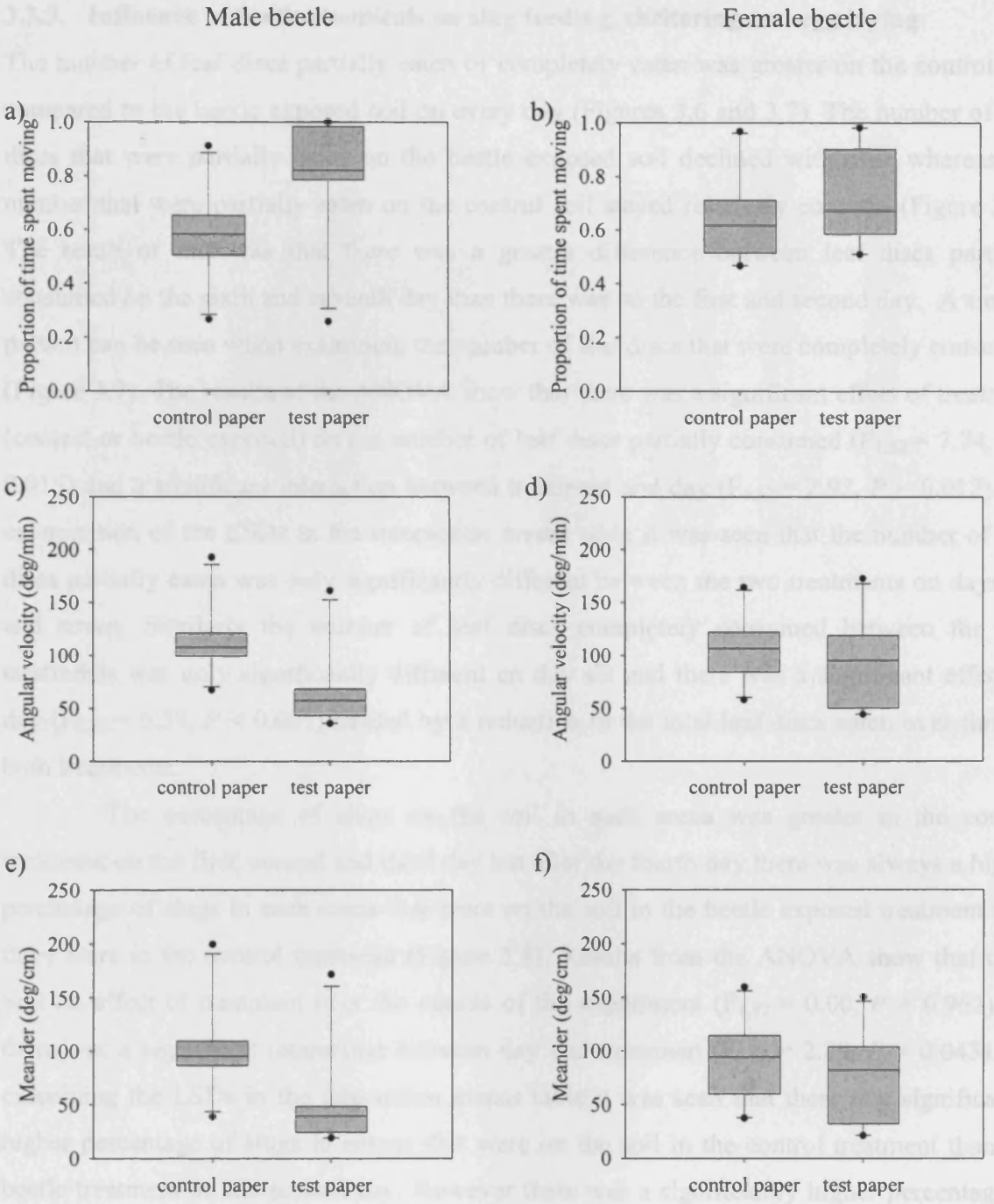

Figure 3.5. Boxplots for slug movement parameters. Proportion of time spent moving (a, b), mean absolute angular velocity (c, d) and mean absolute meander (e, f) for slugs in arenas with male beetle exposed paper and control paper; and for female beetle exposed paper and control paper respectively. The median is indicated by the horizontal bar, the 1st and 3rd quartile by the box, 1.5 times the interquartile range is represented by the bars and the outliers are represented by closed circles.

3.3.3. Influence of beetle chemicals on slug feeding, sheltering and egg laying

The number of leaf discs partially eaten or completely eaten was greater on the control soil compared to the beetle exposed soil on every day (Figures 3.6 and 3.7). The number of leaf discs that were partially eaten on the beetle exposed soil declined with time whereas the number that were partially eaten on the control soil stayed relatively constant (Figure 3.6). The result of this was that there was a greater difference between leaf discs partially consumed on the sixth and seventh day than there was on the first and second day. A similar pattern can be seen when examining the number of leaf discs that were completely consumed (Figure 3.7). The results of the ANOVA show that there was a significant effect of treatment (control or beetle exposed) on the number of leaf discs partially consumed ($F_{1,82} = 7.74$, $P = 0.015$) and a significant interaction between treatment and day ($F_{6,82} = 2.92$, $P = 0.012$). On examination of the LSDs in the interaction means table it was seen that the number of leaf discs partially eaten was only significantly different between the two treatments on days six and seven. Similarly the number of leaf discs completely consumed between the two treatments was only significantly different on day six and there was a significant effect of day ($F_{6,82} = 6.39$, $P < 0.001$) caused by a reduction in the total leaf discs eaten over time in both treatments.

The percentage of slugs on the soil in each arena was greater in the control treatment on the first, second and third day but after the fourth day there was always a higher percentage of slugs in each arena that were on the soil in the beetle exposed treatment than there were in the control treatment (Figure 3.8). Results from the ANOVA show that there was no effect of treatment over the course of the experiment ($F_{1,82} = 0.00$, $P = 0.962$) but there was a significant interaction between day and treatment ($F_{6,82} = 2.29$, $P = 0.043$). By examining the LSDs in the interaction means table it was seen that there is a significantly higher percentage of slugs in arenas that were on the soil in the control treatment than the beetle treatment on the second day. However there was a significantly higher percentage of slugs that were on the soil in the beetle exposed treatment than the control treatment on the seventh day.

The mean number of eggs laid per live slug per day was about four times greater on the control soil than on the beetle exposed soil (Figure 3.9). A t-test revealed that this difference was significant (t test: $t_{13} = 3.40$, $P = 0.005$).

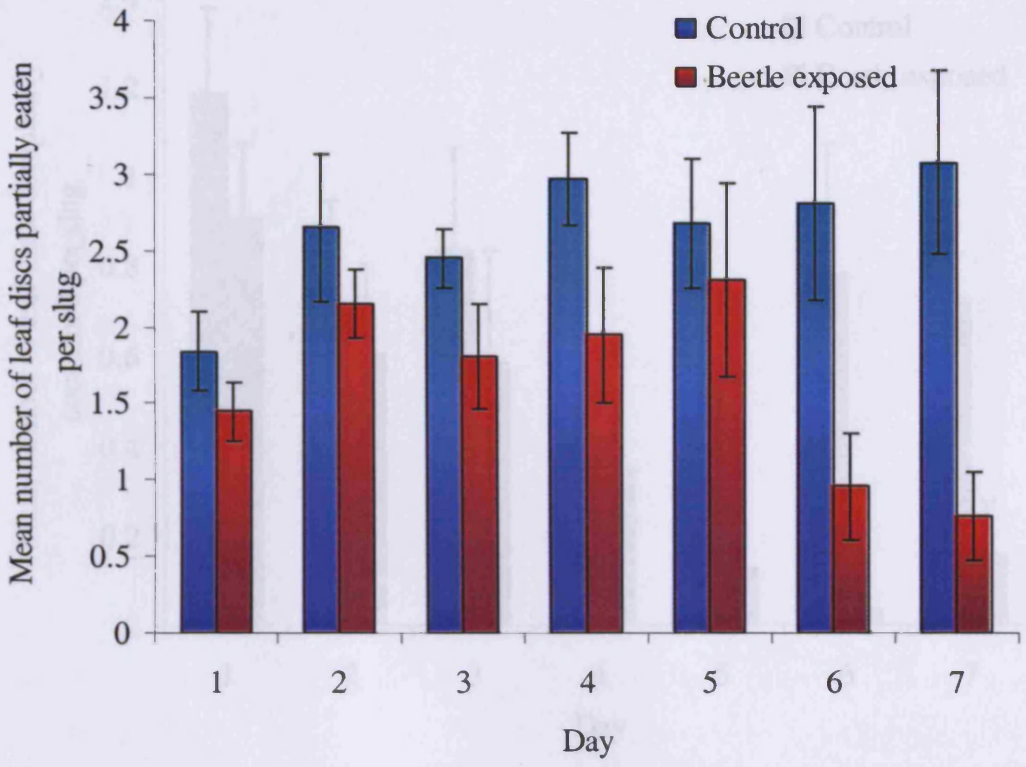

Figure 3.6. Bar graph showing the mean number of leaf discs partially eaten per *Deroceras reticulatum* on each day. A comparison of the number of leaf discs partially eaten by each slug on either soil exposed to *Pterostichus melanarius* or on control soil. Closed bars = standard errors.

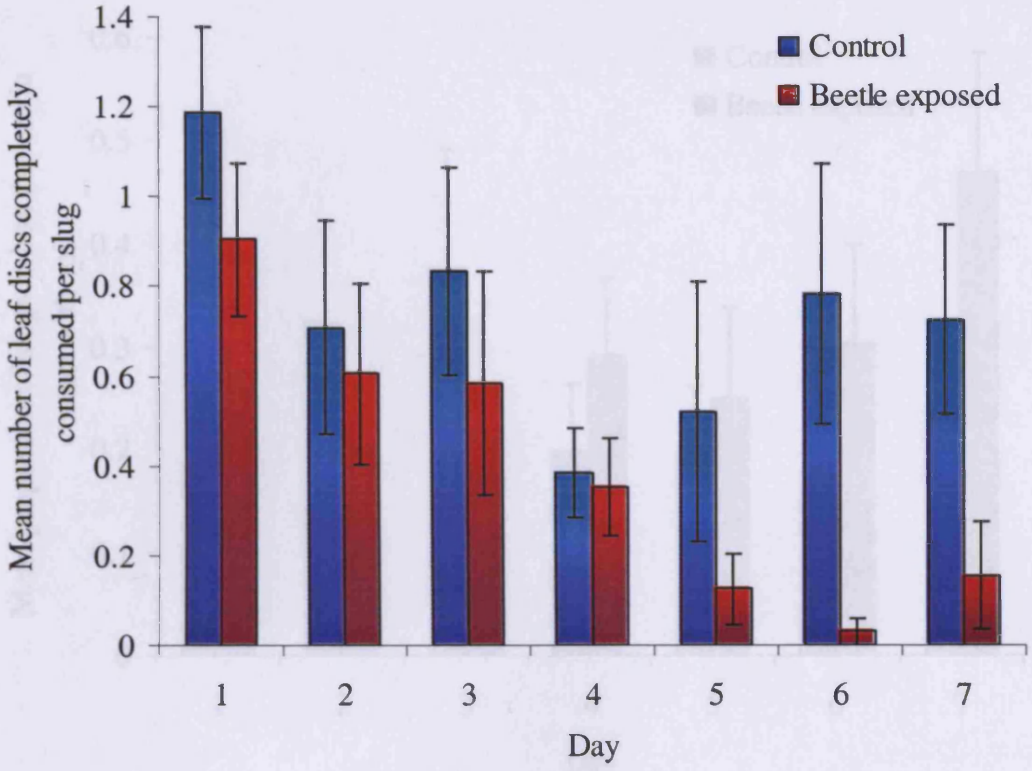

Figure 3.7. Bar graph showing the mean number of leaf discs completely eaten per *Deroceras reticulatum* on each day. A comparison of the number of leaf discs partially eaten by each slug on either soil exposed to *Pterostichus melanarius* or on control soil. Closed bars = standard errors.

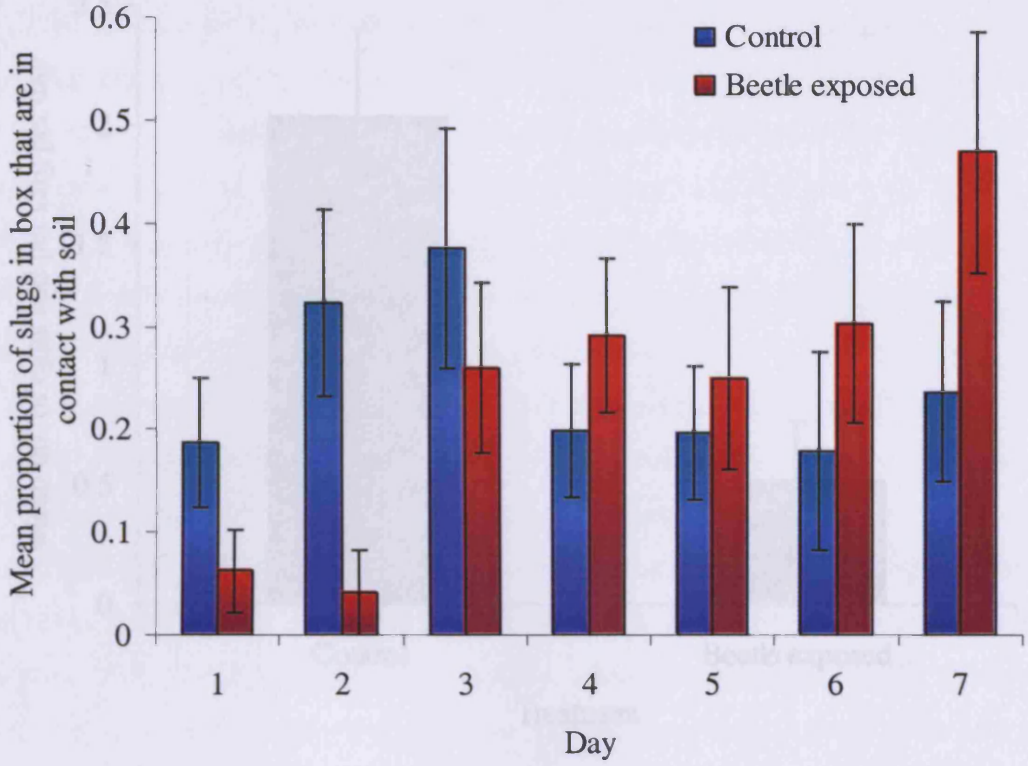

Figure 3.8. Bar graph showing the mean proportions of *Deroceras reticulatum* that are in contact with the soil on each day. A comparison of the proportion of slugs that are in contact with the soil in arenas containing either soil exposed to *Pterostichus melanarius* or control soil. Closed bars = standard errors.

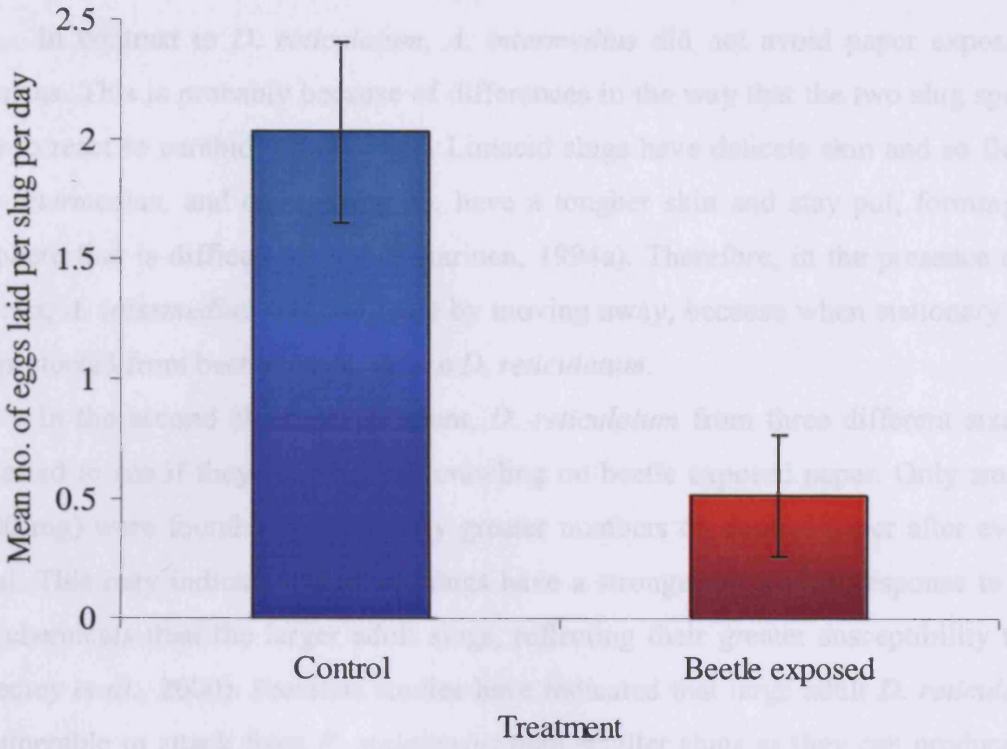

Figure 3.9. Bar graph showing the mean number of eggs laid per *Deroceras reticulatum* per day. A comparison of the number of eggs laid by slugs on either soil exposed to *Pterostichus melanarius* or on control soil. Closed bars = standard errors.

3.4. Discussion

The work in this chapter tested the null hypothesis that slugs do not respond to chemical cues from the slug predator *P. melanarius*, and other carabid beetles present in the field. The results show that *D. reticulatum* but not *A. intermedius*, avoided crawling over paper that had been exposed to *P. melanarius*. Chemical cues from the beetles altered parameters of movement behaviour in *D. reticulatum*. *D. reticulatum* also avoided paper exposed to the closely related *P. madidus* but not paper exposed to *P. cupreus*, also in the same genus as *P. madidus*, or paper exposed to *H. affinis*, a purely phytophagous species. *D. reticulatum* that were stored with soil exposed to *P. melanarius* laid fewer eggs, fed less and, on the first couple of days, spent less time on the soil than slugs that were stored on unexposed control soil.

In contrast to *D. reticulatum*, *A. intermedius* did not avoid paper exposed to *P. melanarius*. This is probably because of differences in the way that the two slug species are known to react to carabid beetle attack. Limacid slugs have delicate skin and so flee attack but *A. intermedius,* and other *Arion* sp. have a tougher skin and stay put, forming a tight hemisphere that is difficult to bite (Pakarinen, 1994a). Therefore, in the presence of beetle chemicals, *A. intermedius* may not react by moving away, because when stationary they are better protected from beetle attack, unlike *D. reticulatum*.

In the second choice experiment, *D. reticulatum* from three different size classes were tested to see if they would avoid crawling on beetle exposed paper. Only small slugs (30-100 mg) were found in significantly greater numbers on control paper after every time interval. This may indicate that small slugs have a stronger avoidance response to residual beetle chemicals than the larger adult slugs, reflecting their greater susceptibility to attack (McKemey *et al.*, 2000). Previous studies have indicated that large adult *D. reticulatum* are less vulnerable to attack from *P. melanarius* than smaller slugs as they can produce greater quantities of defensive mucus (Pakarinen, 1994b). Laboratory studies have shown that *P. melanarius* readily attacks and consumes *D. reticulatum* < 40 mg, but only consumes slugs > 40 mg after prolonged confinement together in Petri dishes (McKemey *et al.*, 2000). In semi-field plots a range of sizes are taken, hence it is possible that relatively greater avoidance behaviour by smaller slugs, under field conditions, counteracts this vulnerability to some extent, leading to predation rates no greater than those on larger size classes (McKemey *et al.*, 2003). Although it may be easier for beetles to overcome the defences of smaller slugs, in a complex field environment, smaller slugs may be more capable of finding and taking refuge from beetles than larger slugs. Even if larger slugs are at a much lower risk of predation by the carabid beetles, it would still be beneficial to avoid areas with a high density of the predators since any eggs that are laid by the slugs are at risk. Other animals showing a decrease in anti-predator behaviour with increasing body size (and hence decreasing vulnerability) include larvae of the salamander *Ambystoma annulatum* (Cope) (Mathis *et al.*, 2003), the whelk *Buccinum undatum* (L.) (Rochette & Himmelmen, 1996) and freshwater snail *Physa gyrina* (Say) (Dewitt *et al.*, 1999).

Male and female beetle exposed paper elicited equally strong avoidance responses in *D. reticulatum*, and consequently it would seem likely that slugs were responding to a chemical cue produced by both male and female beetles.

In the fourth choice test, examining the effects of beetle density, fewer beetles were exposed to test paper and for less time, which probably better represents field conditions. The

avoidance response of *D. reticulatum* in this test was still significant after 24 h for both sexes, and also at 6 h when males were stored with test paper. The slugs were still able to detect and respond to the lower density of beetles but the response was slower than in the previous choice tests.

Five-day-old beetle exposed paper was not avoided by *D. reticulatum* in the fifth choice test suggesting that slugs can assess how long ago the beetles were in an area by the concentration of residual chemicals present on the surface and hence the current risk of predation. It would not be beneficial to initiate potentially costly predator avoidance behaviour or forgo exploiting areas where predation risk is no longer high since this can interfere with reproduction and foraging (Lima & Dill, 1990). Other studies have shown that prey may show graded avoidance responses to varying concentrations of predator chemical cues (Loose & Dawidowicz, 1994; Persons & Rypstra, 2001).

In the sixth choice test more juvenile *D. reticulatum* were counted on control paper at every time interval, but high variability in the data, caused by the effect of arena, meant that none of these differences were significant. Therefore it's possible that juvenile slugs did respond to beetle exposed paper but the response was much weaker than that of the adult slugs.

In the final choice test *D. reticulatum* were shown to avoid paper exposed to *P. madidus*, a close relative of *P. melanarius* and a known predator of slugs (Tod, 1973; Ayre & Port, 1996). Paper exposed to *P. cupreus*, which has been shown to eat slugs under laboratory conditions (Oberholzer & Frank, 2003), but is in a different sub-genus to *P. melanarius*, was not avoided by slugs. The results seem to indicate that *P. melanarius* and *P. madidus* share similar chemistry, possibly because *P. madidus* is closely related to *P. melanarius*. *P. cupreus* is not as closely related to *P. melanarius* as *P. madidus* and it is placed in a different sub-genus. Paper exposed to *H. affinis*, which is a purely phytophagous carabid, was also not avoided by slugs. These choice tests show the specificity of the slug response and that chemicals that slugs avoid are not produced by all carabids. Dodds *et al.* (1997) also found that firing in the olfactory nerve of slug tentacles was only induced by chemicals from predatory *P. melanarius*, and not from the phytophagous carabid *Zabrus tenebrioides* (Goeze).

One could argue that the slug response to beetle exposed paper is simply to a novel cue and not necessarily to the predator specifically, since the control was clean unexposed paper. However, such a response could still be adaptive and the results of the sixth choice

experiment show that not all carabid chemicals are detected or avoided by the slug, the response seems to be specifically associated with beetles that are slug predators.

In contrast to the findings of choice experiment three, *D. reticulatum* responded in the video tracking study only to paper exposed to male beetles. The beetles had been stored for one month before the choice experiments but up to three months before the video tracking experiments and this may have affected the chemicals they deposited on the paper in some way. During these three months beetles would normally be either dying off or overwintering in the field. Consequently, after an extended period in which females are well fed, they might become progressively less active in the laboratory and leave less chemical residue on test papers.

Results from the video tracking study suggests that the accumulation of *D. reticulatum* on the control paper observed in choice experiments two and three was because slugs increased their velocity and time spent moving and reduced their rate of turning (meander and angular velocity) on the beetle exposed side. This behaviour shows that the slugs were responding to the predator cue using klinokinesis and orthokinesis simultaneously (Fraenkal & Gunn, 1940). These responses would enable the slugs to exit the unfavourable environment more rapidly and explain why slugs spent significantly longer periods of time on the control paper. Some marine molluscs also alter their velocity in the presence of predator chemical cues. Marsh periwinkles, *Littoraria irrorata* (Say), crawled faster when they detected mucus produced by the predatory conch *Melongena corona* (Gmelin) (Dix & Hamilton, 1993); direction of travel was random and the purpose of the increase in speed appeared to be for the purpose of escaping by finding a suitable plant stem to crawl up. Limpets, *Acmaea* (*Collisella*) *limatula* (Segal) and *Acmaea* (*Notoacmea*) *scutum* (Rathke) moving upward on a vertical surface increased their rate but maintained the same direction of movement when odour from a predatory starfish *Pisaster* sp. flowed over them (Phillips, 1975). As with this study, these studies of marine molluscs showed that individuals did not respond to the direction of the chemical cue but altered the rate of movement in response to an increased intensity of the stimulus. To the best of my knowledge, this video tracking study is one of the first to measure kinesis quantitatively as a method of avoiding predation and the first to examine the behavioural responses of a terrestrial mollusc to risk associated with predation.

In laboratory bioassays similar to the set-up described in this chapter, Schüder *et al.* (2003) recorded and tracked (using EthoVision) snail movements in an arena with a control zone and a zone coated in a novel molluscicide: cinnamamide. They found that the snails

reduced their velocity and time spent moving when on the treated side of the arena, but spent significantly less time in these areas overall. The repellent effect of cinnamamide in these bioassays resulted in slugs avoiding crawling onto zones coated in it and then less movement when they did. *Deroceras panormitanum* (Lessona & Pollonera) also reduced velocity and time spent moving when placed on compost coated in the repellent molluscicide ureaformaldehyde (Schüder *et al.*, 2003). In the video tracking study described in this chapter, slugs did not avoid crawling on beetle exposed paper (distance moved on either paper was not significantly different) but increased rates of movement when they did. It is interesting to see that slugs are behaving in different ways when encountering two different types of chemical. Slugs probably move less on the surfaces coated in ureaformaldehyde and cinnamamide because of their potential toxic and irritant properties, whereas lots of movement is required on surfaces coated in predator cues because the slug needs to move away from these areas.

The presence of beetle residual chemicals also affected sheltering, feeding and especially egg laying by adult *D. reticulatum*. There was a big difference in the number of eggs laid by slugs on the two treatments, with slugs on beetle exposed soil laying only a quarter of the number of eggs as slugs on control soil. Since oviposition has high energy costs associated with it, then this could be an adaptation to reduce the risk that eggs and young will be eaten by the beetles. Other animals reduce oviposition and reproductive behaviour in the presence of predator chemical cues. Fewer spider mites, *Tetranychus urticae* (Koch), fed on or laid eggs on leaf discs previously exposed to predators or injured conspecifics compared to control discs (Grostal & Dicke, 1999). When searching for aphids to parasitize, the parasitic wasp, *Aphidius ervi* (Haliday), reduces the time it spends in a patch and contacts fewer aphids when this patch has previously been occupied by a ladybird predator of aphids, *Coccinella septempunctata* (L.) (Taylor *et al.*, 1998). This behaviour reduces the chance that aphids it has parasitized will be eaten by another predator. Finally, the snail *Physella virgata virgata* (Gould), reduced reproductive activity and exhibited increased growth rates in the presence of chemicals produced by the predatory starfish *Orconectes virilis* (Hagan) feeding on conspecifics (Crowl & Covich, 1990).

The effect of beetle chemicals on feeding and sheltering was less conclusive. Beetle exposed soil was avoided by *D. reticulatum* in the first three days but feeding was not significantly reduced until after six days had passed. The results of the fifth choice experiment indicated that slugs did not respond to beetle chemicals on paper after five days had elapsed. Although there are differences between the two experiments because in the

feeding/sheltering experiment beetles were stored on soil and for a longer period of time, you would still expect that the effect of chemicals on feeding would be stronger in the first few days rather than the last two. The fact that slugs avoided soil in the first few days would also point toward this expectation. This was not the case however and one possible explanation is that the effect of the chemicals on the slugs was to reduce their fitness overall leading to fewer eggs being laid and by the end of the experiment, less feeding. As slugs on beetle exposed soil still needed to feed, their feeding bouts were likely to have been shorter and hence they spent less time on the soil than slugs on control soil whilst there was not much difference in the quantities of food consumed. Since food was always placed on soil with predator cues then it is inevitable that slugs must venture onto the soil at some point when hunger outweighs predator risk, this phenomenon is described by the 'risk-spreading theorem' (Lima & Dill, 1989; Houston *et al.*, 1993). In the field risk is likely to be less constant and slugs must weigh up the benefits of activity and feeding with the changing risks of predation (Sih, 1992; Lima & Bednekoff, 1999). Other animals have been shown to reduce feeding and activity in response to predator chemical cues. Dogwhelks, *Nucella lapillus* (L.), reduced feeding and increased refuge use in the presence of chemical cues from predatory crabs and injured conspecifics, and well-fed individuals were more risk-averse than starved individuals (Palmer, 1990; Vadas *et al.*, 1994). Spotted cucumber beetles, *Diabrotica undecimpunctata howardi* (Barber), reduced feeding in the presence of chemical and visual cues from the predatory spider *Hogna helluo* (Walckenaer) (Snyder & Wise, 2000). Finally, damselfly larvae, *Ischnura elegans* (van der Linden), reduced feeding rates and increased refuge use in the presence of chemical cues from female predatory water-boatman *Notonecta glauca* (L.) (Heads, 1986).

In a complex field environment slugs may not respond to the chemical cues from *P. melanarius* by moving away across the surface of the soil, but they may choose to take refuge under the soil or in the plants. In the beetle exposed arenas slugs were only 'safe' when on the walls or lids of the arenas and consequently slugs were counted less often on the soil to begin with when the chemical would have been at it's highest concentration. In a laboratory study by McKemey (2000), *D. reticulatum* responded to the presence of *P. melanarius* by aggregating out of reach of the beetles on the control side of arenas. Symondson (1993b) found that slugs were only susceptible to attack by the carabid *A. parallelepipedus*, another generalist predator that includes slugs in its diet, in immature lettuce plants, because in mature plants they responded to beetle presence by climbing up into the leaves out of reach of attack. The results in this chapter may help to explain patterns

of spatial dissociation between beetles and slugs observed by Bohan *et al.* (2000a), as both direct attacks by beetles and the presence of residual chemicals on the soil surface may result in slug mortality and moving away to areas of lower beetle density, respectively.

Other cues upon which slugs might respond are to that of injured or dead conspecifics, which might indicate the presence of a predator in the local area. *Deroceras agreste* (L.), a slug that is closely related to *D. reticulatum*, avoids feeding on lettuce coated in a solution of crushed conspecifics (Niemela *et al.*, 1988). In a choice test *Arion fasciatus* (Nilsson), but not *D. reticulatum*, avoided crawling over an area coated in mucus from stressed conspecifics (Pakarinen, 1992). Other animals show antipredator behaviour in the presence of dead or injured conspecifics (McCarthy & Dickey, 2002; McCarthy & Fisher, 2000; Oku *et al.*, 2003; Wisenden *et al.*, 2001). Cues from dead conspecifics can also come from predator frass, if the predator has recently fed on these prey (Keefe, 1992; Wilson & Lefcort, 1993). It is possible that slugs might use fecal cues from *P. melanarius* to avoid areas where this beetle has recently been present and feeding on slugs.

The common periwinkle, *Littorina littorea* (L.), shows stronger avoidance behaviour towards crab predators reared on a diet of *L. littorea* compared to crabs of the same species reared on a purely fish diet, indicating that chemical cues from the predator varied according to its diet (Jacobsen & Stabell, 1999). It is possible that *P. madidus* and *P. melanarius* are avoided by slugs because the slugs can detect that it has been feeding on conspecifics. It would be interesting to test whether *P. melanarius* are avoided by slugs after the beetles have been fed for long enough on a no-slug diet. This could be another explanation for why female *P. melanarius* exposed paper was not avoided by slugs in the video tracking study, as the beetles had been fed only dogfood for three months, and hence were not 'chemically labelled' as a slug predator. The response of slugs to injured conspecifics, beetle fecal cues and beetles maintained on different diets are potential areas for further study that unfortunately due to time constraints could not be examined for inclusion in this thesis.

The results of the experiments in this chapter suggest that *D. reticulatum* might have been exposed to sufficient predation selection pressure by carabids, for them to have evolved mechanisms for detecting and identifying predator kairomones, and taking avoidance action. Many large carabids are specialised slug predators (reviewed in Symondson, 2002), but it is interesting to see that slugs are responding in a similar way to generalists. Previous work, showing coupled dynamics between *P. melanarius* and *D. reticulatum* (Symondson *et al.*, 2002a), suggested that slugs comprise a substantial

proportion of the total prey available to the beetles, driving changes in the slug population. Under such selection pressure the evolution of avoidance behaviour by the slugs might be selected for. Understanding the mechanics behind exactly what cues slugs use, and how they detect these cues, was the next step to understanding the interaction between these two species in the field.

Chapter 4

Dispersion of juvenile slugs from egg batches and the influence of *Pterostichus melanarius*

4.1. Introduction

Evidence from laboratory behavioural experiments in Chapter 3 shows that *P. melanarius* leave chemicals behind on surfaces and that *D. reticulatum* then avoid these surfaces. The work in this chapter aims to examine the movement and dispersion of juvenile slugs under more natural, semi-field conditions and then compare dispersion in the presence and absence of *P. melanarius*.

Previous work by Bohan *et al.* (2000b), showed that the slope of the variance to mean relationship for populations of *D. reticulatum* and *A. intermedius* in an arable field over one year was similar for both species, and that the variance for a given mean was lower in the summer (for details of grid used see Section 2.2.1). This was coupled with a reduction in the number of zero counts in samples. It was hypothesised that the cause of the reduction was due to dispersion of juveniles from aggregations of both species (probably caused by egg laying in batches) at a spatial scale of 0.25 m. However, in late May to early June, *P. melanarius* begins to emerge from the soil. Density-dependent predation of slugs by *P. melanarius* is evident in June and July (Bohan *et al.*, 2000a). Predation of slugs could have also accounted for the reduction in the number of zero counts and over-dispersion in slug population samples (Bohan *et al.*, 2000b). Spatial dissociation between slugs and *P. melanarius* in July could also be caused by slugs detecting the presence of the beetles and moving away to areas of lower beetle density.

The work described in this chapter is a study of the dispersal of slug juveniles of both species from egg batches, and then an examination of the effect of the presence of predatory *P. melanarius* on dispersal of *D. reticulatum*. Dispersal was measured under simulated field conditions using replicated mini-plots of winter wheat. Due to an infestation of pathogenic nematodes in the stored *A. intermedius* eggs, the influence of *P. melanarius* on juvenile slug dispersal in mini-plots was tested only on *D. reticulatum*.

4.2. Methods

Three experiments were performed. The initial experiment to measure and compare dispersion rates of *A. intermedius* and *D. reticulatum* used small circular mini-plots of winter wheat (300 mm diameter and 300 mm depth). A further two experiments to measure the dispersion rates of *D. reticulatum*, in the presence and absence of *P. melanarius*, were carried out in larger rectangular mini-plots of winter wheat (600 × 800 × 200 mm).

4.2.1. Dispersion of *Arion intermedius* and *Deroceras reticulatum* in small mini-plots

The aim of this first experiment was to compare the dispersion rates of *D. reticulatum* and *A. intermedius*. Twenty mini-plots were sown in total and dispersion of slugs was measured after either one or two days, giving five replicate mini-plots for each species and time treatment. The mini-plots were sown on 17.09.02 and stored on a gravel bed for eight weeks.

Three holes were punched around the base of each mini-plot and the holes were covered with gauze (1 mm diameter) to allow water in during soil flooding and yet prevent slugs and soil from escaping.

Each mini-plot contained straw at a density of 800 g m^{-2} based on an estimated field straw density of 8 tonnes per ha. Straw was cut into 10 - 15 cm lengths. Each mini-plot was filled with unsterilised loam to a depth of 50 mm, then a layer of straw added (45 g), then another layer of loam (50 mm). Twenty-two wheat seeds (cv. Riband) were sown evenly in each mini-plot and at no less than 2.5 cm from the edge. The remaining 11 g of straw was incorporated into the top layer of soil.

Mini-plots were watered and stored outside on a gravel bed sprinkled with molluscicidal pellets (to prevent contamination of the mini-plots by other slugs). After eight weeks the seedlings had reached approximately 100 mm in height and the mini-plots were then moved to an unheated greenhouse for the start of the experiment.

Mini-plots were moved to an unheated greenhouse because the outside temperature was very cold but the temperature in the greenhouse was more representative of conditions that slugs would experience when hatching in the spring. Temperature in the greenhouse was monitored throughout the two days of the experiment using a Tinytalk (Gemini Dataloggers Ltd.) data logger, which took a temperature reading every 10 min.

Adult *D. reticulatum* were collected from patches of clover in Long Ashton Research Station fields using cover traps. Cover traps were constructed from upturned 10-

inch diameter flowerpot bases. Each trap was baited with a handful of chicken layers mash. Adult *A. intermedius* were collected from samples of arable soil, flooded over a period of 8-10 days (South, 1992). Some adults were found under baited cover traps. Both species were stored in groups of 20, in Perspex boxes lined with moist cotton wool and containing Chinese cabbage leaves. The boxes were stored in a consistently dark incubator at 15 °C (± 2 °C) and cleaned out once a week. Eggs were collected and stored in Perspex boxes lined with moist cotton wool and incubated at 15 °C (± 2 °C) until development of the juvenile slugs inside the eggs became visible. The eggs were then transferred to a consistently dark incubator 5 °C (± 2 °C) until required for the experiments. Fully developed eggs (identifiable by a darkening of the egg interior) were used for the experiment.

A small depression was made in the centre of each mini-plot and batches of 30 fully developed slug eggs of either *A. intermedius* or *D. reticulatum* were placed into the depression. This number is within the size range of egg batches laid in the field, which are reported to be in the range 9-49 eggs (South, 1992). Eggs were covered over with a glass microscope coverslip painted black. Twenty-four hours after addition of the eggs, any unhatched eggs were removed and counted. This was to ensure that dispersion was only measured for slugs that had hatched on the first day of the experiment.

The surfaces of ten of the mini-plots (five that contained *A. intermedius* and five that contained *D. reticulatum*) were examined for slugs. Slugs were collected from under straw and leaves as well as on the soil surface itself, and a record was made of where each slug was found. Slugs were removed and the distance from the central egg batch was recorded and slugs were then allocated to a particular radial zone. Each of the mini-plots were split into four concentric zones, comprising different radial distances around the central egg batches. Zone 1 closest to the egg batch covered a radial distance of up to 38 mm away from the eggs. Each zone thereafter covered a further 38 mm radiating outwards. Slugs that had moved down into the soil were obtained by flooding the soil slowly over a period of 8-10 days. Mini-plots were segmented into the concentric zones by pushing Perspex barriers into the soil to a depth of 80 mm. Three barriers of diameter 77 mm, 154 mm and 231 mm were created from PVC sheeting cut into strips and stapled into tubes (165 mm height) and the joining edges were sealed using silicon sealant. Each barrier was separated with 38 mm space between them. The barriers prevented horizontal movement of slugs in the soil during flooding. The number of slugs surfacing in each zone could then be recorded. This procedure was repeated for the remaining ten mini-plots after a further 24 h.

The soil flooding procedure follows that described in South (1992). The mini-plots were flooded in metallic troughs with a single drainage hole in the base (Figure 4.1.). Stoppers were placed over the drainage holes, and water was added from a hosepipe until the level had reached the bottom of the gauze-covered holes in the base of the mini-plots. A drip-feed nozzle attached to an irrigation system was then placed over each trough and the drip rate was set at approximately 19 drips per 10 s (this raised the water level by approx 1 cm a day). Each day slugs found on the surface were collected and a record made of the zone in which they had surfaced. Soil flooding was complete after 8-10 days.

Figure 4.1. Photograph showing the extraction of slugs from circular mini-plots in the first experiment. The mini-plots were flooded gradually using drip-feed irrigation into the metal troughs. The first mini-plot experiment measured dispersal of *Arion intermedius* and *Deroceras reticulatum*.

The percentages of the total number of slugs recovered in each zone were calculated. These data were angular transformed to normalise the residuals. Slug distribution

across the four zones was compared between the four zones, the two different slug species and after the two time intervals using a three-way ANOVA. The mean percentages of slugs in each zone for each species were plotted on two graphs, for distribution over either 24 or 48 h.

4.2.2. Dispersal of *Deroceras reticulatum* in the presence and absence of *Pterostichus melanarius*

A preliminary experiment was carried out to measure the dispersion of *D. reticulatum* in larger rectangular mini-plots over 24, 48 and 72 h in the absence of beetles. Eighteen mini-plots were sown, with six replicates for each time. The aim of the preliminary experiment was to calculate the speed of dispersion of the slugs and to test the viability of the method for the main mini-plot experiment.

For the main experiment, comparisons were made between *D. reticulatum* dispersing in mini-plots previously exposed to or not previously exposed to *P. melanarius*. Dispersion was measured over 24 and 48 h and there were ten mini-plots for each time treatment, five that had contained beetles and five unexposed controls. Each beetle mini-plot had previously contained one male and one female *P. melanarius* giving a density of 4.17 beetles per m^2. This value falls within the range of field densities reported in the literature: 13.5 beetles per m^2 (Purvis & Fadl, 1996) and 0.26 beetles per m^2 (Thomas *et al.*, 1998) in winter sown cereals.

Rectangular mini-plots of winter wheat were created from plastic trays (600 mm × 800 mm × 200 mm) with holes in the base for drainage. A 150 mm high copper gauze barrier (1 mm diameter) surrounded the top of each mini-plot. Gaps between the barrier and the plastic tray were filled using silicon sealant and duck tape. Winter wheat seeds (cv. Riband) were sown at a density of 320 m^{-2} and the mini-plots were stored outside over winter on raised palettes over a gravel bed. A rain cover protected the gravel bed and mini-plots were watered daily using an irrigation system. The surrounding gravel bed was treated with molluscicidal pellets to prevent the mini-plots becoming contaminated by slugs. Two weeks before the start of each experiment weeds were removed and the plants in the outer 50 mm of each mini-plot were trimmed back as this could form a bridge over the gauze by which slugs/beetles could escape.

In preparation for the main experiment, *P. melanarius* were collected three days before slug eggs were added to the mini-plots. Live beetles were captured in dry pitfall traps

in a field of winter wheat. Twenty-five traps were dug five paces apart and five paces from the field margin. Each trap was protected from flooding by a rainfall cover. Male beetles were separated from females and were kept in groups of ten in Perspex boxes containing a 40 mm deep layer of soft peat, a sheet of moistened paper towel and a small Petri dish lid containing distilled water. Boxes were stored overnight in a dark incubator at 10 °C (± 2 °C). Beetles were added to the mini-plots 48 h before adding the eggs. This time interval would allow the beetles to habituate to the new environment and leave residual chemicals on the soil surface, which have been shown to affect slug behaviour (Armsworth *et al.*, 2005; Chapter 3). The mini-plots were searched before the addition of slug eggs and all of the beetles were removed.

Mini-plots were randomly assigned to treatment groups and labelled. Forty *D. reticulatum* eggs were added to each of the mini-plots in both experiments. The procedure for both of the experiments closely follows that described in Section 4.2.1. Slugs were collected after the relevant time intervals (24 or 48 h) and then barriers were inserted into the soil. Since the mini-plots were larger than the circular ones described in Section 4.2.1., a further three barriers of diameter 308 mm, 385 mm and 462 mm were inserted into each of the mini-plots to create seven concentric zones around the central egg batches with 38 mm between them.

The soil flooding procedure was modified from Section 4.2.1. for the large rectangular mini-plots as they were too large to be flooded in the troughs. A new method of soil flooding was designed to accommodate these mini-plots (Figure 4.2). Inflatable paddling pools (1300 mm × 2200 mm) were laid out flat in a line on a length of thick black irrigation matting. The matting protected the base from punctures. A 1300 mm × 1700 mm rectangle of matting was also placed inside each paddling pool. Mini-plots were moved on their palettes using a forklift, and placed onto the base of each pool. Each pool accommodated a maximum of four mini-plots. The pools were then inflated around the mini-plots using an electric pump. Water was added to the pools from a hose until it reached 20 mm above the base of the soil level in the mini-plots. Each day, the water level in each pool was raised by 20 mm using the hose. The flooding process took 11 days until the soil in each pool was completely submerged. Mini-plots were examined every day for the presence of slugs on the surface. Slugs were removed and the zone that it was collected from was recorded.

The percentages of the total number of slugs recovered in each mini-plot that were in each zone were calculated. The data were modelled separately for each day using

regression analysis. The modelling fitted polynomials of increasing order (linear, quadratic, cubic) to assess the significance of curvature in the data with increasing distance (zone) and the effect of treatment (presence or absence of beetles). For this modelling, an angular transformation of the data was required to normalise the residuals. The mean transformed percentages of slugs in each zone for each treatment were plotted on two graphs (one for each day) and lines of best fit added.

Figure 4.2. Photograph showing the extraction of slugs from rectangular mini-plots in the second experiment. The mini-plots were flooded gradually in paddling pools by adding water from a hosepipe every day. The second mini-plot experiment measured dispersal of *Deroceras reticulatum*.

4.3. Results

4.3.1. Dispersal of *Arion intermedius* and *Deroceras reticulatum* in small mini-plots

Sixty-nine *A. intermedius* and 112 *D. reticulatum* were recovered from mini-plots before and after soil flooding. Although fewer *A. intermedius* were recovered from mini-plots than *D. reticulatum*, fewer *A. intermedius* eggs had hatched after 24 h. The percentage of hatched slugs that were recovered was higher for *A. intermedius* than for *D. reticulatum* after 24 and 48 h (Table 4.1.). The data were analysed in a two-way ANOVA. There was no significant difference in the recovery of slugs from mini-plots after 24 and 48 h (ANOVA: $F_{1,16} = 0.69$, $P = 0.418$), no difference in recovery of the two species (ANOVA: $F_{1,16} = 2.88$, $P = 0.109$) and no significant interaction between species and time treatment (ANOVA: $F_{1,16} = 0.22$, $P = 0.815$).

Table 4.1. The number of *Arion intermedius* and *Deroceras reticulatum* eggs that did not hatch, slugs that were recovered and the percentage of hatched slugs that were recovered under each treatment in the first mini-plot experiment. The first mini-plot experiment measured dispersal of *Arion intermedius* and *Deroceras reticulatum*.

Species and time after hatching that slugs were sampled	Eggs not hatched	Slugs recovered	Percentage of hatched slugs that were recovered
A. intermedius			
24 h	71	40	51
48 h	79	29	41
D. reticulatum			
24 h	42	59	55
48 h	37	53	47

Temperature over the two days reached a maximum of 15.6 °C during the day and a minimum of 11.0 °C at night (Figure 4.3).

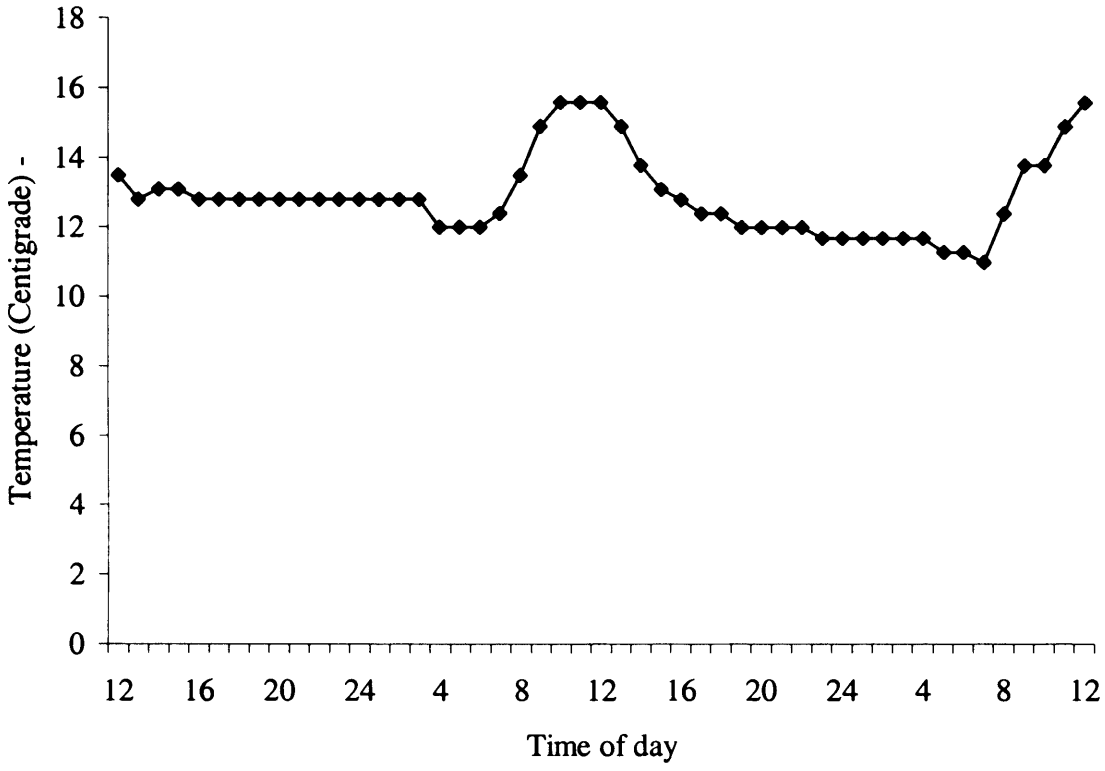

Figure 4.3. Line graph showing the temperature in the unheated greenhouse over two days in the first mini-plot experiment. The first mini-plot experiment measured dispersal of *Arion intermedius* and *Deroceras reticulatum.*

For each mini-plot, the percentage of the total slugs recovered that was in each zone was calculated. The mean percentage of slugs in each zone for each treatment was then plotted (Figures 4.4.a and 4.4.b). Twenty-four hours after hatching, most slugs of both species were still found in zone one and had not moved far from the egg batch. A few individuals of both species had managed to reach the outer zone. The patterns of distribution across the four zones were similar for both species. *D. reticulatum* was slower to disperse 24 h after hatching than *A. intermedius*, but this difference was not significant. After hatching, *D. reticulatum* were observed feeding on egg remains before dispersing, but this was not observed for *A. intermedius*. Egg feeding may account for the high numbers of *D. reticulatum* found in the egg batch up to 24 h after hatching. There was a significant interaction between time, species and zone (ANOVA: $F_{3,64} = 4.07$, $P = 0.010$). By examination of the LSDs it was possible to determine that although there were fewer *A. intermedius* in zone one after 24 h than *D. reticulatum*, this difference was not significant. Similarly although there were more *D. reticulatum* in zone four after 48 h than *A.*

intermedius, this difference was also not significant. Significant differences occurred between zones (i.e. slugs of both species were not evenly distributed across the four zones on either day) and time treatments, which would be expected as both species were moving away from the egg batch. There was no significant difference in the rate of dispersion of the two species over a period of 48 h.

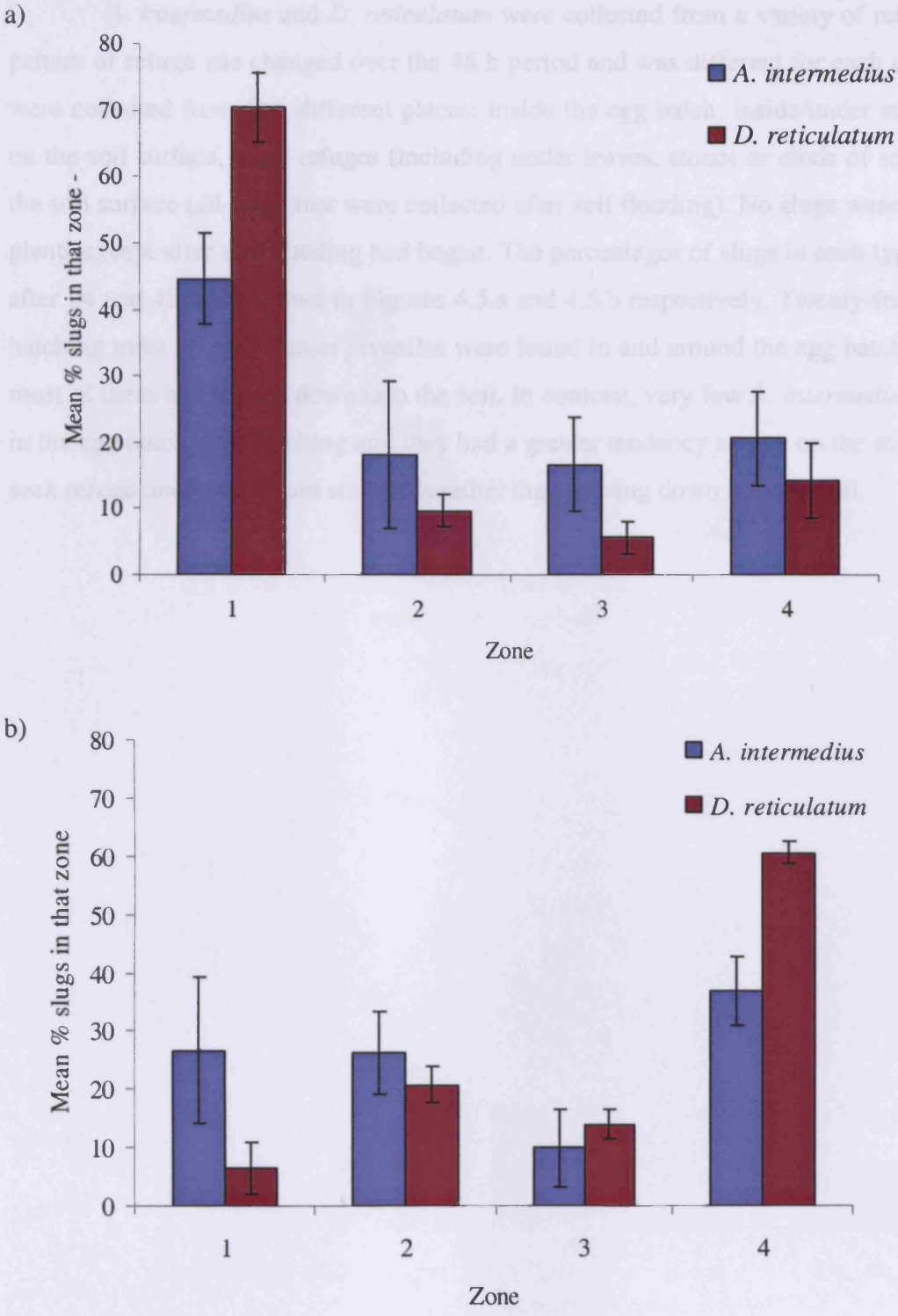

Figure 4.4. Graphs showing the distribution of *Arion intermedius* and *Deroceras reticulatum* across the four concentric zones in circular mini-plots in the first mini-plot experiment. Distribution of slugs a) 24 h after hatching and b) 48 h after hatching. Closed bars represent standard errors.

A. intermedius and *D. reticulatum* were collected from a variety of refuges, but the pattern of refuge use changed over the 48 h period and was different for each species. Slugs were collected from five different places: inside the egg batch, inside/under straw, exposed on the soil surface, other refuges (including under leaves, stones or clods of soil) and under the soil surface (all slugs that were collected after soil flooding). No slugs were found on the plants except after soil flooding had begun. The percentages of slugs in each type of location after 24 and 48 h are shown in Figures 4.5.a and 4.5.b respectively. Twenty-four hours after hatching most *D. reticulatum* juveniles were found in and around the egg batch, but by 48 h most of them had moved down into the soil. In contrast, very few *A. intermedius* were found in the egg batch after hatching and they had a greater tendency to stay on the soil surface and seek refuge under straw and soil clods rather than moving down into the soil.

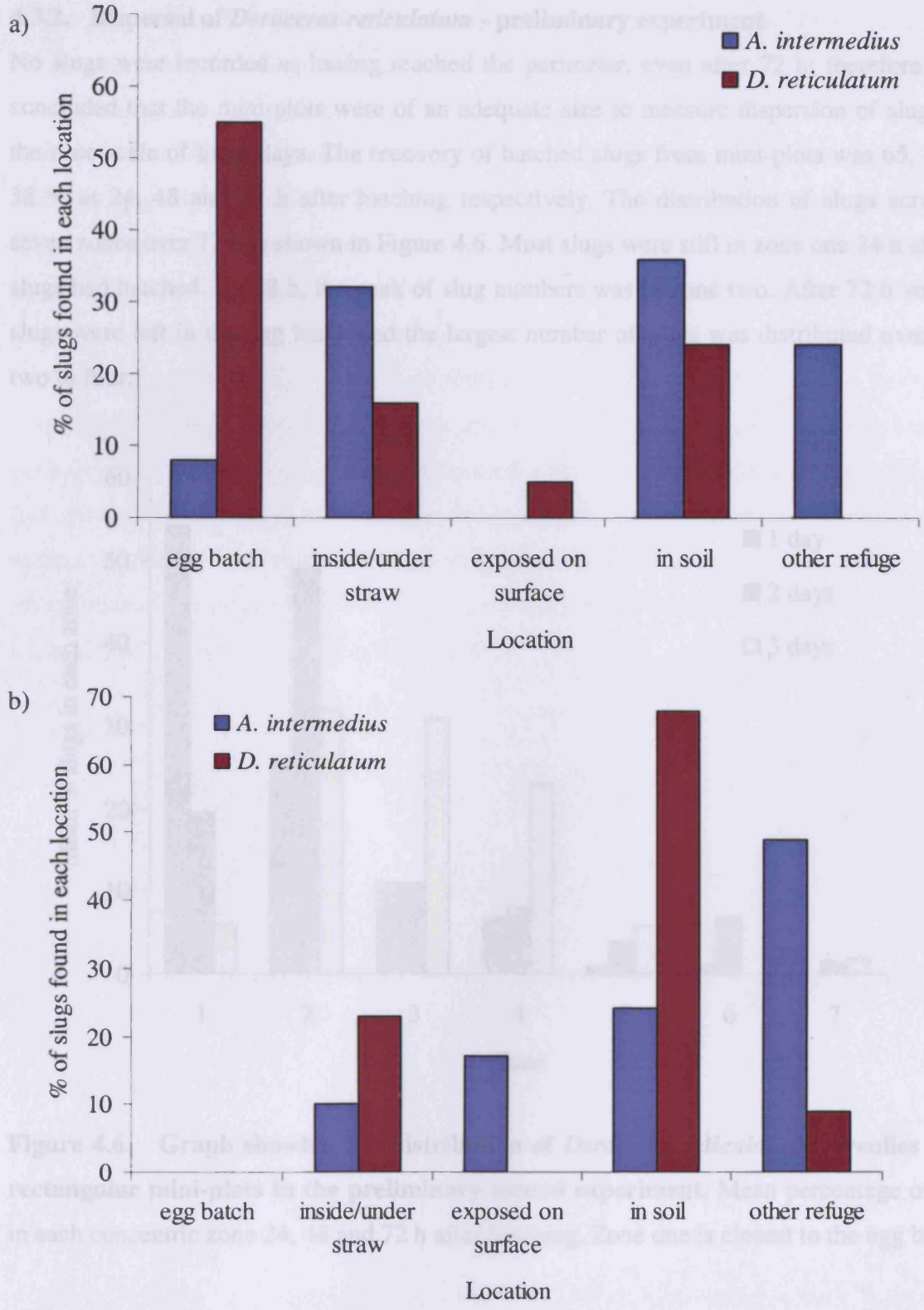

Figure 4.5. Bar graphs showing the percentage of *Arion intermedius* and *Deroceras reticulatum* in each location in mini-plots in the first experiment. Percentage of slugs in each location a) 24 h after hatching and b) 48 h after hatching. The category 'other refuge' includes slugs found under leaves, stones and soil clods.

4.3.2. Dispersal of *Deroceras reticulatum* - preliminary experiment

No slugs were recorded as having reached the perimeter, even after 72 h; therefore it was concluded that the mini-plots were of an adequate size to measure dispersion of slugs over the time scale of three days. The recovery of hatched slugs from mini-plots was 65, 43 and 38 % at 24, 48 and 72 h after hatching respectively. The distribution of slugs across the seven zones over 72 h is shown in Figure 4.6. Most slugs were still in zone one 24 h after the slugs had hatched. By 48 h, the peak of slug numbers was in zone two. After 72 h very few slugs were left in the egg batch and the largest number of slugs was distributed over zones two to four.

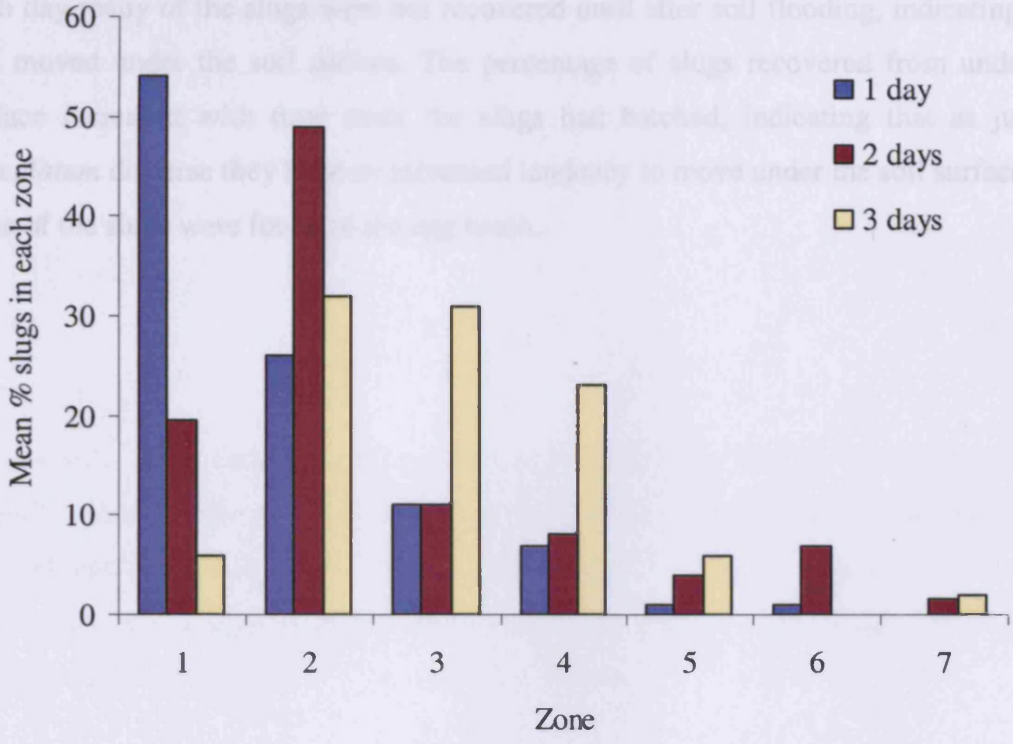

Figure 4.6. Graph showing the distribution of *Deroceras reticulatum* juveniles in the rectangular mini-plots in the preliminary second experiment. Mean percentage of slugs in each concentric zone 24, 48 and 72 h after hatching. Zone one is closest to the egg batch.

The mean distance that *D. reticulatum* dispersed in each 24 h period was calculated. The exact distance that each slug moved was not known, only the zone in which they were found. The distance to the centre of each zone was therefore used to calculate the mean distances moved, and it was assumed that this was the mean distance to which a slug found

in that zone had moved. Zone one therefore became 19 mm, zone two = 57 mm, zone three = 95 mm etc. After 24 h slugs had moved on average 43.6 mm (speed = 2.03 mm/h). After 48 h slugs had moved on average 78.1 mm from the egg batch (speed = 1.63 mm/h) and 34.5 mm in the last 24 h (speed = 1.44 mm/h). After 72 h slugs had moved on average 93.8 mm from the egg batch (speed = 1.30 mm/h) and 15.7 mm in the last 24 h (speed = 0.65 mm/h). The speed of dispersal was therefore reduced over time since the eggs hatched. For the whole three days, the total sum of slug speeds was 1005 cm day^{-1} (n = 243), yielding an estimated per capita dispersal speed of 4.14 cm day^{-1}.

The location where slugs were found changed over 72 h (Figure 4.7). Twenty-four hours after hatching many of the juvenile slugs were found in and around the egg batch. On each day many of the slugs were not recovered until after soil flooding, indicating that they had moved under the soil surface. The percentage of slugs recovered from under the soil surface increased with time since the slugs had hatched, indicating that as juvenile *D. reticulatum* disperse they have an increased tendency to move under the soil surface. By 72 h none of the slugs were found in the egg batch.

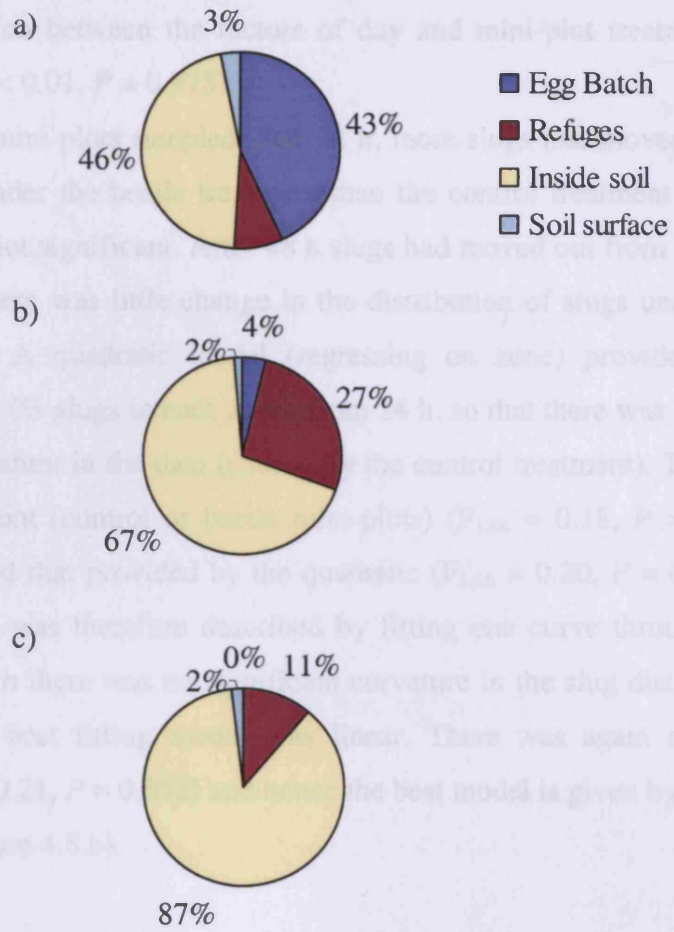

Figure 4.7. Pie charts showing the percentage of *Deroceras reticulatum* that were found in each location in rectangular mini-plots in the preliminary second experiment. The percentage of slugs found in each location a) 24 h after hatching, b) 48 h after hatching and c) 72 h after hatching. Slugs were found in the egg batch, in refugia (under leaves, soil clods and stones), under the soil and on the soil surface.

4.3.3. Dispersal of *Deroceras reticulatum* in the presence and absence of *Pterostichus melanarius*

Most of the eggs hatched during the first 24 h, although recovery of slugs from mini-plots was low at 59.0 % for mini-plots sampled after 24 h and 59.1 % for mini-plots sampled after 48 h. No slugs had reached the outer zone by the end of 48 h, therefore it is unlikely that any would have escaped. Recovery of slugs was lower in mini-plots that had contained beetles but this difference was not significant (ANOVA: $F_{1,16} = 1.28$, $P = 0.274$). There was also no difference in the recovery of slugs between the two days (ANOVA: $F_{1,16} < 0.01$, $P = 0.975$)

and no interaction between the factors of day and mini-plot treatment (beetle or control) (ANOVA: $F_{1,16} < 0.01$, $P = 0.975$).

In the mini-plots sampled after 24 h, more slugs had moved out from the centre of the mini-plot under the beetle treatment than the control treatment (Figure 4.8.a.) but this difference was not significant. After 48 h slugs had moved out from the centre in the control treatment but there was little change in the distribution of slugs under the beetle treatment (Figure 4.8.b.). A quadratic model (regressing on zone) provided the best fit for the distribution data (% slugs in each zone) from 24 h, so that there was significant ($F_{1,67} = 5.13$, $P = 0.027$) curvature in the data (caused by the control treatment). There was no significant effect of treatment (control or beetle mini-plots) ($F_{1,66} = 0.18$, $P = 0.670$) and no further curvature beyond that provided by the quadratic ($F_{1,66} = 0.20$, $P = 0.653$ for a cubic term). The best model was therefore described by fitting one curve through all the data (Figure 4.8.a). After 48 h there was no significant curvature in the slug distribution data ($F_{1,67}$, $P = 0.319$) and the best fitting model was linear. There was again no significant effect of treatment ($F_{1,66}$ 0.21, $P = 0.652$) and hence the best model is given by fitting one line through all the data (Figure 4.8.b).

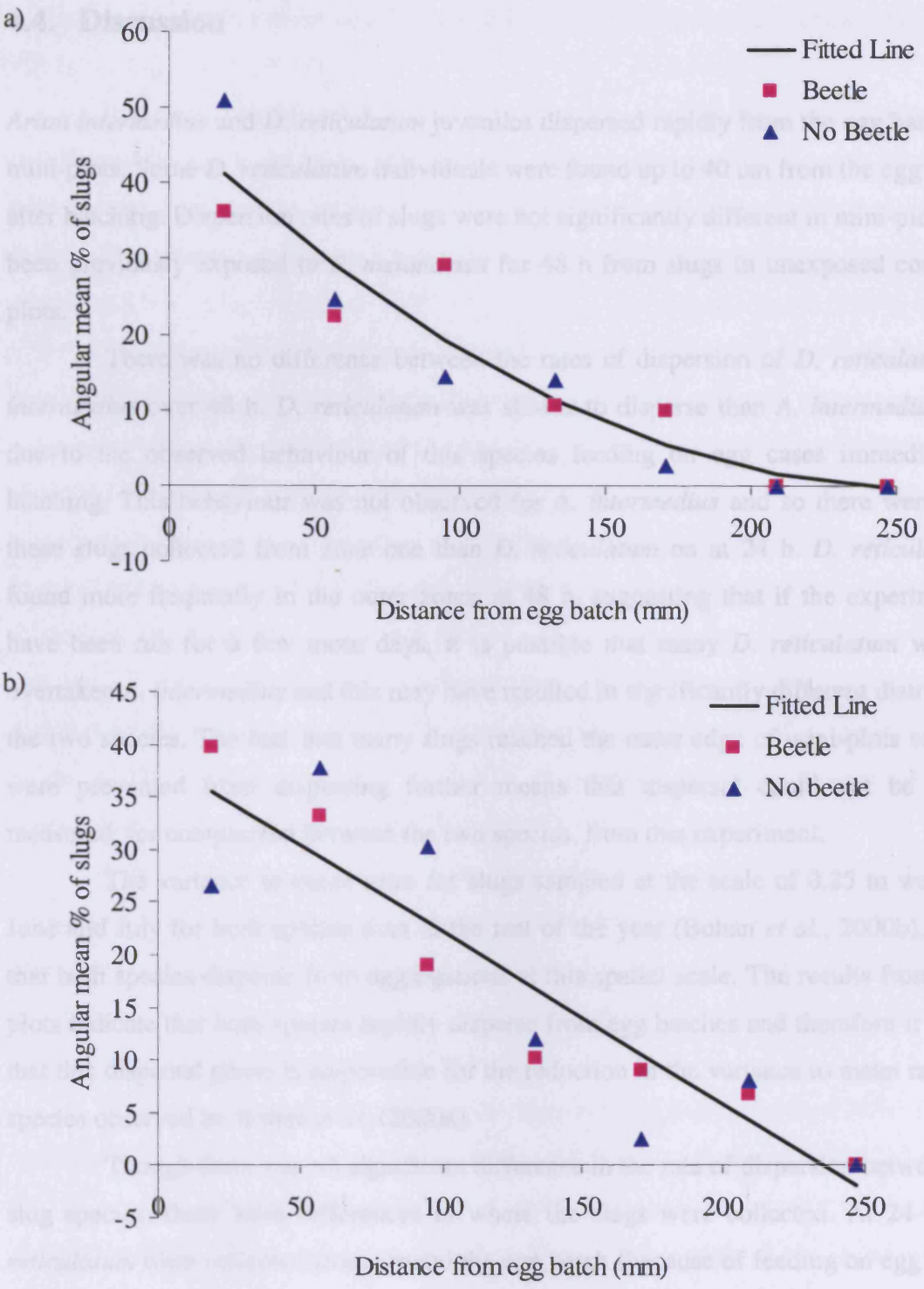

Figure 4.8. Line graphs showing the distributions of *Deroceras reticulatum* in rectangular mini-plots in the second experiment. Angular mean percent of slugs in each concentric zone (a) 24 h and (b) 48 h after hatching in mini-plots previously or not previously exposed to *Pterostichus melanarius*. Lines for the best model were fitted to the data set using regression analysis.

4.4. Discussion

Arion intermedius and *D. reticulatum* juveniles dispersed rapidly from the egg batches in the mini-plots. Some *D. reticulatum* individuals were found up to 40 cm from the egg batch 72 h after hatching. Dispersion rates of slugs were not significantly different in mini-plots that had been previously exposed to *P. melanarius* for 48 h from slugs in unexposed control mini-plots.

There was no difference between the rates of dispersion of *D. reticulatum* and *A. intermedius* over 48 h. *D. reticulatum* was slower to disperse than *A. intermedius* initially, due to the observed behaviour of this species feeding on egg cases immediately after hatching. This behaviour was not observed for *A. intermedius* and so there were fewer of these slugs collected from zone one than *D. reticulatum* on at 24 h. *D. reticulatum* were found more frequently in the outer zones at 48 h, suggesting that if the experiment could have been run for a few more days, it is possible that many *D. reticulatum* would have overtaken *A. intermedius* and this may have resulted in significantly different distributions of the two species. The fact that many slugs reached the outer edge of mini-plots so soon and were prevented from dispersing further means that dispersal could not be accurately measured, for comparison between the two species, from this experiment.

The variance to mean ratio for slugs sampled at the scale of 0.25 m was lower in June and July for both species than in the rest of the year (Bohan *et al.*, 2000b), indicating that both species disperse from aggregations at this spatial scale. The results from the mini-plots indicate that both species rapidly disperse from egg batches and therefore it is possible that this dispersal phase is responsible for the reduction in the variance to mean ratio in both species observed by Bohan *et al.* (2000a).

Though there was no significant difference in the rate of dispersion between the two slug species, there were differences in where the slugs were collected. At 24 h most *D. reticulatum* were collected from around the egg batch (because of feeding on egg cases) and *A. intermedius* were collected from a variety of refuges, but none were on the soil surface. At 48 h neither species were found in the egg batch and most *D. reticulatum* were not collected until after soil flooding (indicating that they had moved down into the soil). *A. intermedius* were much more surface active and were collected from under surface refuges such as straw and soil clods. This was unexpected as adult *A. intermedius* are reported to be largely

subterranean and *D. reticulatum* is reported to be a highly surface active species (South, 1965).

The small circular mini-plots proved to be too small to measure effectively juvenile slug dispersion. 24 h after the eggs had been added to the mini-plots, some of the slugs of both species had already reached the perimeter and were prevented from dispersing any further. It is possible that some slugs moved back towards the egg batch after reaching the perimeter.

The large outdoor mini-plots proved to be a satisfactory small-scale field simulation for the study of slug dispersion from egg batches. Unlike in the small circular mini-plots, no slugs had reached the perimeter by the end of the experiment; therefore dispersion was not restricted in any way. Caution must be extended when applying the results from semi-field, controlled conditions to the observed patterns in the field. Replication of the exact conditions experienced by slugs in the field using mini-plots is very difficult. These differences were minimised by sowing the wheat at a time of year that seeds are normally sown in the field and leaving them outside over the winter. The experiment was also carried out in May, which is the time of year that many *D. reticulatum* juveniles hatch in the field (Barker, 1991; South, 1992). Weather and crop conditions experienced by slugs during the experiment would therefore have matched those of slugs hatching in the field.

The plastic barriers appeared to have been effective in preventing slugs moving through the soil during the flooding procedure. There was no difference in the numbers of slugs found in the outer zones before and after soil flooding. The tubes were pushed 10 cm into the soil, which is reported to be the maximum depth at which slugs are found in moist soil conditions in the field (South, 1964).

In the main experiment most of the eggs hatched during the first 24 h, although the recovery of slugs from mini-plots was low at 59.0 % for mini-plots sampled after 24 h and 59.1 % for mini-plots sampled after 48 h. These figures compare poorly to the high recovery rates of between 89 and 100 % reported in South (1992) using the original soil flooding method. Lower recovery is probably due to the size of the slugs, which at approximately 2-3 mm are difficult to find and may have been missed. Also, pushing the circular barriers into the soil before flooding may have caused significant slug mortality.

No difference in the distribution of *D. reticulatum* in mini-plots was detected when mini-plots had been previously exposed to *P. melanarius* compared to unexposed control mini-plots. Other than the possibility that juvenile slugs do not disperse more quickly (or more slowly) in areas previously exposed to the predator, there may be other explanations

for why no difference in slug dispersion was detected. Dispersion was only measured over 48 h and it is possible that a difference would only become apparent over many days of dispersion. Clearly if dispersion of juveniles is to be studied over longer time periods then field studies would be required. Hatching slugs may also be stimulated to move away from the egg batch initially to avoid competition with its siblings, find refuge and/or find food and these factors may override any responses to predators. Beetles also demonstrate a type of behaviour known as thigmotaxis - whereby they move around the edge of a mini-plot or arena without regularly crossing the centre. This behaviour was observed in the video tracking study (Section 6.3.1. Chapter 6), although the arenas in that experiment were much smaller than the mini-plots. If beetles rarely moved across the centre of the mini-plot then it is unlikely they would have left behind much chemical trace around the area where the slugs were hatching, and this could be why the slugs did not increase dispersion in mini-plots where beetles had once been present.

There have been few studies of the dispersal of ground dwelling invertebrates and studies of the dispersal of terrestrial molluscs have tended to focus on the movements of the adults. Most studies report a low dispersal rate in slugs and snails compared to other invertebrates, which has been related to the low movement rates of individuals, the difficulty of finding mates and suitable food/refugia. This behaviour usually results in animals of the same species aggregating in suitable areas for long periods of time. For example, the land snails *Mastus butoti* and *M. cretensis* moved randomly and dispersed on average only 50 cm and 100 cm a month respectively, because population density was low and suitable habitat scarce (Parmakelis & Mylonas, 2004). The rock dwelling land snail, *Albinaria coerulea*, had a small mean dispersal rate of 162.4 cm a month, depending on neighbourhood size and habitat structure. Spatial distribution was highly aggregated around areas of suitable refugia (Giokas & Mylonas, 2004). Adult land snails, *Arianta arbustorum* (Frauenfeld), moved randomly within the habitat in which they were found, were highly aggregated (Kleewein, 1999) and moved an average 0.58 m a day in forest clearings and only 0.40 m a day in tall grass (Baur & Baur, 1993). In contrast, the dispersal rates of the large land slug *Arion luscitanics* were much larger with home ranges in the region 12 - 45 m^2, depending on population density. Like *D. reticulatum* (Bohan *et al.*, 2000a), this slug tended to lay eggs in batches and juvenile slugs were found to be aggregated around egg hatch sites (Grimm & Paill, 2001). Dispersal rates of different terrestrial molluscs appear to depend on their population size, habitat suitability/structure and body size. South, (1965) found that adult *D. reticulatum* dispersing from a single point covered only 46 cm in 5 days. Release of adult *D.*

reticulatum into a single location resulted in several days dispersive movement and slugs then developed a home range (South, 1965), or rested for a few days under a suitable refuge (South, 1992). Although movement observed under laboratory conditions is very rapid (South, 1992), previous work has suggested that adult *D. reticulatum* do not move over large distances in the field (Fleming, 1989). However, Bohan *et al.* (2000a) showed that the distribution of *D. reticulatum* changes from month to month. It is possible therefore that during previous studies of the dispersal and distribution of *D. reticulatum*, the effects of predation have not been taken into account.

Juvenile *D. reticulatum* dispersed at an average speed of 4.14 cm day^{-1} in mini-plots not previously exposed to beetles, which is faster than anticipated. If juvenile slugs keep dispersing at a similar rate to that found in mini-plots then this alone would result in a less aggregated population in the summer regardless of the influence of carabid beetles. Carabid beetles may have more influence on slug movements by encouraging slugs to find refuge in the local area, rather than by increasing rates of dispersal. Results from laboratory experiments (Armsworth *et al.*, 2005; Section 3.3.1.2. Chapter 3) showed that slugs > 30 mg avoided areas coated in beetle chemicals, but in the field this may translate to slugs moving down into the soil or up into plants rather than horizontally across the soil surface where they will remain susceptible to attack. Slugs exposed to the predatory carabid *A. parallelepipedus* avoided attack by moving up into cabbage plants (Symondson, 1993b). Evidence from laboratory experiments (Section 3.3.1.6. Chapter 3) indicated that juvenile slugs may have avoided paper previously exposed to *P. melanarius* but because the response was much weaker than that observed in the adult slugs, the response was not significant. The response could be weaker in juvenile slugs because they are less susceptible to beetle predators, being smaller and more difficult to detect (Mckemey *et al.*, 2003; Symondson, 2004). There are examples in the literature of other animals showing graded avoidance behaviour depending on their size and susceptibility to predation (see Chapter 3 discussion).

It is possible that a reduction in the variance to mean ratio observed in the slug population in summer by Bohan *et al.* (2000b) could have partly been caused by beetles aggregating in areas of high slug density and encouraging dispersal of adult slugs, rather than juvenile slugs. The next step would be to design an experiment where the effects of beetles on adult slug movement could be tested in the field and not just in the laboratory. Mini-plot experiments are unlikely to be suitable as larger slugs disperse more rapidly and the mini-plots would have to be very big; this would cause problems when attempting to flood them.

In the field it would also be practically very difficult as slugs are primarily subterranean and small slugs such as *D. reticulatum* are not amenable to mark- recapture experiments.

It can be concluded from these experiments that *D. reticulatum* is less surface active than thought, though this might only be the case for juveniles as adult dispersal was not studied. Juvenile *A. intermedius* and *D. reticulatum* disperse from eggs at a similar rate, though there are some differences in refuge use between the two species. Juvenile slugs disperse faster than expected and was different in mini-plots previously exposed to *P. melanarius*. It is likely that the rapid dispersal of juvenile slugs, regardless of the presence of predators, could alone be responsible for the reduction in the variance to mean ratio for slug populations in summer observed by Bohan *et al.* (2000b).

Chapter 5

The chemical basis of slug anti-predator responses to *Pterostichus melanarius*.

5.1. Introduction

Behavioural studies show that *D. reticulatum* avoids areas recently exposed to the predator *P. melanarius* (Armsworth *et al.*, 2005; Chapter 3). It is likely therefore that beetles leave traces of chemicals behind on surfaces where they have walked and that slugs have exploited this as a means of avoiding areas where there is a higher risk of predation. The work in this chapter is an investigation into the chemical interaction between beetles and slugs, in particular to attempt to isolate chemicals from the exterior of the beetles and then identify them.

Although carabid beetles are important predators of pests, little is known about whether they interact chemically with any of their prey, probably because carabid beetles are typically considered generalists that might not be expected to exert sufficient selection pressure for anti-predator responses to evolve in their prey. Slugs, however, form a major constituent of the diet of *P. melanarius* and this species is known to affect the temporal and spatial dynamics of slugs (Symondson *et al.*, 1996, 2002a; Bohan *et al.*, 2000a; Chapter 2). The evidence now available shows that *D. reticulatum* can detect chemicals produced by *P. melanarius*, distinguish these from non-predatory carabids (Dodds *et al.*, 1997; Chapter 3) and respond by moving away (Armsworth *et al.*, 2005; Chapter 3).

One group of chemicals from carabid beetles that have received extensive study is defensive compounds for protection against predators and diseases. Carabid beetles produce a variety of defensive chemicals that have been reported to perform several different functions, such as repellents, toxicants, insecticides and antimicrobics (Dettner, 1987). Carabids, as well as other terrestrial adephagan beetles, possess a pair of pygidial glands that open onto the abdomen, close to the anus, through which secretions can be vented. Defensive secretions appear universal throughout the Carabidae though there is variation in their chemical constituents and size of pygidial glands (Moore & Wallbank, 1968).

Chemicals from carabids are either secreted onto the cuticle or ejected by muscular contraction of gland reservoirs. Three distinct classes of compounds have been identified in

the defensive secretions of Carabidae: aldehydes, acids and quinones (Moore & Wallbank, 1968). Pterostichini have been separated into three groups that secrete either: formic acid and alkanes, high molecular weight carboxylic acids (primarily methacrylic and tiglic acid), or salicylaldehyde (Moore, 1979; Dettner, 1987; Will *et al.*, 2000). *P. melanarius* are known to produce both methacrylic and tiglic acid (Jacobson, 1966). These compounds probably serve the purpose of making them distasteful, both in taste and smell, to predators such as birds and mammals. Although a lot is known about carabid defensive chemicals, no one has investigated the effects of these chemicals on their prey.

Since *P. melanarius* has not been reported to spray defensive chemicals, it is likely that these compounds are secreted onto their cuticle. If enough of the defensive compound(s) (or other compounds that the beetles might be producing) are left behind on surfaces where the beetles have been present then it is possible that slugs might have adapted to recognise the chemical(s) and exploit it as a cue for avoiding the predator. This would explain avoidance of paper exposed to *P. melanarius* by slugs in behavioural bioassays (Armsworth *et al.*, 2005; Chapter 3). In this chapter, the chemicals on the exterior of *P. melanarius* were isolated and analysed. The null hypothesis that chemicals isolated from the cuticle of *P. melanarius* are detected and avoided by *D. reticulatum* was tested using leaf disc choice bioassays. The work in this chapter was carried out in collaboration with members of the Biological Chemistry (BCH) division at Rothamsted Research, Harpenden, U.K.

5.2. Methods

Isolation of the chemicals responsible for eliciting anti-predator behaviour in slugs involved three important stages. Firstly the chemicals present on the exterior of the beetles were dissolved in solvent to create a beetle chemical extract. The chemicals in the extract were then separated using high performance liquid chromatography (HPLC) and thin layer chromatography (TLC). Finally the chemicals in two of the extract fractions were analysed using mass spectrometry and nuclear magnetic resonance (NMR) spectroscopy. At each stage the extracts were tested in leaf disc choice bioassays for repellent and antifeedant effects against slugs.

5.2.1. Creating a beetle chemical extract

20 male and 20 female live *P. melanarius* were collected from dry pitfall traps in a field of winter wheat close to Rothamsted. The males were immersed in freshly distilled diethyl ether (20 ml) for four hours. The procedure was repeated for the female beetles.

The extracts were decanted from the beetles, dried with anhydrous magnesium sulphate, filtered through cotton wool and then evaporated under reduced pressure until approximately 0.5 ml of each solution remained. The solutions were analysed using TLC, which showed that male and female extracts contained the same chemicals, although there may have been slight differences in the quantities of each chemical that the beetles produced (Hasse Rasmussen, pers. comm.). Since both solutions contained the same chemicals, they were combined to produce an extract of *P. melanarius*.

5.2.2. Leaf disc choice bioassay one - testing the crude beetle extract

Six Petri dishes (90 mm diameter) were lined with moistened cotton wool. Using a marker pen a line was drawn on each of the Petri dish lids to separate the dishes into two equal halves. Each half was labelled with either 'test' or 'control' (three with the control on the left and three with the control on the right) and the dishes were numbered 1-6.

36 leaf discs of 11 mm diameter were cut from leaves of Chinese cabbage using a leaf disc cutter. Control discs were created by adding 20 μl of ether to the exact centre of 18 of the discs using a pipette. The discs were then placed on a sheet of moist filter paper for five minutes until all the ether had evaporated. Test discs were created by 20 μl of the beetle extract to the exact centre of each of the remaining leaf discs using a pipette. The discs were then placed on a separate sheet of moist filter paper for five minutes until all the ether had evaporated. Three of the control leaf discs were arranged in a vertical line on the cotton wool in the control half of each Petri dish. Three of the test discs were then also arranged in a vertical line down the test half of each Petri dish.

Six adult *D. reticulatum* were collected from cover traps baited with chicken layers mash from a field of winter wheat close to Rothamsted. A slug (300-600 mg) was placed into the exact centre of each of the Petri dishes using a paintbrush. The lids were then placed over the dishes and the dishes were then stored in a dark incubator at 5 °C (+/- 1 °C) for 16 h. After this time period the slugs were removed from the dishes. Each leaf disc was compared to a template disc (11 mm diameter) cut from 1 mm graph paper and the area of each leaf disc that had been consumed was recorded. The proportions of leaf discs consumed were compared between test and control discs using a one-way ANOVA with blocking for dishes.

5.2.3. Separating the beetle extract into fractions based on polarity

50 male and 50 female *P. melanarius* were immersed in 50 ml of freshly distilled diethyl ether for 48 h. The solvent was then decanted and a further 50 ml of diethyl ether was added. The combined washings were evaporated under reduced pressure. The crude residue was subjected to vacuum liquid chromatography (Coll & Bowden, 1986; Pelletier *et al.*, 1986) over TLC grade silica (Merck silica gel 60H - Merck & Co, Inc.), eluting with 9:1 hexane:ether mixture, moving to 100 % ether, to yield 25 fractions. Liquid chromatography separates compounds on the basis of their polarity: earlier fractions contained the most apolar compounds, and the later fractions contained compounds of higher polarity. All the fractions were analysed by TLC and the fractions were pooled based on polarity. This left five fractions that contained different compounds: A, B, C, D and E (Appendix D). Fraction A was not tested because it contained cuticular wax, not a substance that the beetles would leave behind on surfaces to be detected or avoided by slugs (Hasse Rasmussen pers. comm.).

5.2.4. Leaf disc choice bioassay two - testing the beetle extract fractions

Samples from each of the extracts B-E were tested in leaf disc choice bioassays. The protocol follows that of the previous bioassay (Section 5.2.2) with the exception that eight replicate dishes were set up for each of the four fractions B-E.

The proportions of leaf discs consumed were compared between test and control discs for each extract using one-way ANOVAs with blocking for dishes.

5.2.5. Identifying compounds in extract fractions using mass spectrometry

Fractions C and D were analysed by electron impact mass spectrometry. This technique involved the bombardment of molecules with electrons, leading to the cleavage of the molecule and formation of positively charged fragments, which are accelerated though a mass analyser before reaching a detector (Wilson & Walker, 2000).

Each sample was introduced into the heated source area of a MAT95XP mass spectrometer via a probe-tip containing a sample cup. The sample was vaporised and bombarded with electrons produced by a heated filament. The electrons caused the molecule to disintegrate by losing electrons, producing fragment positive ions. These ions were unstable and so disintegrated into smaller ions, which themselves were unstable and fragmented further into a series of product ions. The ions and product ions were accelerated out of the source and into a mass analyser. The mass analyser recorded the mass to charge ratio (m/z) for each ion, where m is the mass and z is the number of charges carried by the ion

(in this case z = 1). The *m/z* is what is represented in the spectrum and there is a peak representing each of the ions and product ions.

The mass spectrum of each sample was compared to known mass spectra of chemicals stored on a database (NIST, 2002) to find the closest possible match to the mass spectra of the chemicals being identified.

5.2.6. Identifying compounds in extract fractions using NMR spectroscopy

The compounds in fractions C and D were also analysed using NMR spectroscopy, and the output from these experiments is still being examined. Fraction C was analysed using both proton ^{1}H NMR and carbon C NMR. Fraction D was analysed using ^{1}H NMR only as there was not enough of the sample to analyse it using C NMR.

5.3. Results

5.3.1. Leaf disc choice bioassay one - testing the crude beetle extract

Coating leaf discs with the beetle extract significantly reduced feeding by slugs compared to control discs (ANOVA: $F_{1,29} = 5.96$, $P = 0.021$) (Table 5.1.).

Table 5.1. The mean proportions of control and test leaf discs eaten by each *Deroceras reticulatum*. Test discs were coated with the crude *Pterostichus melanarius* extract (n = 6).

Slug/Dish	Extract coated test discs	Control discs
A	0	0.563
B	0.330	0.317
C	0	0.850
D	0.350	0.080
E	0.590	0.593
F	0	0.397
Mean total	0.212	0.467

5.3.2. Leaf disc choice bioassay two - testing the extract fractions

All of the fractions tested reduced feeding on the treated leaf discs compared to the control discs (Figure 5.1.). Fraction C did not significantly reduce feeding upon treated discs compared to control discs (ANOVA: $F_{1,47} = 1.22$, $P = 0.276$). Fraction B reduced feeding upon test discs compared to control discs but this difference was only just significant

(ANOVA: $F_{1,47} = 4.14$, $P = 0.049$). Fractions D and E were most active against slugs and significantly reduced feeding compared to control discs (ANOVA: $F_{1,47} = 8.07$, $P = 0.007$ and ANOVA: $F_{1,47} = 4.31$, $P = 0.045$ respectively). Less area of the control discs in dishes with discs coated in extract D and E were also eaten compared to dishes containing discs coated in fractions C and B (Figure 5.1.). It seems that D and E had an antifeedant effect on the slugs as well, causing them to feed less on test and control discs in their dishes.

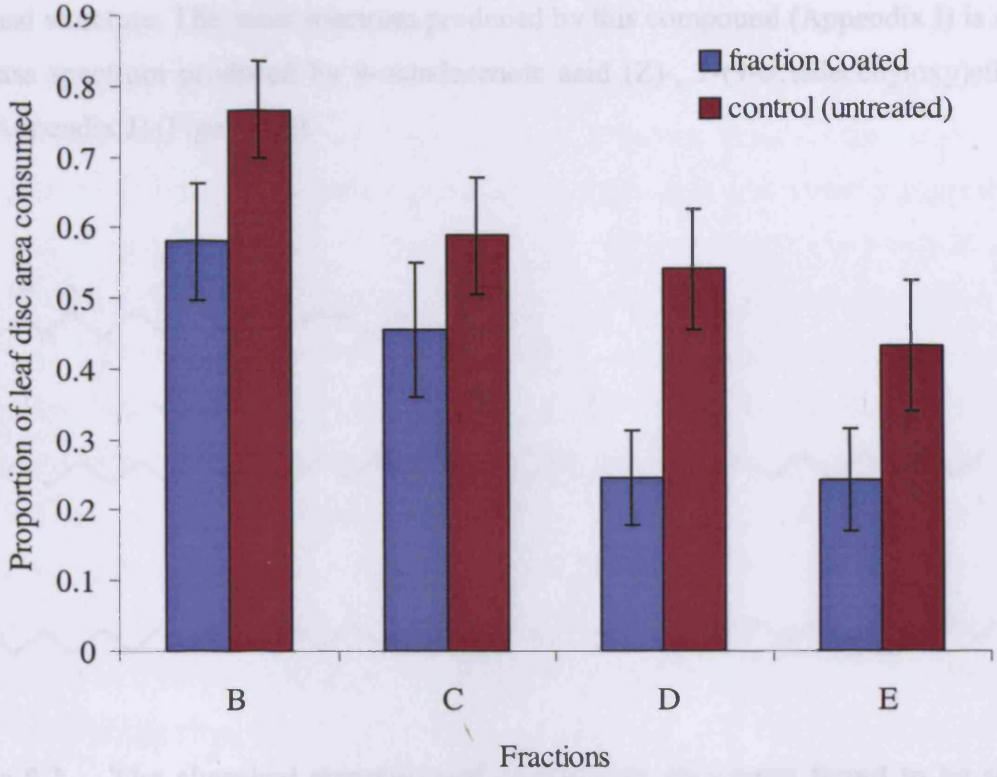

Figure 5.1. Bar graph showing the areas of control and treated discs eaten by *Deroceras reticulatum*. Test discs were coated in either fractions B, C, D or E. Closed bars represent standard errors.

5.3.3. Identifying compounds in extract fractions using mass spectrometry

The electron impact ionisation spectrum of fraction C is given in Appendix E. The percent relative abundance is a scale whereby the largest peak (the base peak) in the spectrum (55 *m/z* in this case) was set at 100 %, and all the others were calculated in proportion as a percentage. The mass spectrum of the chemical in fraction C was similar to the chemical 9-octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester (Appendix F). The chemical structure is lipid derived because it contains a fatty group, similar to glycerol, attached to a

string of 18 carbon atoms (with a double bond connecting carbon 9 and carbon 10) (Figure 5.2).

Fraction D produced two peaks. This could have arisen through degradation under the harsh conditions in the source area of the mass spectrometer. The first peak was produced by a straight-chain hydrocarbon, similar to the chemical 17-pentatriacontene (Appendix G and H). 17-pentatriacontene contains a chain of 35 carbon atoms, with a double bond between carbon 17 and carbon 18 (Figure 5.2). The second compound has a more complex chemical structure. The mass spectrum produced by this compound (Appendix I) is similar to the mass spectrum produced by 9-octadecenoic acid (Z)-, 2-(9-octadecenyloxy)ethyl ester, (Z)- (Appendix J) (Figure 5.2).

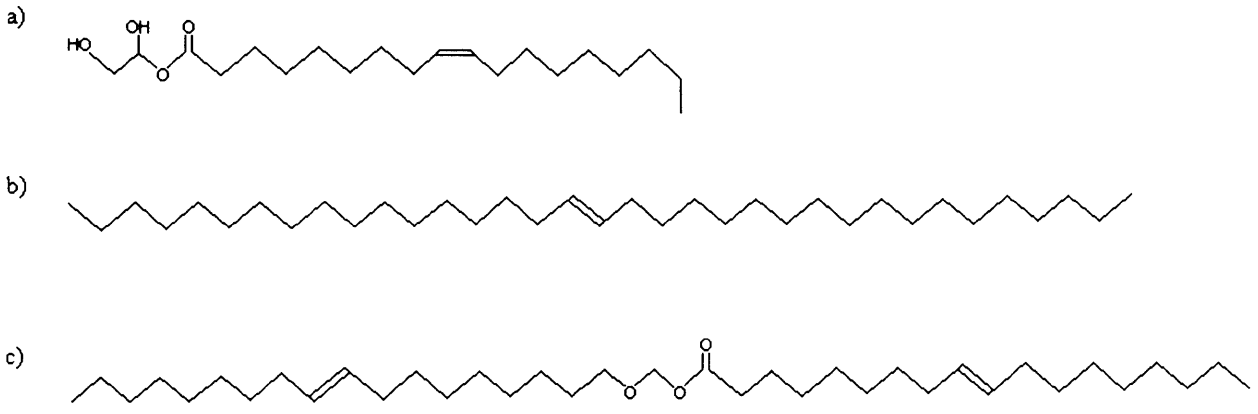

Figure 5.2. The chemical structures of compounds that were found to be similar in structure to compounds found in fractions C and D. Chemical structure of a) chemical 9-octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester; b) 17-pentatriacontene and c) 9-octadecenoic acid (Z)-, 2-(9-octadecenyloxy)ethyl ester, (Z)-

5.3.3. Identifying compounds in extract fractions using NMR

The output from these experiments is still under examination. It is hoped that the results from this combined with the information collected with mass spectrometry will aid identification of the compounds.

5.4. Discussion

Chemicals from the exterior of *P. melanarius* were successfully isolated and analysed. Furthermore, two of the chemicals, in fractions D and E, were shown to have antifeedant effects on slugs in leaf disc bioassays. These findings support the behavioural work in Chapter 3 (Armsworth *et al.*, 2005 - Appendix A), in particular the findings that residual chemicals from *P. melanarius* on soil reduced feeding by slugs on leaf discs compared to discs that had been placed on control soil (Section 3.3.3. Chapter 3).

Fractions C and D were analysed using NMR and mass spectrometry. Work is currently underway to identify the chemicals in each fraction based on the output from these techniques. Preliminary examination of the output from mass spectrometry suggests that the compound in fraction C is lipid derived and is very similar in structure to 9-octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester. Slugs did not avoid eating leaf discs coated in fraction C. The compound in fraction D split into two separate molecules during analysis making it difficult to determine the original structure of the compound. One of the molecules is a simple straight chain hydrocarbon similar in structure to 17-pentatriacontene and the other is a more complex molecule similar to the compound 9-octadecenoic acid (Z)-, 2-(9-octadecenyloxy)ethyl ester, (Z)-. It is hoped that analysis of the NMR output will aid the identification of the entire compound, especially since it was the chemical in this fraction that was found to be active against slugs in leaf disc choice bioassays.

Although these specific chemicals are not mentioned in conjunction with carabid beetles in the literature, a possible function for 9-octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester (fraction C) was found. In the defensive secretions of some staphylinid beetles, ethylesters of octadenoic acid act as solvents for toxic quinones (Dettner, 1987). Since not all the components of the *P. melanarius* extract have been identified it is possible that one of them might have been a quinone. Quinones are known to be produced by many carabids as a component of their defensive secretions (Moore & Wallbank, 1968; Dettner, 1987; Will *et al.*, 2000), usually in conjunction with nonpolar components such as hydrocarbons, ketones or esters. Dettner (1987) suggests that the nonpolar components might facilitate penetration of the toxic quinones into epicuticles.

Given time it would be interesting to isolate a crude extract from other carabid beetles and compare the chemical profiles with *P. melanarius*. It is likely that *P. madidus* also produces the same active chemical as *P. melanarius* as residual chemicals from these

beetles were also avoided by *D. reticulatum* in choice tests (Chapter 3). Biochemical analysis of *P. madidus* could determine if this was the case. Closely related species of Carabidae are reported as usually having a common mode of chemical defence (Moore & Wallbank, 1968). If the chemical(s) that are active against slugs have a defensive role for *P. melanarius* then this could explain why *P. melanarius* and *P. madidus* exposed papers were both avoided by slugs. Another carabid beetle worth testing would be *P. cupreus*, which is in the same genus as *P. madidus* and *P. melanarius*, but slugs did not avoid paper exposed to this beetle. Possibly this species does not produce the active chemical(s), or produces them in lower quantities.

Chapter 6

Analysis of the searching and feeding behaviour of the slug predator *Pterostichus melanarius*

6.1. Introduction

Most of the work in this thesis has focussed on the slug side of the predation interaction, and results have shown that slugs avoid sources of chemical cues from *P. melanarius* (Chapters 3 and 5). It has been suggested that this may help explain spatial dissociation between the two species observed in late summer (Bohan *et al.*, 2000a; Chapter 2), combined with the effects that density-dependent predation will have upon slug numbers in areas of high beetle density. However, to understand better the interaction, it is important also to investigate how beetles respond to their slug prey and how this might influence the spatial interaction in the field. Firstly, how do the beetles find aggregations of slugs in the field that might explain patterns of spatial association between the two species in early summer? Secondly, is there any aspect of the beetle's behaviour that might explain why they do not remain spatially associated with slugs throughout the summer?

Research into the olfactory responses of carabid beetles to prey, including responses by *P. melanarius* to slugs and other prey, shows that some beetles can detect and respond to chemical cues from certain prey items (Wheater, 1989; Digweed, 1994; Ayre, 1995; Kielty *et al.*, 1996; McKemey *et al.*, 2004). *P. melanarius* has been shown to be spatially associated with aphids in the field (Winder *et al.*, 2001, 2004) and work by other scientists shows that *P. melanarius* detects an aphid alarm pheromone (Kielty *et al.*, 1996) and aggregates in areas of a field where the chemical has been released (Kirkland, 1999). The specialist slug-feeding carabid, *Carabus nemoralis* (Müller), has been shown to orientate towards slug mucus trails but only early in the summer; by late June the response had disappeared (Digweed, 1994). Ayre (1995) showed that *Carabus problematicus* (Herbst), *Carabus nemoralis* (Müller), *Cychrus caraboides* (Linné), *Pterostichus niger* (Schaller) and *P. madidus* responded to patches of slug mucus by slowing down and stopping in these areas. *C. problematicus*, *C. nemoralis* and *P. madidus* demonstrated an orthokinetic response and *C. caraboides* demonstrated a klinokinetic response to mucus patches. Wheater (1989) showed that three

other species of carabid beetles orientate towards volatile cues from the mucus of the slug *Arion subfuscus* (Draparnaud): *Carabus violaceous* (L.), *C. caraboides*, and *C. problematicus*, whilst *P. melanarius* did not respond. Recently McKemey *et al.* (2004) showed that *P. melanarius* did respond to patches of slug mucus via contact with these areas but not when volatile chemicals from slugs were blown over beetle antennal preparations. According to McKemey *et al.* (2004) the contact response to mucus disappeared when the antennae were removed indicating a role for these in mucus detection, and beetles exhibited kinesis when encountering these patches. Wheater (1989) found the palps to be important in mucus detection by carabid beetles as the response was lost when either the labial or maxillary palps were coated in glycerol. There seems to be some contention therefore over the mechanism by which beetles detect mucus.

Using the experimental arenas and design developed for studying the behaviour of slugs encountering patches of beetle-exposed paper, a similar experiment to McKemey *et al.* (2004) was attempted whereby movements of beetles with different organ amputation treatments in mucus and control zones were compared. The purpose of this experiment was to compliment the slug video tracking study (Chapter 3; Armsworth *et al.*, 2005) by looking at the opposite side of the predation interaction and measure movements of beetles in response to slug chemical cues. A further aim was to elucidate further the mechanism by which beetles detect and respond to mucus, and validate or contest the results from the study by McKemey *et al.* (2004).

The second part of this chapter focuses on whether beetle diet choice might influence the spatial relationship with slugs (Bohan *et al.*, 2000a). Results from Chapter 2 indicated that foregut as a proportion of the body mass of beetles was greater in June when there was spatial association than in July when there was dissociation. The foregut as a proportion of the body mass was also greater in slug-positive than slug-negative beetles in June, but there was very little difference in July. These results seem to indicate that slugs were not such an important prey item in July as in June, and that beetles ate more alternative prey in July. It is possible that in July beetles ate a wider range of prey because of increasing availability of other prey and/or decreasing availability of slugs. Evidence from laboratory work suggests that carabid beetles do better on a mixed diet, in terms of putting on weight and producing more eggs (Wallin *et al.*, 1992; Bilde & Toft, 1994). It might be expected therefore that in order to maximise their fitness beetles should choose a mixed diet when a variety of prey are available. A laboratory feeding study was carried out whereby male and female *P. melanarius* were offered a choice of several prey items after different feeding

regimes (one of which was slugs only). The purpose of the experiment was to investigate how beetles respond to prey diversity and whether previous feeding experience influences prey choice. Several null hypotheses were tested:

- Beetles do not select a particular prey item when several are available, but choose a mixed diet.
- There is no difference in weight change between beetles fed on different diets.
- Previous feeding experience does not influence prey choice.

6.2. Methods

6.2.1. Beetle movements in response to areas of slug mucus

D. reticulatum were collected from baited cover traps and stored in boxes containing moist cotton wool and food (Chinese cabbage, clover, dandelion and carrot) for up to two weeks before the experiment.

P. melanarius were collected in pitfall traps and stored in peat filled boxes in a CE room (L: 16 h; D: 8 h) at 15 °C for up to two weeks before the experiment and fed on dog food. Amputations of beetle sensory organs were made using nail clippers. Each beetle was held firmly in a pair of tweezers and the antennae were quickly clipped off at the base. For the palps, both pairs were clipped off at the base. Beetles were then isolated in individual Petri dishes lined with moist cotton wool for 24 h at 18 °C (+/-2 °C) before video tracking.

A set of test papers coated in slug mucus were created for each recording. The sides of three Perspex boxes (26 × 14 × 9 cm) were painted with FLUON to prevent slugs climbing, and left to dry overnight. Once dry they were each lined with moist cotton wool. On top of the wool, the boxes were lined with two sheets of filter paper (14 × 13 cm), one at each end, so that they met in the middle and covered the cotton wool. Six adult slugs were randomly assigned to each box, three on each piece of paper. The boxes were stored in a dark incubator at 15 °C (± 2 °C) for 24 h, after which the slugs were removed.

Six arenas were filmed simultaneously over a video-tracking period of 30 mins. Recordings were made for male and female beetles with the following amputation treatments: intact (no amputation), beetles with the palps removed, beetles with the antennae removed and beetles with both the antennae and the palps removed (no organs). There were

ten replicate arenas for intact beetles, beetles with palps removed and beetles with antennae removed, and twelve replicate arenas for beetles with both sensory organs removed.

Arenas were made from Perspex boxes (similar to those described above but not painted with FLUON), and were lined with moist cotton wool. Half of each arena was lined with control paper, and the other half was lined with test mucus paper. The arenas were arranged on a bench in a controlled environment room (18 ± 1 °C) under a monochrome video camera so that three of the arenas had the control paper on the left and the other three arenas had the control paper on the right (Figure 6.1.). The room was illuminated with a red strip light with an additional desk lamp fitted with a red bulb mounted directly overhead to provide uniform lighting over the arena floors. The video camera was connected to a VCR.

A single *P. melanarius* was placed in each arena; for each amputation treatment one of the beetles was placed onto a control paper and the other onto a mucus coated paper in the other arena. Beetles were allowed two minutes to settle and then the recording was started. Each beetle and each piece of control and slug-exposed paper was only used once and arenas were washed thoroughly after each use.

The video data were analysed using EthoVision Pro, (see Chapter 3). The program calculated parameters describing the movement and activity of the beetles directly from the recordings. For each recording all six test arenas and "zones" for the control and slug-exposed papers, within each arena, were defined on the computer screen. The digitised images were sampled at the rate of five per s and 30 mins of real-time footage was analysed each time.

The movement parameters selected for analysis included time spent in the zone, distance moved, mean velocity, mean absolute angular velocity, mean absolute meander and time spent moving (see Section 3.2.2., Chapter 3). The time spent moving was the total time spent moving in each zone and included any time when the beetle moved more than 0.2 cm between image samples. This threshold was set to avoid slight movements caused by body wobble being interpreted as movement. The time spent moving by beetles in each zone was then divided by the time spent in each zone to get the proportion of time in each zone that was spent moving.

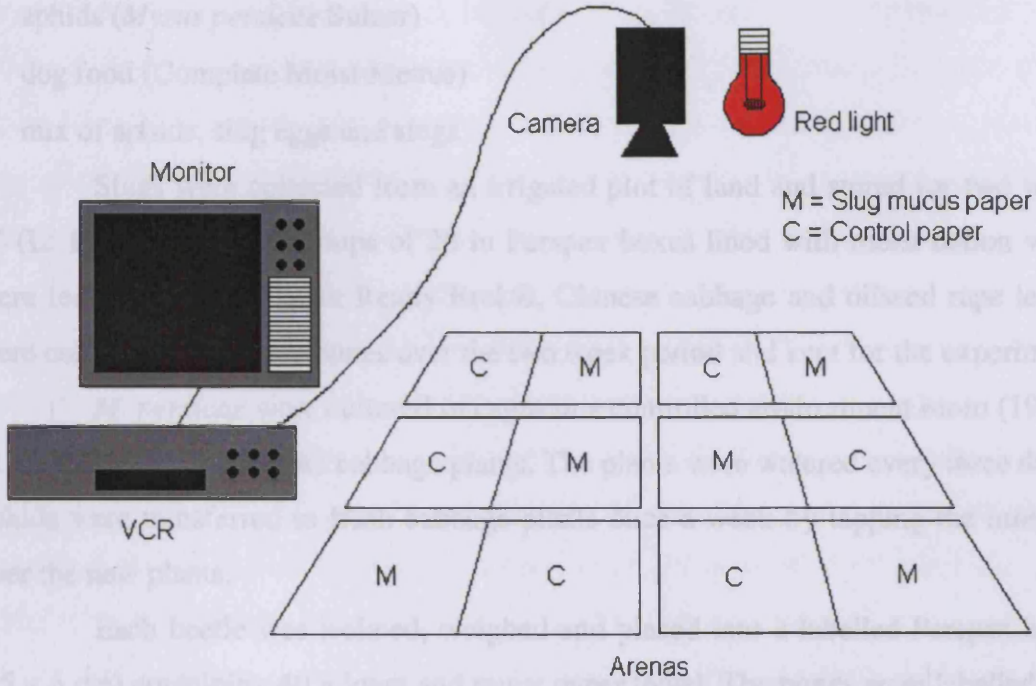

Figure 6.1. The experimental design used in the *Pterostichus melanarius* video tracking study. Video footage was recorded of beetles moving on slug exposed and control areas within arenas. Six arenas were filmed simultaneously.

Some tracks had to be discarded because beetles had either not moved at all during the tracking period or they had managed to crawl underneath the paper during recording.

Each parameter of movement was analysed using a three-way ANOVA with factors for beetle sex (male or female), beetle organ amputation treatment (intact, no antennae, no palps or no antennae and no palps) and zone (control or mucus-coated).

6.2.2. Beetle diet choice study

70 beetles (35 males and 35 females) were collected from pitfall traps up to two weeks before the experiment. During this period they were fed on dog food twice a week and stored in boxes at 18 ºC with moist loam and moist paper towel.

The beetles were separated into groups of either ten males or ten females. Each group of ten male and ten female beetles were given a different preliminary diet after a starving period. The five diets were as follows:

- live slugs (*D. reticulatum*)
- slug eggs (*D. reticulatum*)

- aphids (*Myzus persicae* Sulzer)
- dog food (Complete Moist Menus)
- mix of aphids, slug eggs and slugs

Slugs were collected from an irrigated plot of land and stored for two weeks at 10 °C (L: 16 h; D: 8 h) in groups of 20 in Perspex boxes lined with moist cotton wool. Slugs were fed twice a week with Ready Brek®, Chinese cabbage and oilseed rape leaves. Eggs were collected from these boxes over the two week period and kept for the experiment.

M. persicae were cultured in cages in a controlled environment room (19 °C ± 2 °C; L: 16 h; D: 8 h) on Chinese cabbage plants. The plants were watered every three days and the aphids were transferred to fresh cabbage plants once a week by tapping the infested leaves over the new plants.

Each beetle was isolated, weighed and placed into a labelled Perspex box (13.5 × 7.5 × 6 cm) containing 40 g loam and moist paper towel. The boxes were labelled to indicate the sex of the beetle and the feeding regime. The beetles were stored in this way for seven days at 18 °C (± 2 °C; L: 16 h; D: 8 h) and not fed.

The different feeding regimes were started after the seven day starving period. Aphids and slug eggs were provided in high numbers to begin with to ensure that the beetles would not go hungry, and the numbers of eggs and aphids were topped up when needed. Beetles fed on slugs were each given one slug (100 - 500 mg) to begin with, which was replaced whenever it had been eaten overnight. Beetles fed on dog food were each given 0.3 g Complete Moist Menus® dog food, which was replaced whenever necessary. Food that wasn't eaten was replaced with fresh food regularly. Beetles were fed these regimes for three weeks and supplied with fresh paper towel once a week.

After the three week diet regime each beetle was weighed and then put into a separate Petri dish. Each dish was labelled with the sex of the beetle and the feeding regime that they had been given. Into each Petri dish a choice of diet was provided: one slug (100 - 500 mg), twenty fully mature non-winged aphids and ten slug eggs. The dishes were stored overnight at 18 °C (± 2 °C; L: 16 h; D: 8 h) and a record was made of what had been eaten by each beetle after a period of 24 h.

6.2.2.1. Statistical analysis of beetle diet choice study

For each food type (slugs, eggs and aphids) the proportions eaten by beetles under each treatment were modelled using generalized linear models for univariate analyses assuming a

binomial distribution (McCullagh & Nelder, 1989). This analysis was selected in favour of ANOVA as the data were non-continuous and binomially distributed. Only one slug was fed to each beetle, therefore there was no accurate way of determining the proportion of each slug eaten. The proportion of each slug eaten was therefore taken out of two; zero being no slug eaten, one being partially eaten and two being completely eaten.

In each model a constant term was fitted for the mean proportion of the food type eaten. Beetle sex, previous diet and sex × previous diet were fitted as factors in each model to see if there were any significant effects. Over-dispersion (see, for example, Collett, 1991) of the data, given that expected for a proposed binomial distribution, was accounted for where necessary by setting the dispersion parameter to the residual mean deviance rather than assuming unity in the calculation of standard errors. All analyses were run using the GenStat (2003) (GenStat 7th ed. - VSN International Inc.) statistical package.

Since the proportions of each food type eaten are not independent of each other (for example if a beetle eats a whole slug then this will probably affect how many aphids and eggs are eaten by this beetle too) the data were also analysed using canonical variates analysis (CVA) (see, for example, Krzanowski, 1998). This analysis allows the simultaneous comparison of more than one variate and so in this case allows comparison of the amounts of all three foods eaten by male and female beetles fed the five different diets. CVA looks for directions of variability maximising differences between the groups (sex, previous diet and sex × previous diet). The proportion of each food type was analysed, rather than the counts because the beetles were fed different numbers of each prey type.

There are three variables (proportions of eggs eaten, slugs eaten and aphids eaten) and so the data can be visualised as a 3D graph. CVA produces a linear combination of the three variables to plot on a 2D axis. A first line, or vector, through the data, is produced called the first canonical variate. This is given by the equation CV1 = α (proportion eggs eaten) + β (proportion slugs eaten) + γ (proportion aphids eaten). CVA calculates the values of α, β and γ (the loadings) so that they provide the new axis (i.e. CV1) that maximises the differences between the groups (the between group variability relative to the within group variability). The total number of canonical variates (CVs) is given by minimum (G-1, p) (whichever is lowest), where G is the number of levels of a factor (for this analysis: two for sex, five for previous diet and ten for sex × previous diet) and p is the number of variables (for this analysis there are three). Therefore, the maximum number of CVs we can have for this analysis is one for the sex factor, three for the previous diet factor and three for the sex × previous diet factor. The CVA calculates the CV1 score (and CV2 and CV3 scores if

appropriate) for each original data point using the loading values, and so transposes them onto these new co-ordinate axes. Hence, for each beetle:

$(\alpha, \beta, \gamma) \times$ (proportion aphid eaten, proportion slug eaten, proportion eggs eaten)$^{\text{Transposed}}$ = CV score for CV1

Plotting the scores on the first CV or on the first two CVs when there is more than one CV allows visualisation of the differences between groups. The group means for the CVs are then also plotted on the new axis and a 95 % CI is drawn around them. If the circles from the group means all overlap then there is no difference between them at the $P = 0.05$ level.

6.3. Results

6.3.1. Beetle movements in response to slug mucus

On visual examination of all the beetle tracks, a lot of movement occurred around the edges of the arenas (see example in Figure 6.2.).

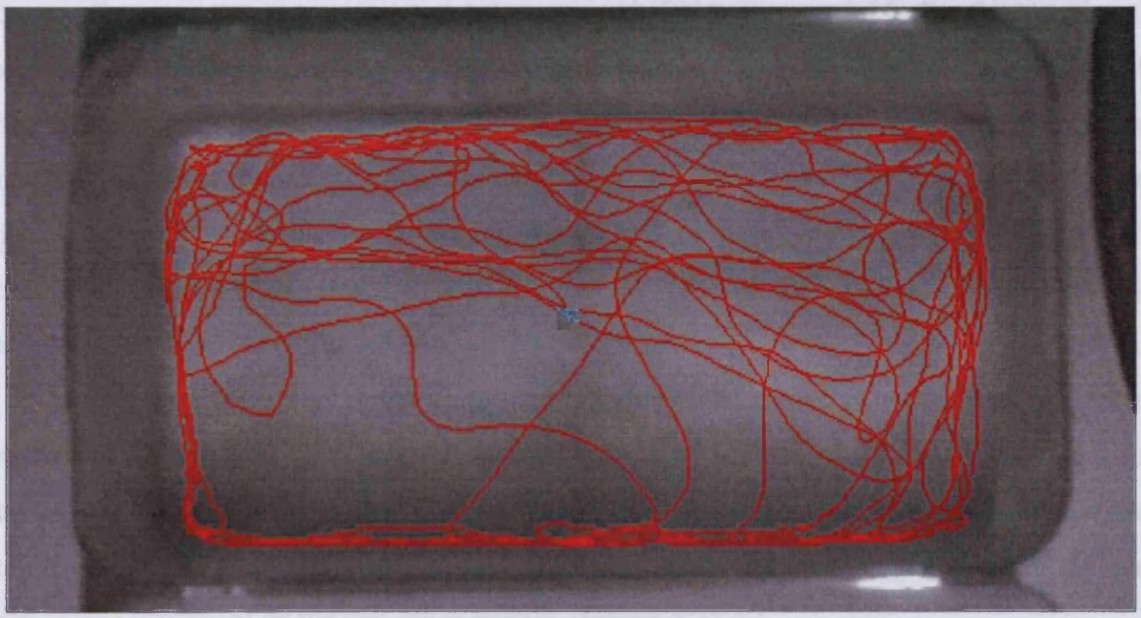

Figure 6.2. Example of a *Pterostichus melanarius* track in an arena. The beetle has not had any organs amputated. The control paper is on the left and the *Deroceras reticulatum* mucus coated paper on the right. The track is highlighted in red and the starting point of the beetle is marked by a blue portion of the track.

There was a significant interaction between time spent in each zone and amputation treatment (ANOVA: $F_{3,126} = 6.61$, $P < 0.001$) but no other factors or interactions were significant for this movement parameter. Comparisons using LSDs showed that both male and female beetles with only their antennae removed spent significantly more time in mucus zones than in control zones (Figure 6.3.). No other comparisons were significantly different.

Male beetles moved significantly greater distances than females in arenas (ANOVA: $F_{1,126} = 18.77$, $P < 0.001$). No other factors or interactions were significant when analysing the distances moved (Figure 6.4.).

For the velocity of beetles, sex was a significant factor, with males beetles moving at greater velocity than females overall (ANOVA: $F_{1,126} = 5.19$, $P = 0.024$). Zone was a significant factor (ANOVA: $F_{1,126} = 5.10$, $P = 0.026$) and the interaction between zone and amputation treatment was also significant (ANOVA: $F_{3,126} = 5.65$, $P < 0.001$). By comparing LSDs, velocity was significantly greater in control zones than in mucus zones for both male and female beetles that had only their antennae removed. For no other organ amputation treatment was this difference significant (Figure 6.5.).

When beetle angular velocity was analysed, there was a significant interaction between the amputation treatment and the zone (ANOVA: $F_{3,126} = 3.26$, $P = 0.024$). No other factors or interactions were significant. Comparison using LSDs showed that male beetles with only their antennae removed had significantly greater angular velocity in mucus zones than in control zones. Female beetles with only their antennae removed also had greater angular velocity in mucus zones though the difference was not significant. There were no other significant differences between beetles in control and mucus zones with other amputation treatments (Figure 6.6.).

There was a significant interaction between meander in zones and amputation treatment (ANOVA: $F_{3,126} = 3.66$, $P = 0.014$). Comparisons using LSDs revealed that male beetles with only their antennae removed had significantly greater meander in mucus zones than in control zones; this difference was not significant for females with the same amputation treatment or for any other beetles (Figure 6.7).

When analysing the proportion of time spent moving there was a significant interaction between zone and amputation treatment (ANOVA: $F_{3,126} = 5.25$, $P = 0.002$). By comparison of LSDs, both male and female beetles with only their antennae removed spent a significantly greater proportion of time moving on control paper than mucus paper. For no other amputation treatments was this difference significant (Figure 6.8.).

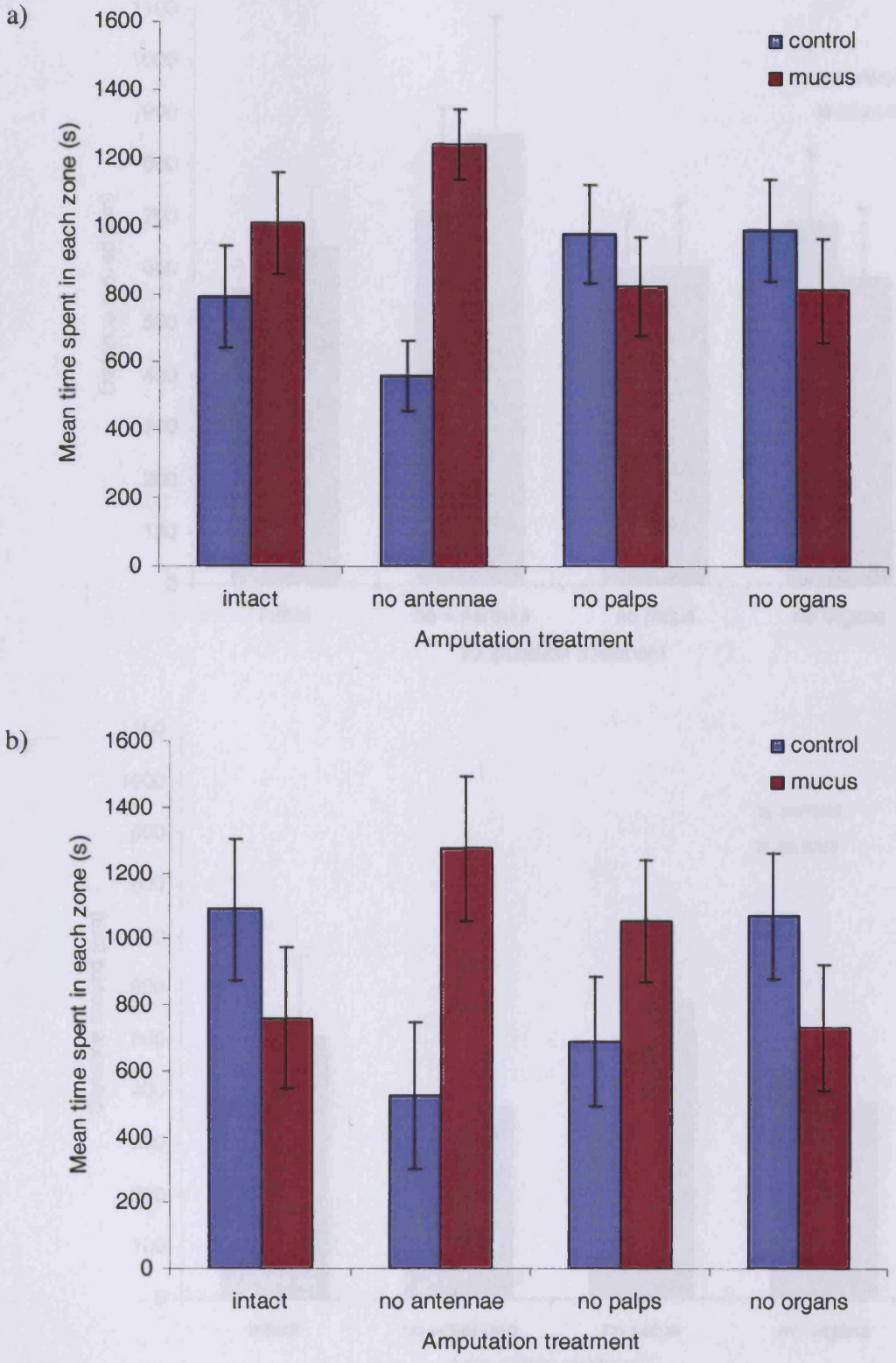

Figure 6.3. Bar graphs showing the mean time spent by *Pterostichus melanarius* in the control and mucus coated zones of arenas. Male (a) and female (b) beetles with different organ amputation treatments. Closed bars = standard errors.

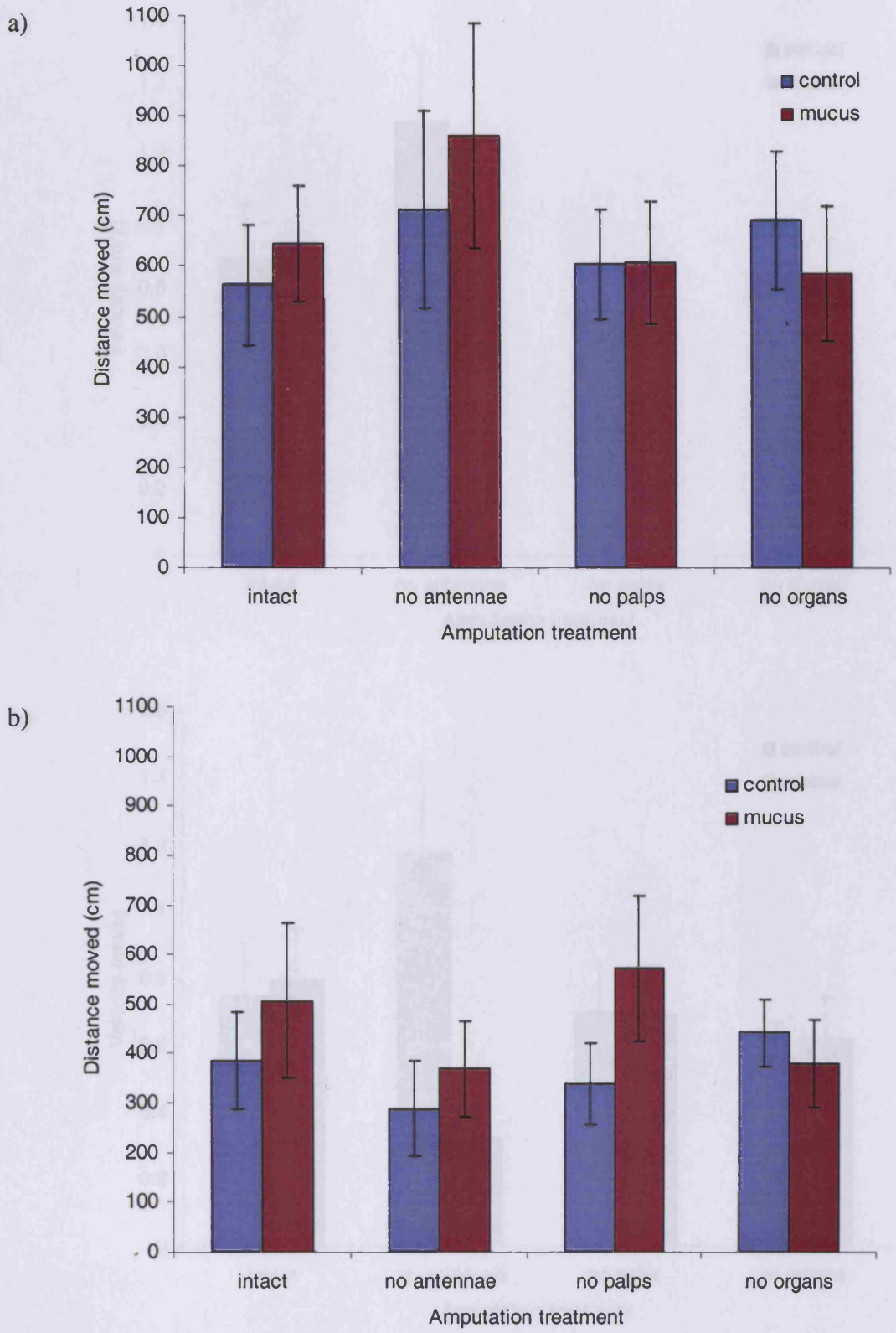

Figure 6.4. Bar graphs showing the mean distances moved by *Pterostichus melanarius* in control and mucus coated zones of arenas. Male (a) and female (b) beetles with different organ amputation treatments. Closed bars = standard errors.

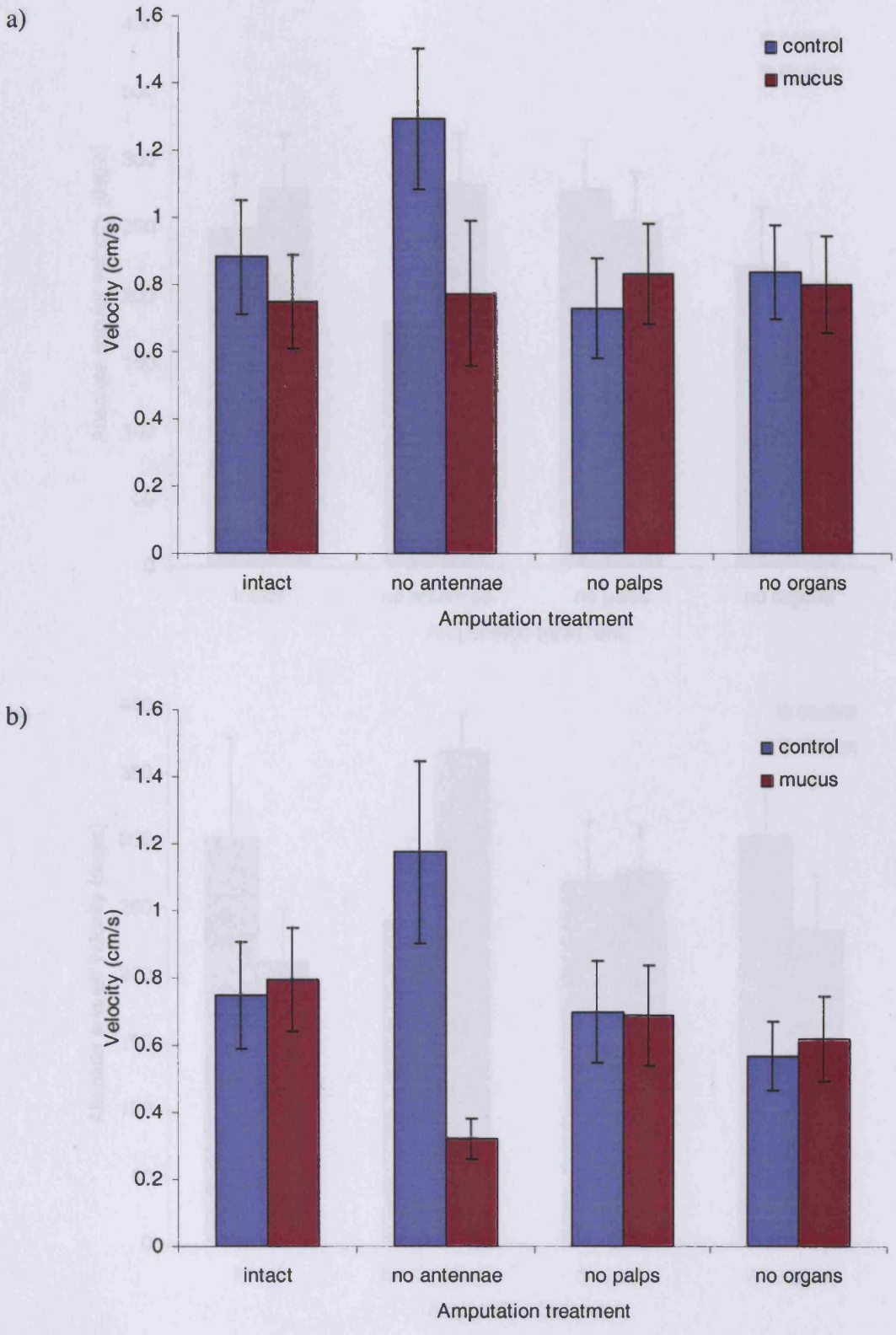

Figure 6.5. Bar graphs showing the mean velocities of *Pterostichus melanarius* in control and mucus coated zones of arenas. Male (a) and female (b) beetles with different organ amputation treatments. Closed bars = standard errors.

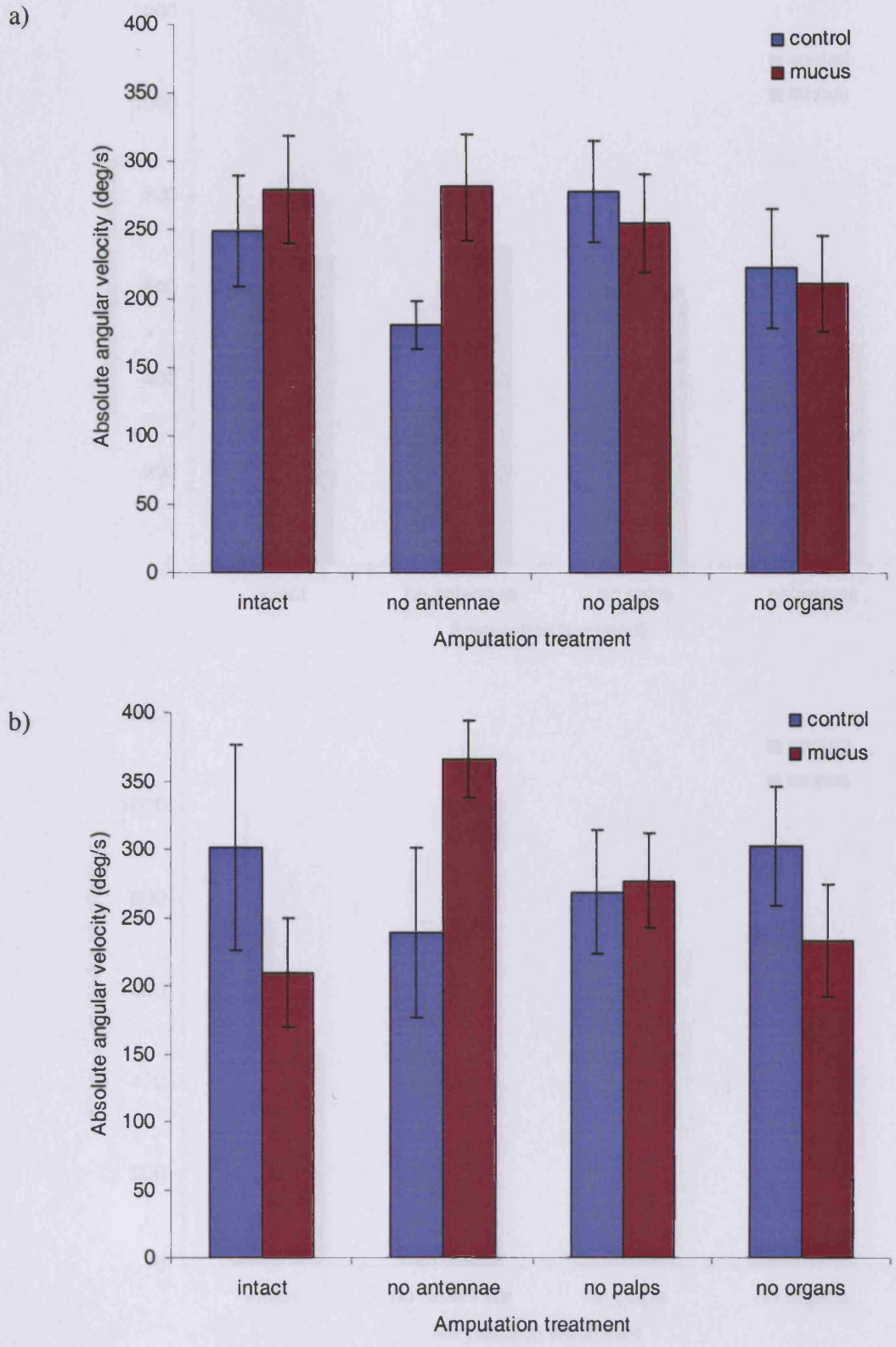

Figure 6.6. Bar graphs showing the mean absolute angular velocities of *Pterostichus melanarius* in control and mucus coated zones of arenas. Male (a) and female (b) beetles with different organ amputation treatments. Closed bars = standard errors.

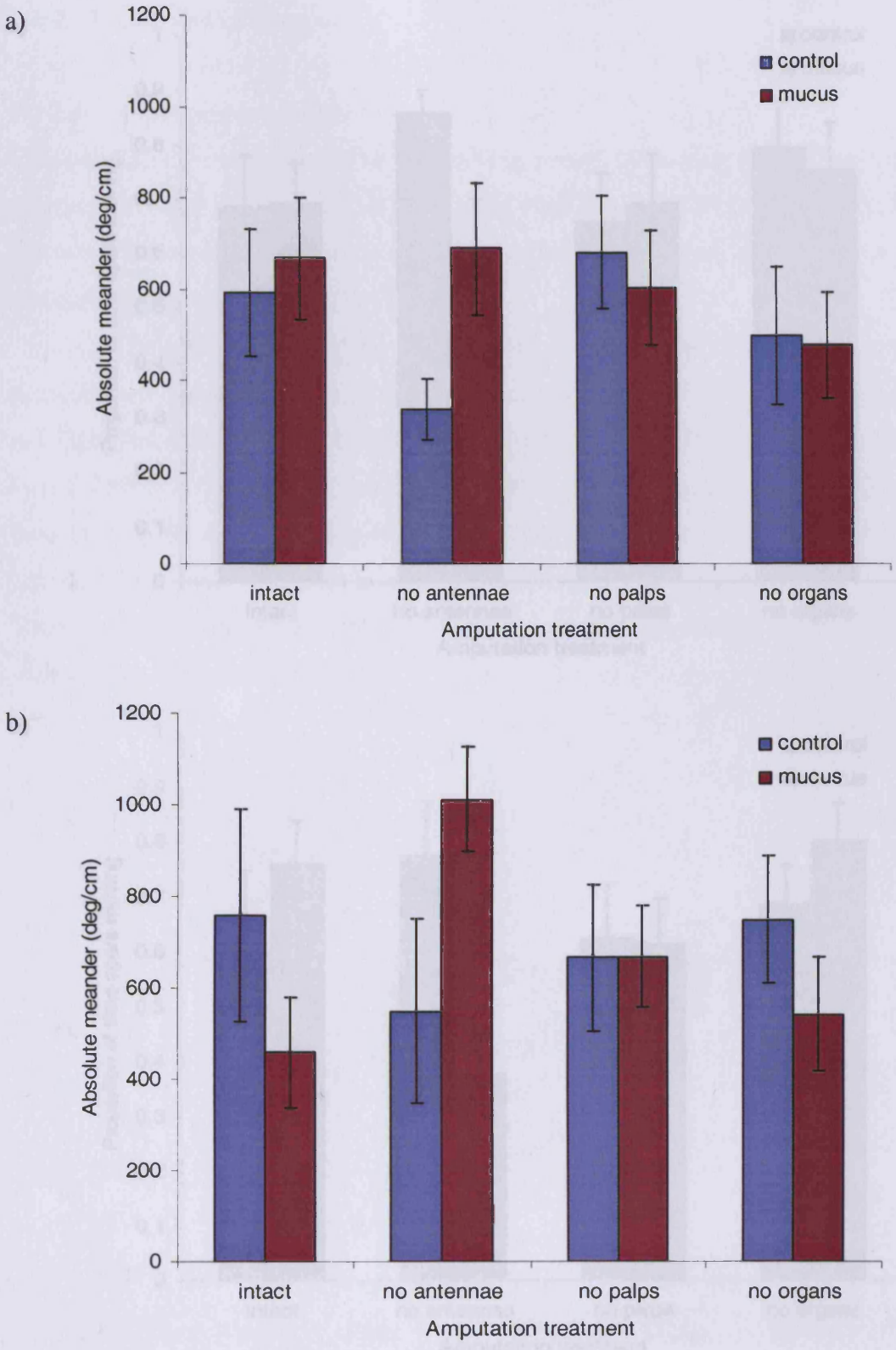

Figure 6.7. Bar graphs showing the mean absolute meander of *Pterostichus melanarius* in control and mucus coated zones of arenas. Male (a) and female (b) beetles with different organ amputation treatments. Closed bars = standard errors.

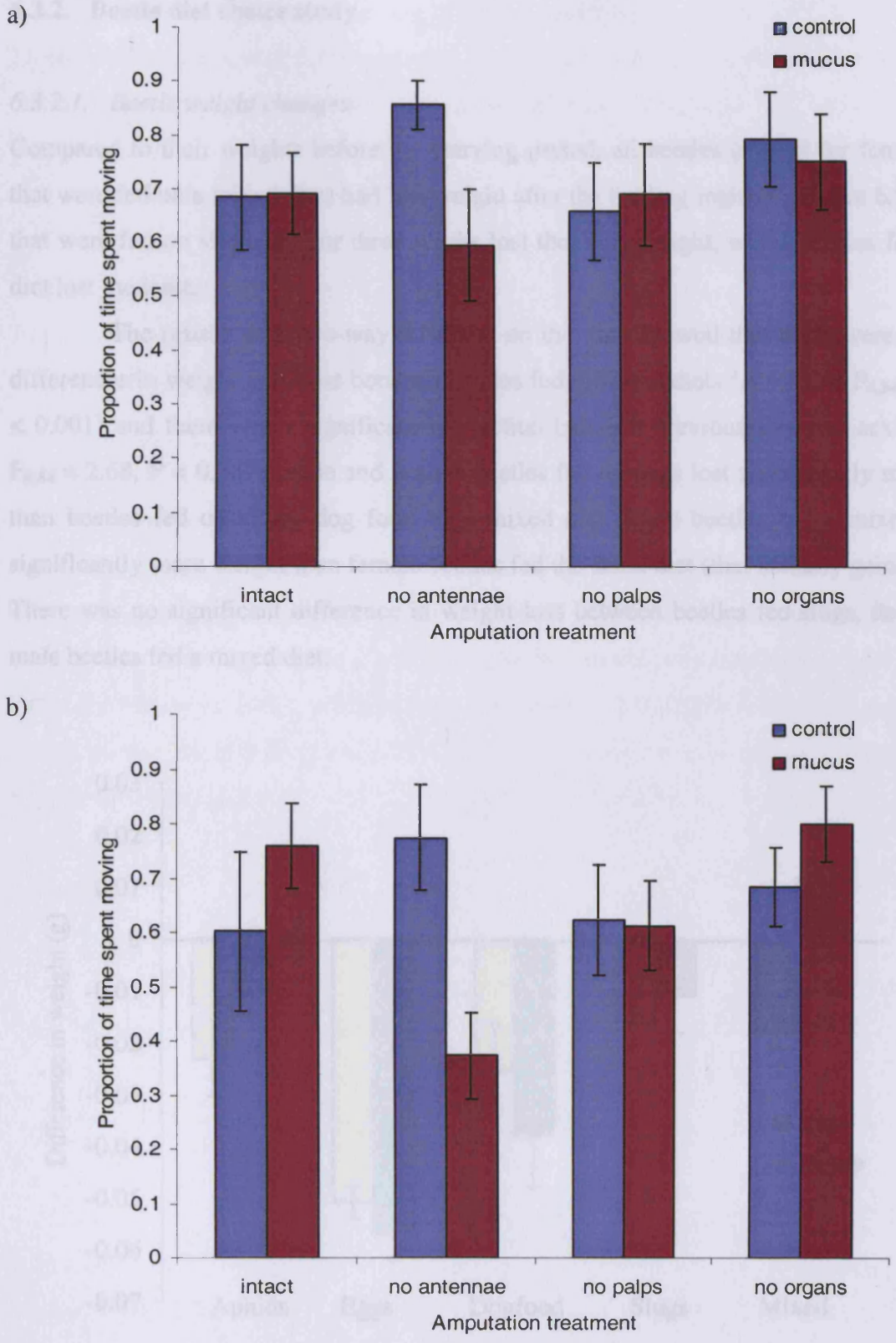

Figure 6.8. Bar graphs showing the mean proportion of time spent moving by *Pterostichus melanarius* in control and mucus coated zones of arenas. Male (a) and female (b) beetles with different organ amputation treatments. Closed bars = standard errors.

6.3.2. Beetle diet choice study

6.3.2.1. Beetle weight changes

Compared to their weights before the starving period, all beetles (except for female beetles that were fed on a mixed diet) had lost weight after the feeding regimes (Figure 6.9.). Beetles that were fed on slug eggs for three weeks lost the most weight, whilst beetles fed a mixed diet lost the least.

The results of a two-way ANOVA on the data showed that there were significant differences in weight gain/loss between beetles fed different diets (ANOVA: $F_{4,84} = 15.24$, $P < 0.001$) and there was a significant interaction between previous diet and sex (ANOVA: $F_{4,84} = 2.68$, $P = 0.037$). Male and female beetles fed on eggs lost significantly more weight than beetles fed on slugs, dog food or a mixed diet. Male beetles fed a mixed diet lost significantly more weight than female beetles fed the same diet (that actually gained weight). There was no significant difference in weight loss between beetles fed slugs, dog food and male beetles fed a mixed diet.

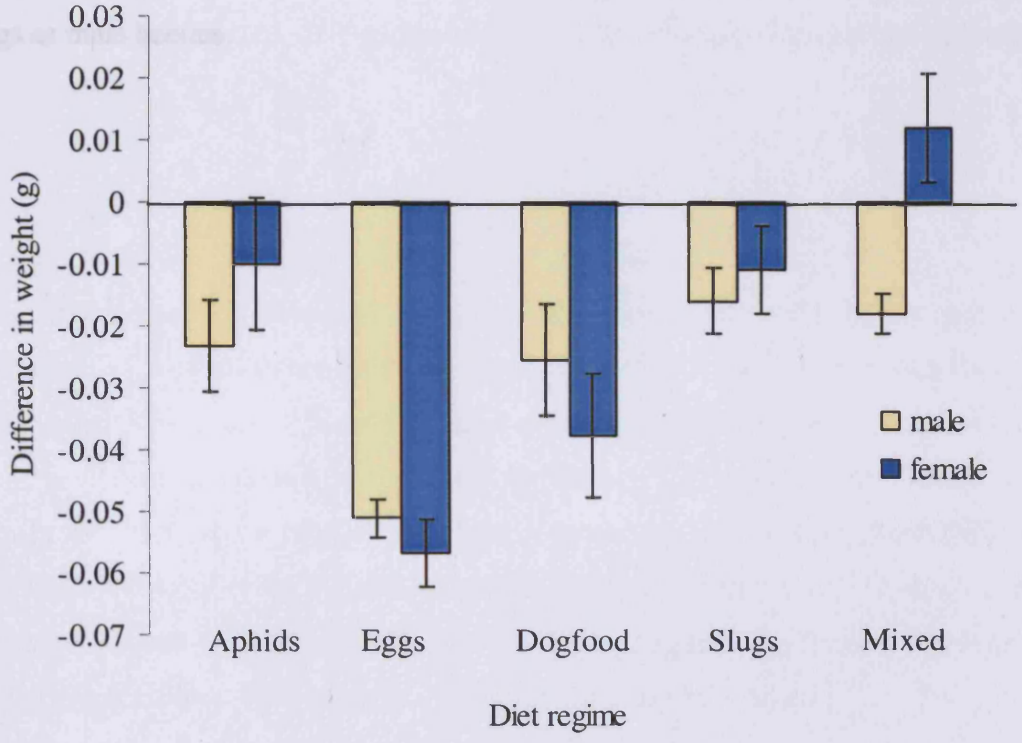

Figure 6.9. Bar graph showing the mean differences in weight of *Pterostichus melanarius* in the beetle feeding study. Difference in weight of male and female beetles before starving and after diet regime for beetles fed on different diets.

6.3.2.2. Generalised linear modelling of prey proportions eaten data

Three beetles died during the experiment and so data were collected from 67 beetles. Over-dispersion of the data given a binomial distribution was high, especially for the aphid data, therefore an approximate F-distribution was used to do the tests on the factors here, i.e ratio of mean deviances to residual mean deviance.

- Slug eggs eaten

The mean proportion of slug eggs eaten by male and female beetles under each previous diet treatment is given in Figure 6.10. Except for beetles previously fed on slugs, females ate more slug eggs than males. This difference is particularly strong for beetles previously fed on eggs or aphids.

When all factors were fitted in the model, the only factor having a significant effect was beetle sex ($F_{1,57} = 4.52$, $P = 0.038$). Previous diet was not a significant factor affecting how many eggs were eaten ($F_{4,57} = 0.60$, $P = 0.667$) and there was no interaction between sex and previous diet ($F_{4,57} = 0.76$, $P = 0.557$). The best model was fitted (a constant term and a factor for sex only), which predicted that males ate 11.8 % (s.e = 3.46) of the eggs and that female beetles ate 21.5 % (s.e = 2.75) of the eggs. Female beetles ate almost twice as many eggs as male beetles.

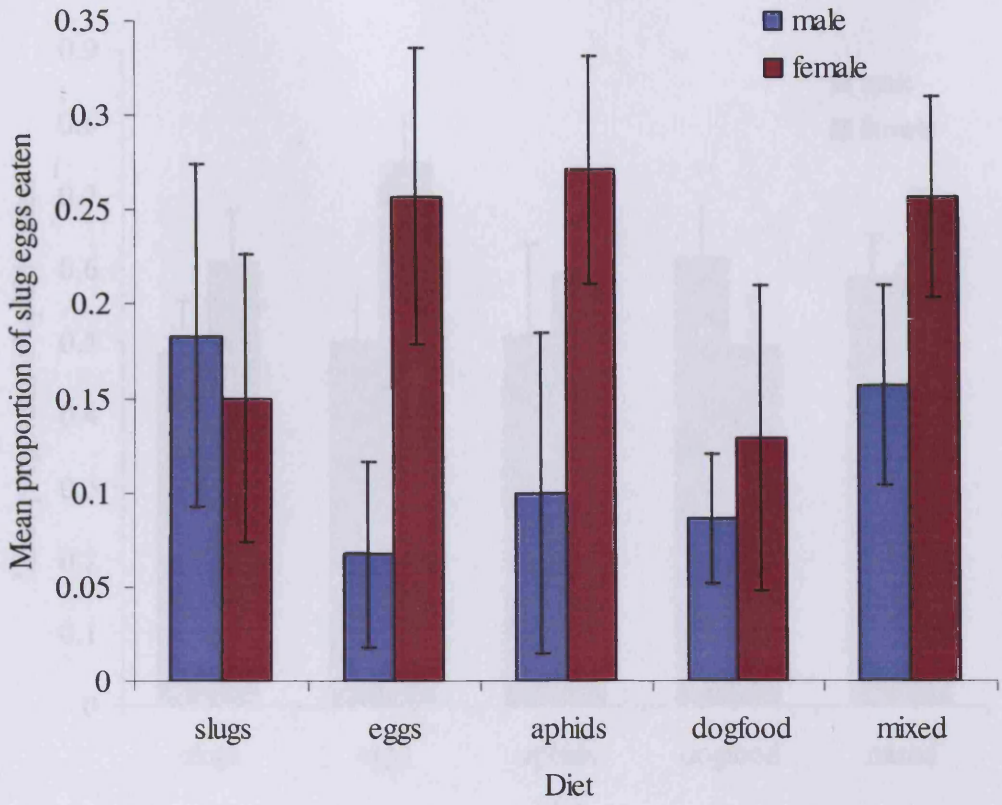

Figure 6.10. Bar graph showing the mean proportion of *Deroceras reticulatum* eggs eaten by *Pterostichus melanarius* in the beetle feeding study. Mean proportion of slug eggs eaten by male and female beetles fed different diets. Closed bars represent standard errors.

- Aphids eaten

The mean proportion of aphids eaten by male and female beetles under each previous diet treatment is given in Figure 6.11. Except for beetles previously fed on dog food, females ate more aphids than males. This difference is only significant for beetles previously fed on eggs.

When all factors were fitted in the model, none of the factors tested had a significant effect on the proportion of aphids eaten. Sex ($F_{1,57} = 1.98$, $P = 0.165$) and previous diet ($F_{4,57} = 0.44$, $P = 0.776$) were not significant, and there was no interaction between sex and previous diet ($F_{4,57} = 1.13$, $P = 0.353$). The best model was fitted (a constant term only), which predicted that beetles ate 58.1 % (s.e = 2.82) of the aphids.

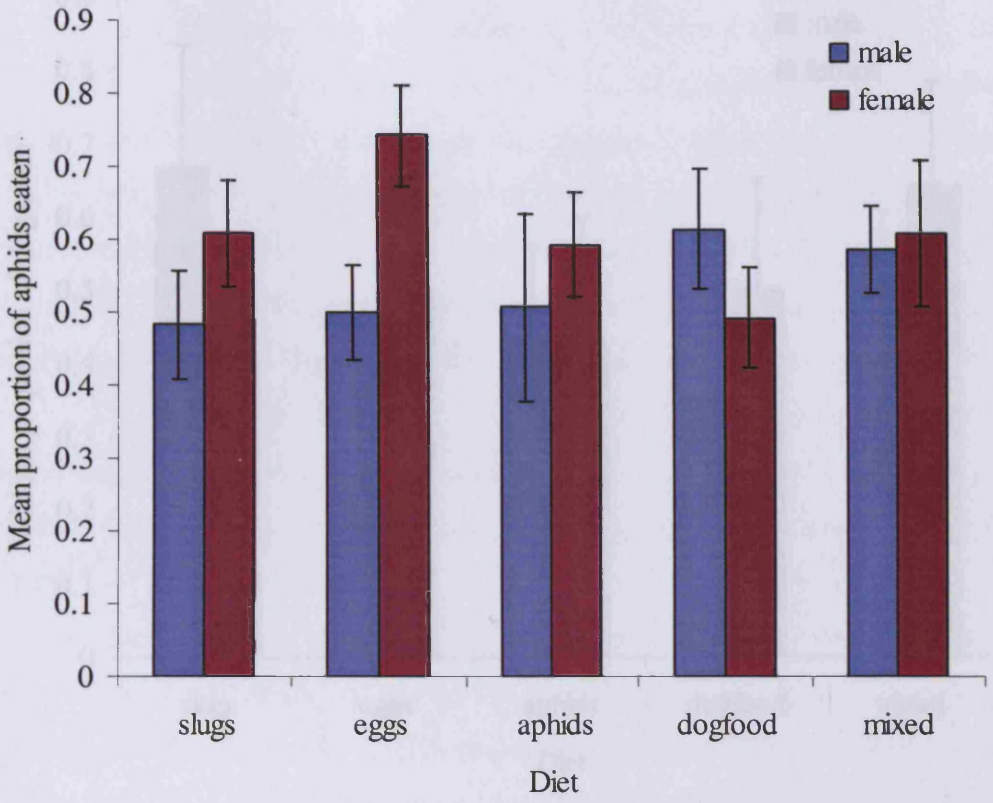

Figure 6.11. Bar graph showing the mean proportion of *Myzus persicae* eaten by *Pterostichus melanarius* in the beetle feeding study. Mean proportion of aphids eaten by male and female beetles fed different diets. Closed bars represent standard errors.

- Slugs eaten

The mean proportion of each slug eaten by male and female beetles under each previous diet treatment is given in Figure 6.12. Out of 67 beetles, 20 did not eat the slug, 31 partially ate the slug and 16 completely consumed the slug.

When all factors were fitted in the model, none of the factors tested had a significant effect on the proportion of slug eaten. Sex ($F_{1,57} = 0.31$, $P = 0.582$) and previous diet ($F_{4,57} = 0.44$, $P = 0.777$) were not significant, and there was no interaction between sex and previous diet ($F_{4,57} = 0.60$, $P = 0.663$). The best model was fitted (a constant term only), which predicted that beetles ate 47.0 % (s.e = 5.29) of the slug.

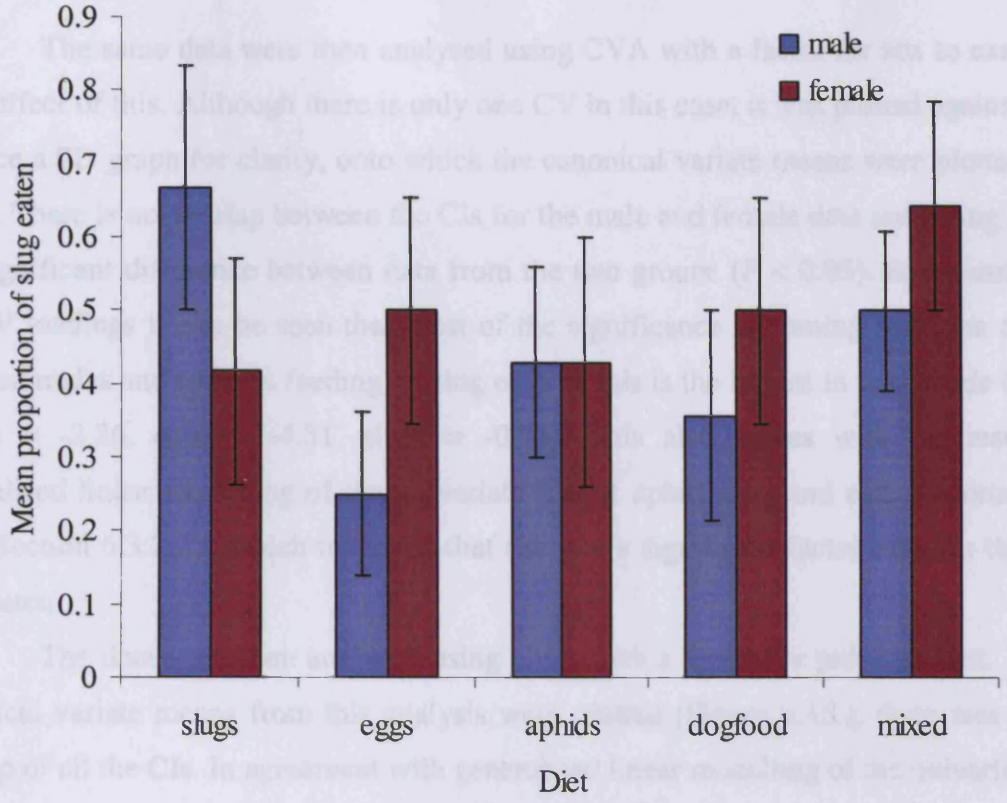

Figure 6.12. Bar graph showing the mean proportion of *Deroceras reticulatum* eaten by *Pterostichus melanarius* in the beetle feeding study. Mean proportion of slugs eaten by male and female beetles fed different diets. Closed bars represent standard errors.

Under most feeding regimes, females tended to eat more prey in the choice test than male beetles but only for consumption of egg prey was this difference significant. The previous diet of the beetles had no effect on the proportions of any of the prey consumed.

6.3.2.3. Results of canonical variates analysis

A CVA was run for the full model (factor = sex × previous diet). By examination of the eigenvalues (the proportion of variance accounted for by the correlation between the respective canonical variates) it can be seen that the first two CV's take up 90.3 % of the variation in the data (eigenvalues: CV1 = 67.8 %, CV2 = 22.5 %). The canonical variate means are plotted in Figure 6.13. There is complete overlap of all CI's therefore there is no significant difference between the groups ($P > 0.05$). The interaction between sex and previous diet is not significant and this agrees with the results from generalised linear modelling of the univariate sets of aphid, slug and egg proportions eaten data (Section 6.3.2.2.).

The same data were then analysed using CVA with a factor for sex to examine the main effect of this. Although there is only one CV in this case, it was plotted against itself to produce a 2D graph for clarity, onto which the canonical variate means were plotted (Figure 6.14.). There is no overlap between the CIs for the male and female data indicating that there is a significant difference between data from the two groups ($P < 0.05$). By examination of the CV loadings it can be seen that most of the significance is coming from the difference between males and females feeding on slug eggs as this is the largest in magnitude (loadings: aphids = -2.26, eggs = -4.31, slugs = -0.74). This also agrees with the results from generalised linear modelling of the univariate sets of aphid, slug and egg proportions eaten data (Section 6.3.2.2.), which indicated that sex was a significant factor only for the data on eggs eaten.

The data were then analysed using CVA with a factor for previous diet. When the canonical variate means from this analysis were plotted (Figure 6.15.), there was complete overlap of all the CIs. In agreement with generalised linear modelling of the univariate sets of aphid, slug and egg proportions eaten data (Section 6.3.2.2.), previous diet did not significantly influence prey choice in the feeding choice test ($P > 0.05$).

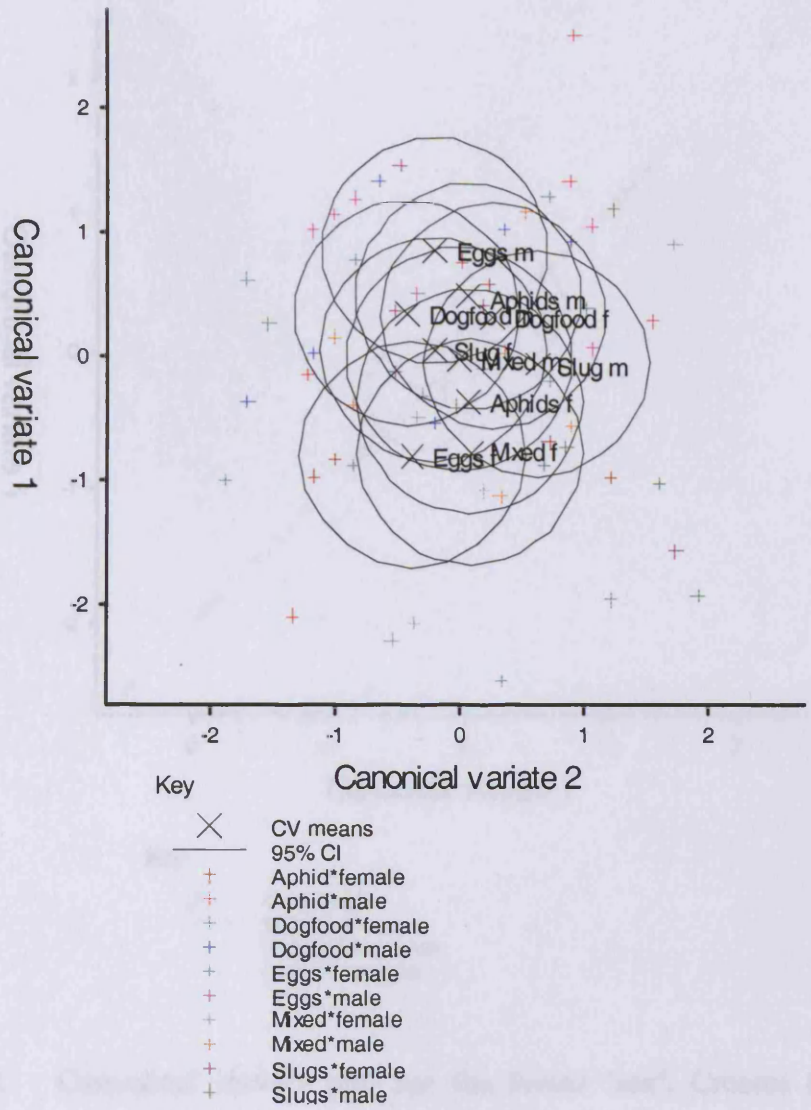

Figure 6.13. Canonical variate plot for the factor 'sex × previous diet' (full model). Crosses (X) indicate mean canonical variate for each group (all combinations of diet and sex) and circles around mean canonical variates represent the 95 % CI for each data group.

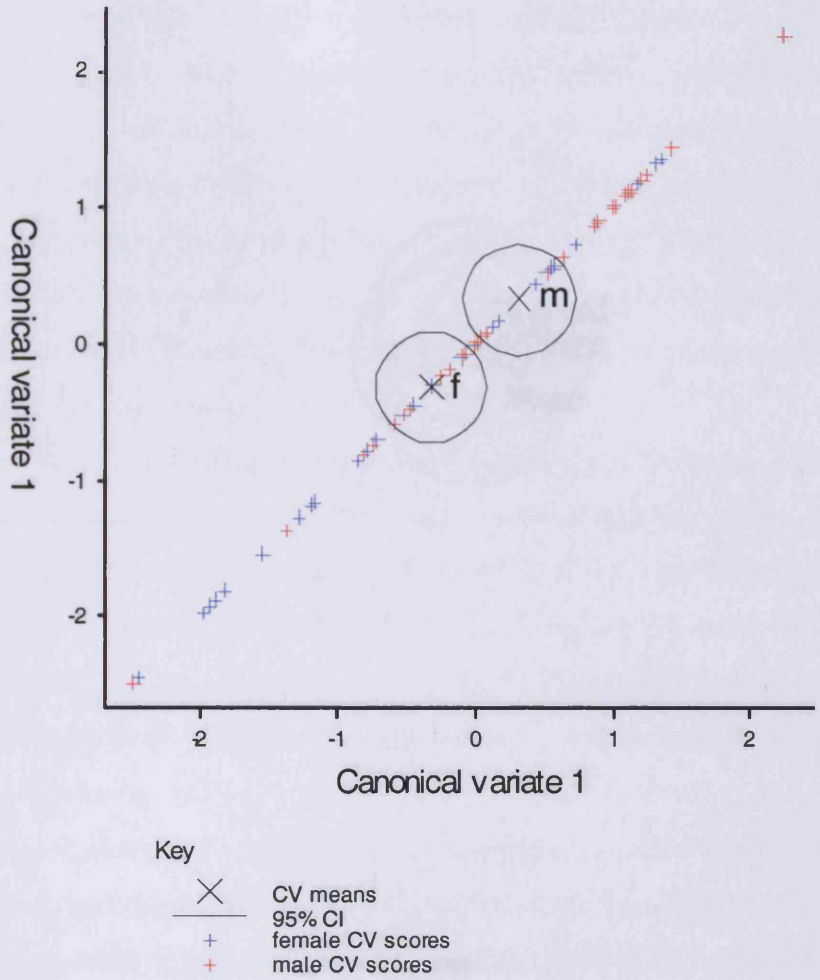

Figure 6.14. Canonical variate plot for the factor 'sex'. Crosses (X) indicate mean canonical variate for each data group (male or female beetles) and circles around mean canonical variates represent the 95 % CI for each group.

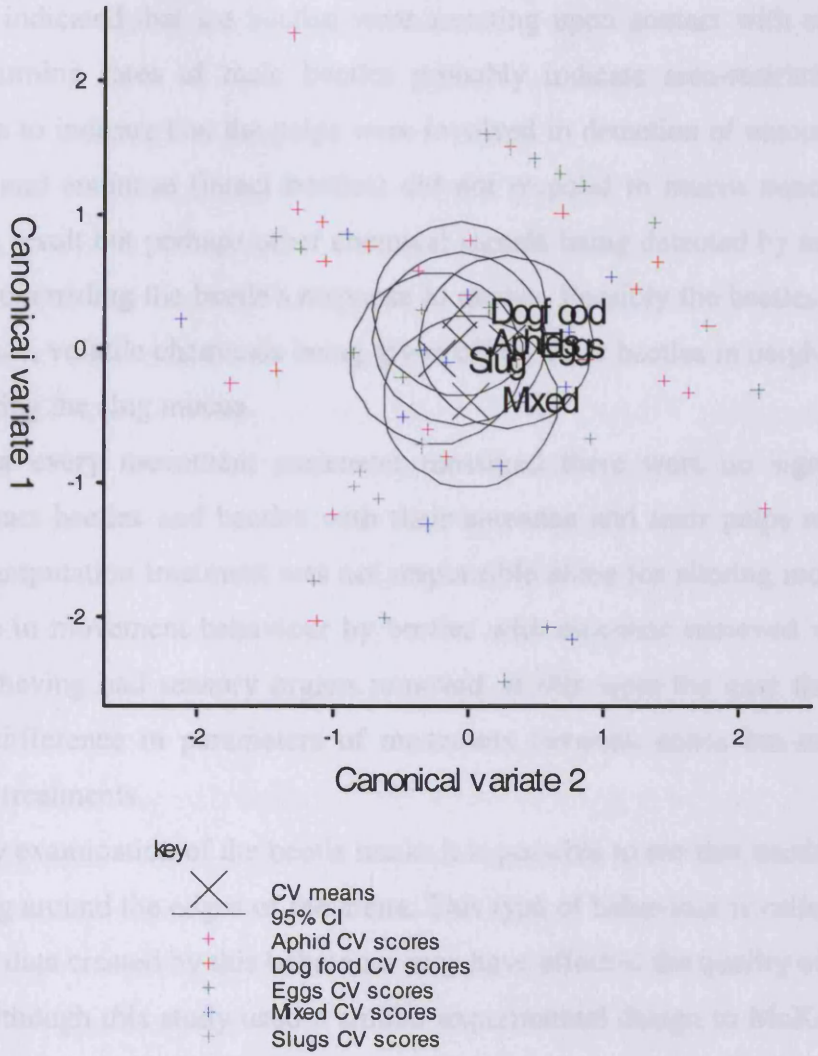

Figure 6.15. Canonical variate plot for the factor 'previous diet'. Crosses (X) indicate mean canonical variate for each data group (diets: aphids, dog food, slug eggs, mixed and slugs) and circles around mean canonical variates represent the 95 % CI for each group.

6.4 Discussion

For nearly all the parameters tested, beetles with no antennae but intact palps altered movement behaviour when entering the mucus-coated half of the arenas. Movement parameters were not significantly different on the mucus paper for any other amputation treatment tested. Male and female beetles with antennae removed spent less time moving, reduced their velocity and spent more time in total on mucus zones than on control zones. Male beetles also increased meander and angular velocity on mucus coated paper. These

behaviours indicated that the beetles were arresting upon contact with slug mucus and the increased turning rates of male beetles probably indicate area-restricted searching. The results seem to indicate that the palps were involved in detection of mucus, however, beetles with palps and antennae (intact beetles) did not respond to mucus zones. It is difficult to explain this result but perhaps other chemical signals being detected by antennae but not the palps were overriding the beetle's response to mucus. Possibly the beetles could detect using their antennae, volatile chemicals being given off by other beetles in neighbouring arenas and hence ignoring the slug mucus.

For every movement parameter measured there were no significant differences between intact beetles and beetles with their antennae and their palps removed, indicating that organ amputation treatment was not responsible alone for altering movement behaviour. The change in movement behaviour by beetles with antennae removed was therefore not a product of having had sensory organs removed. If this were the case then you would also expect no difference in parameters of movement between zones but only between organ amputation treatments.

By examination of the beetle tracks it is possible to see that beetles spent a lot of the time moving around the edges of the arena. This type of behaviour is called thigmotaxis. The noise in the data created by this behaviour may have affected the quality of the data.

Although this study used a similar experimental design to McKemey *et al.* (2004), the results of their study seemed to indicate that the antennae were involved in the detection of mucus. The results of the electroantennagram (EAG) work in McKemey *et al.* (2004) demonstrated that this was probably not a volatile chemical cue as the odour of live slugs blown over antennal preparations did not result in nerve firing in the antennae. However, their behavioural work showed that beetles responded to patches of mucus by increasing their turning rate, reducing speed and spending less time moving, but that the response disappeared when the antennae were amputated. It was suggested that beetles might have been using touch (gustatory) chemoreceptors on the antennae rather than olfactory chemoreceptors, unless the olfactory chemoreceptors responsible for detecting slug mucus are located on the very tip of the antennae (which is cut off during EAG and may explain the lack of results). The results in this chapter show the opposite because the beetles did not respond to mucus unless they had had their antennae removed. Some of the movement parameters measured by McKemey *et al.* (2004) did not disappear on removal of the antennae, for example, male beetles spent less time moving in mucus zones than in control zones whether their antennae had been removed or not. The results of both studies support

the hypothesis that beetles can detect and respond to slug mucus, but contrasting results regarding the effects of sensory organ amputation raise questions over the mechanism that beetles use for detecting the mucus.

Evidence points towards the beetles not locating slugs by long-range olfaction but encountering slugs and/or chemical cues from mucus results in area restricted searching for more slugs. Area-restricted searching occurs when prey are often aggregated. After a prey item has been found and consumed the predator increases its' rate of turning in search of further prey (Hassall, 1978; Kareiva & Odell, 1987). Carabid beetles have been shown to elicit this behaviour. Mitchell (1963) conducted feeding trials with two adult carabid beetles: *Bembidion lampros* (Herbst) and *Trechus quadristriatus* (Schrank). It was found that beetles located immobile *Erioischia brassicae* (Bouché) (cabbage root fly) eggs by random chance encounter but that both species changed movement behaviour after finding and eating eggs. Prior to finding and eating an egg, beetles moved swiftly around the experimental arena in a directed manner. For 10-15 seconds after eating an egg the beetle would reduce its velocity and increase its turning rate and meander, which resulted in an increased likelihood of the beetle finding neighbouring eggs. If other eggs were not found in this time then the beetles would revert back to normal movement. Since eggs were arranged in patches in experimental arenas, the eggs were eaten group by group. Mitchell (1963) concluded that beetles were not attracted to the eggs from a distance as many of them were passed by unnoticed, but chance encounter of eggs resulted in area restricted searching. In the field this could translate to beetles aggregating in food patches by 'kinesis'. Hughes (1959) suggested that this type of behaviour could have been responsible for the patterns of cabbage root-fly egg loss due to predation observed during field experiments. During feeding studies Mitchell (1963) also noted that on contact with a prey item, chemical stimulus sensed through the beetle's palps was important in food recognition. Larvae of both species relied on contact between the maxillary palps and food before the prey item was eaten.

Beetle movements have been shown to depend on hunger levels. Hungry *P. melanarius* show a more directed walking pattern whereas satiated *P. melanarius* show a more tortuous walking pattern with a higher frequency of turning (Grüm 1971; Chiverton, 1984; Wallin & Ekbom, 1994). Wheater (1991) found that hungry *P. melanarius* are more active than satiated individuals, and that immediately after feeding starved beetles a single prey item, the beetle would initiate tortuous walking (increased meander) for several minutes, possibly in the search for other prey in the vicinity. Mols (1986) used experiments combined with mathematical modelling in developing a model to describe the effects of prey spatial

distribution on predatory behaviour of the carabid *Pterostichus coerulescens* (L.). Three types of walk based on the relative gut contents of beetles were described: straight walking when the gut is almost empty (hence beetle is hungry), normal searching when gut is partly full and tortuous walking when beetles have recently fed. If this model could be applied to *P. melanarius* then this might explain why beetles became aggregated with slugs in June (Bohan *et al.*, 2000a). Beetles with the heaviest guts were associated with the highest slug densities in June (Chapter 2), hence these beetles exhibit more tortuous walking patterns than beetles in other parts of the field that have emptier guts. Combined with the effect of a higher probability of encountering slugs and slug mucus in these areas, the tortuous walking and area restricted searching would result in beetles arresting in the areas that initially have high slug densities. It is possible that slug dispersion during the summer, indicated by a reduction in the variance to mean ratio (Bohan *et al.*, 2000b), is in part an adaptation to avoid predation by beetles, as clusters are more easily detected and exploited.

In the beetle feeding study, the diet regime had a significant effect on the weight of the beetles. All beetles, except females fed on a mixed diet, lost weight after being starved and then fed on a diet regime for three weeks. It is likely that the beetles lost weight during the seven day starving period but did not put as much weight on as they had reached after they had been stored in the lab for two weeks and fed regularly on dog food.

Beetles fed exclusively on slug eggs lost the most weight. During the feeding choice bioassays, beetles also ate few eggs, preferring to eat aphids and slugs. Each beetle ate on average only 16.7 % of the eggs, compared to approximately 47 % of the slug and 58 % of the aphids. One might expect beetles to eat more eggs than they did because the handling time for eggs, which are small and do not move, would probably be shorter than for aphids and slugs. However, since beetles lost the most weight on a diet of slug eggs, they are probably nutritionally poor and this might explain why beetles preferred to eat aphids and slugs in the food choice bioassay. Most beetles chose a mixed diet of at least two different prey types.

Beetles fed on a mixed diet lost the least weight, suggesting that this diet is the best nutritionally. Evidence from other studies shows that mixed diets help to increase the fitness of generalist predators. Wallin *et al.* (1992) found that female *P. melanarius* fed on a mixed diet of cereal aphids, carbohydrate-rich food and maggots were able to produce larger eggs and build up more fat reserves than those fed exclusively on cereal aphids. *Agonum dorsale* (Pontoppidan), another carabid beetle, also produces more eggs when fed on a mixed diet as opposed to a diet consisting purely of aphids or earthworms (Bilde & Toft, 1994). Toft

(1999) argued that mixing of prey types (maximising nutrition) in a spider diet was more important for fitness that maximising energy intake by consuming lots of one type of low-quality prey.

It might have been expected that previous diet would affect prey choice in some way. One could form two hypotheses: either a beetle learns how to tackle a certain prey type from feeding extensively on this for three weeks and then chooses this prey in the choice test, or a beetle chooses to maximise the nutrition in its' diet by eating the alternative prey options available and not the prey it has been eating for the past three weeks. However, the results from CVA and generalised linear modelling of the data on proportions of prey eaten both showed that previous feeding experience had no effect on prey selectivity in the food choice bioassay. When fed on a certain prey type it neither avoided this prey nor fed exclusively on this prey in the choice test. It seems that regardless of previous feeding experience, all beetles preferred to eat a mixed diet when it was offered, and in general showed no preference for any of the prey types. Female beetles ate more slug eggs than male beetles but there was no difference between the sexes in the amounts of slugs or aphids consumed.

The results indicate that beetles exert limited prey choice when a variety of prey is available, even when they have extensive experience of one particular prey type. Beetles preyed upon slugs to a greater extent in June than in July (Bohan *et al.*, 2000a; Chapter 2) but slugs were present in high numbers in the field throughout summer. This drop in the level of slug predation is more likely to be due to slug behavioural responses to beetles (Armsworth *et al.*, 2005; Chapter 3) and a reduction in the surface activity of slugs in July, than prey switching by beetles. In other words, it is not likely that beetles reduced feeding on slugs because they needed a more diverse diet (beetles fed on a slug-only regime did not avoid eating slugs in the prey choice test), but because of a reduction in the availability of slugs and/or an increase in the availability of alternative prey.

An interesting area for further research would be to investigate the range of prey taken in the field by carabids at different points throughout the season and then attempt to relate this to seasonal and spatial abundances of these prey. It would now be possible to identify the range of prey eaten in a predator's gut using PCR techniques (Zaidi *et al.*, 1999; Symondson, 2002b).

Chapter 7

General Discussion

7.1. Introduction

Several hypotheses were created for testing in this project with the aim of investigating factors that influence the survival and dispersal of slug pests, with particular reference to the effect of predation by the generalist, *Pterostichus melanarius*. Two common arable slug species were selected for study: *Arion intermedius* and *Deroceras reticulatum*, because they are the most abundant slug species in winter wheat and display contrasting patterns of spatial arrangement. The results from previous chapters will be discussed with reference to how they answer each of the hypotheses. Further questions raised by this work and possible areas for future research are also discussed.

7.2. Accepting or rejecting hypotheses

7.2.1. Hypothesis 1: The presence of *Pterostichus melanarius* causes increased dispersion of juvenile and adult slugs.

Although adult *D. reticulatum* were found to change movement rates and avoid areas previously exposed to *P. melanarius*, juvenile *D. reticulatum* did not avoid such areas (Chapter 3) or disperse more rapidly from egg batches (Chapter 4) in response to areas previously exposed to *P. melanarius*. The possible reasons for this are discussed in detail in Chapter 4 but the results suggest that *P. melanarius* presence probably does not lead to increased juvenile slug dispersal. However, the work does suggest that *P. melanarius* might influence the dispersal rates of adult *D. reticulatum* and consequently their spatial dynamics.

The choice bioassay work in Chapter 3 showed that when *P. melanarius* had been present on soil or paper, the beetles deposited chemicals on these surfaces. Adult *D. reticulatum*, but not *A. intermedius*, avoided areas where *P. melanarius* had recently been present. Also, in the video tracking study (Chapter 3), slugs on *P. melanarius* exposed paper increased their velocity and time spent moving, and decreased their rate of turning. In the

field this behaviour might result in slugs making directed movements away from areas where the beetles are in higher densities.

These results might help to explain some of the spatial patterns observed by Bohan *et al.* (2000a, 2000b). Bohan *et al.* (2000a) found that *P. melanarius* was aggregated in areas of high slug density in early summer. There were probably higher concentrations of beetle residual chemicals on soil and higher rates of beetle attack in these areas. Slugs that are attacked by carabid beetles yet survive also increase their velocity and move away (Pakarinen, 1994a). Hence, direct attack by carabids and the presence of their chemicals on the soil could result in slugs dispersing away from aggregations to areas of lower slug and beetle densities. This might explain why Bohan *et al.* (2000a) found that slug and beetle populations were spatially dissociated later in the season and why Bohan *et al.* (2000b) found that the slug population spread out when carabid beetles were present in the field.

7.2.2. Hypothesis 2: *Arion intermedius* and *Deroceras reticulatum* differ in parameters of dispersion and/or their behaviour towards *Pterostichus melanarius*.

Juvenile *A. intermedius* and *D. reticulatum* dispersed from egg batches at a similar rate in mini-plots (Chapter 4). Although the effect of *P. melanarius* on dispersion of *A. intermedius* was not tested, exposing mini-plots to beetles did not increase dispersion of *D. reticulatum* juveniles. With regard to adult slugs, *A. intermedius*, unlike *D. reticulatum*, did not respond to *P. melanarius* exposed paper by moving away to control paper (Chapter 3), even though this beetle preys on both species in the field. This is probably because, as shown by Pakarinen (1994a), *Arion* sp. have thicker skin and are better protected from beetle attack when stationary. These results suggest that there may be differences in the way that the two slugs respond to beetles, and potentially in the way that they disperse in the field.

Bohan *et al.* (2000b) showed that *A. intermedius* and *D. reticulatum* shared similar patterns of parametric intensity, and a reduction in the variance to mean ratio when carabid beetles were present was observed in both slug populations. These findings suggest that beetle presence might have influenced the change in the variance to mean ratio. A reduction in the ratio might have been caused by density dependent predation. When carabid beetles aggregated in areas of high slug density, they would probably have eaten more slugs in these areas than in areas of low slug density, causing the population of slugs to become more evenly spread in the field. This is supported by the work of Symondson *et al.* (1996) because they found that beetle guts contained greater quantities of slug protein and that a larger

proportion of their diet was slug in areas of high slug density, indicating greater predation rates on slugs in these areas. Another possibility, and one that was tested in this project, is that the presence of carabid beetles promoted increased dispersal of slugs from aggregations. As mentioned above, adult *D. reticulatum* moving away from areas of high beetle density might have resulted in the slugs spreading out, but in mini-plots juvenile *D. reticulatum* did not increase dispersal in mini-plots previously exposed to *P. melanarius*. Also, *A. intermedius* does not respond to *P. melanarius* by moving away, yet as with juvenile *D. reticulatum*, the distribution of *A. intermedius* became more evenly spread in summer (Bohan *et al.*, 2000b). Juvenile dispersion might have contributed to the reduction in the variance to mean ratio in summer observed by Bohan *et al.* (2000b). Aggregations of slugs, particularly juveniles, were present at a spatial scale of 0.25 m in March but slugs were spatially random at this scale in June (Bohan *et al.*, 2000b). In the mini-plot experiments presented in Chapter 4, the mean displacement of *D. reticulatum* was calculated to be > 4 cm away from the egg batch per day and some slugs exceeded a dispersal rate of 10 cm day^{-1}. If slugs maintained this rate of dispersal then it is possible that juvenile dispersal might contribute to population spread, explaining the change in the spatial distribution of slugs in summer observed by Bohan *et al.* (2000b).

Differences in the spatial arrangements of *A. intermedius* and *D. reticulatum* observed by Bohan *et al.* (2000b) probably arose as a result of their different behaviour towards carabid beetles and differences in the level of surface activity between the two species. The distribution of *D. reticulatum* changed from month to month (Bohan *et al.*, 2000b) probably due to density dependent predation by carabid beetles on this species and adult slugs moving away to areas of lower beetle density. The distribution of *A. intermedius* resolved to a patch in the west of the field that in June was spatially associated with a high density of carabid beetles (Bohan *et al.*, 2000a). Unlike *D. reticulatum* however, the patch appeared to persist in this area as it was detected again in the following March, although it had been somewhat depleted during the summer when carabid beetles were present. *A. intermedius* is known to be a primarily subterranean species and *D. reticulatum* a highly surface active species (South, 1965). *A. intermedius* probably spends much of it's time under the soil and does not increase activity in the presence of *P. melanarius* but *D. reticulatum* is highly surface active and activity did increase in the presence of *P. melanarius* (Chapter 3). These differences might explain why Bohan *et al.* (2000b) found that the *A. intermedius* distribution did not change over time but the *D. reticulatum* distribution constantly changed.

7.2.3. Hypothesis 3: Beetles respond to and aggregate in areas of higher slug density.

Results from the video tracking experiment in Chapter 6 indicated that beetles' palps might be involved in the detection of slug mucus. However, these results do not agree with findings from a similar experiment done by McKemey *et al.* (2004), which indicated that the antennae were probably involved in slug mucus detection. Further study is needed to determine whether beetles actually use chemical detection of slug mucus as a method by which they find aggregations of slugs in the field.

It is likely that beetles aggregate in areas of high slug density because of an increased encounter rate with slugs in these areas, resulting in some type of arresting behaviour. Using the data from Bohan *et al.* (2000b), it was found that beetle foregut biomass was greater in areas of high slug density than low slug density when slugs and beetles were spatially associated (Chapter 2). Satiated beetles are known to move shorter distances with a higher degree of meandering than hungry beetles (Mitchell, 1963; Grüm 1971; Chiverton, 1984; Wheater 1991; Wallin & Ekbom, 1994). This behaviour would result in the beetles arresting in areas of higher prey/slug density, and over time, an accumulation of beetles in these areas. It is possible that this is the reason that *P. melanarius* has been found to be spatially associated with it's prey at certain times in the year (Bohan *et al.*, 2000; Winder *et al.*, 2001; 2004).

However, if an arresting behaviour were responsible for beetle's accumulating in areas of high prey density then the beetles in this area would be less likely to fall into pitfall traps. Some care must be taken in interpreting pitfall trap data as they actually sample activity-density rather than true density. Many factors influence beetle activity including the sex of the beetles (Thomas *et al.*, 1998), pesticide use, reproductive status, prey density (Chiverton, 1984; Wallin & Ekbom, 1994), temperature (Honek, 1997), rainfall (Thomas *et al.*, 1998), hunger (Chiverton, 1984; Pollet & Desender, 1990; Fournier & Loreau, 2001), soil topography and vegetation density (Honek, 1988). Slug positive beetles may have been underrepresented by pitfall trapping as this technique is biased in favour of more hungry beetles, potentially the reason why only 11 % of beetles caught tested positive for slug protein. Despite the drawbacks of using pitfall traps, they may still give a good indication of beetle density. However, it must be borne in mind that beetles in areas of high prey density (i.e. those that have heavier foreguts) may be underrepresented in the samples. Therefore the degree of spatial association between slugs and beetles in early summer, observed by Bohan *et al.* (2000a), might have been stronger than the results indicated.

An important question, and one that has been raised by other researchers (Mair *et al.*, 2001), is that if *P. melanarius* is truly responding to slug density, why did Bohan *et al.* (2000a) find that the beetles did not remain spatially associated with the slug distribution throughout summer? The spatial patterns observed probably do indicate density dependent predation on slugs because if there was only opportunistic predation operating then you would expect no spatial association at all rather than the spatial dissociation that was observed. Guillemain *et al.*, (1997) found that although forest dwelling carabids were found in lower densities when soil prey density was low, the beetles were spatially dissociated from prey availability in leaf litter. They hypothesised that this was both caused by prey depletion by carabids and biased sampling of hungry carabids in preference to satiated carabids. In areas of low prey density where a higher proportion of beetles will be hungry and thus more active, these beetles have an increased probability of falling into pitfall traps, thus over-estimating carabid density. A possible explanation for the spatial patterns observed by Bohan *et al.* (2000a) is that in June when spatial association was observed, carabid beetles would have only just emerged from the soil and would not have had time to significantly reduce the slug numbers in areas of high slug density. However, by July, spatial dissociation could have been caused by higher rates of slug depletion, slugs moving away and beetles reduced activity levels in areas of high slug density.

It is not known how long beetles would arrest for after eating large quantities of prey, but presumably if beetles could still find slugs or other prey to eat then there would be no need for them to move on. There is evidence that in July, when slugs and beetles were spatially dissociated, that beetles fed on a wider range of prey (Chapter 2). Beetle foregut weight was no longer associated with the slug distribution, and there was also no longer a difference in foregut as a proportion of beetle weight between slug-positive and slug-negative beetles in July. Therefore a reduction in the availability of slugs but an increase in the availability of other prey might have kept the beetles from moving on to other areas. Pollet & Desender (1985) found that *P. melanarius* concentrated their feeding on whichever prey item was most abundant at that time, for example, a slight increase in aphid numbers in August coincided with a temporary higher number of beetles feeding on this prey. Carabid beetles have also been shown to have fairly static distributions over time, with local aggregations persisting throughout the sampling season (Thomas *et al.*, 1998; 2001).

7.3. Conclusions

This project provides further evidence that predation upon slugs by carabid beetles is both direct and dynamic rather than opportunistic. Further analysis on the data set of Bohan *et al.* (2000a) in Chapter 2 was valuable in extending our understanding of the spatial dynamics of the interaction. There is now greater evidence that predation upon slugs acts mainly upon immature slugs in the region 25 - 100 mg. This could be an important factor affecting the population dynamics of slugs, as predation by beetles will affect the number of slugs that are able to reach sexual maturity and reproduce. Probably one of the most important findings from this project is that *D. reticulatum* responds to and avoids chemical cues from *P. melanarius* (Chapters 3 and 5). This provides further evidence that predation on slugs is not just opportunistic, since beetles must have exerted significant selection pressure on slugs for anti-predator behaviour to have evolved. These findings have further implications in the field of behavioural ecology since this is the first detailed study of predator avoidance behaviour by a terrestrial mollusc and is significant in that it shows the slugs are responding to a predator that is a generalist and not a mollusc specialist. It is hoped that further analysis of the biochemical data presented in Chapter 5 will identify the chemical to which slugs respond, and potentially the discovery of a novel slug repellent. The implications of this could be very exciting but obviously this depends greatly on the properties of the chemical before any commercial applications could even be considered.

In order for a predator to be a successful biological control agent, some factors have in the past been considered to be critical to their ability to suppress a pest. One of these assumptions is that a biocontrol agent should be highly prey specific, therefore targeting the pest successfully without being diverted by other prey. Other attributes considered to be essential include a high degree of voracity for the prey (including high searching capacity and attack rate) and a functional and numerical response to prey density (behavioural, as in aggregating to prey density, and reproductive) (Luff, 1983). These attributes are more commonly associated with specialists rather than generalists. However, generalists are receiving increasing attention with regards to developing pest management strategies (Chang & Kareiva, 1999; Symondson *et al.*, 2002). Generalists as biocontrol agents may have some advantages over specialists. Generalists can switch to alternative prey when pest densities are low, therefore maintaining a high population density in the field when another pest resurgence occurs (Murdoch *et al.*, 1985; Boer, den 1986; Settle *et al.*, 1996; Symondson *et*

al., 2000). Generalists predators of arable land may be temporally adversely affected by local stochastic events such as ploughing or insecticide spraying, but they can re-colonise from adjoining unaffected areas (Symondson *et al.*, 2002b). Some generalists have also been found to show aggregative responses to prey, particularly if that prey forms a substantial component of their diet (Bohan *et al.*, 2000a; Winder *et al.*, 2001, 2004; Warner *et al.*, 2003). Finally assemblages of natural enemies may be more effective than single species of specialists as many life stages of the pest can be attacked simultaneously and at different times of the year (Symondson *et al.*, 2002b). Some researchers have found that in areas where polyphagous predators were artificially enhanced, prey removal rates increased (Riechart & Bishop, 1990; Winder, 1990; Lang *et al.*, 1999; Menalled *et al.*, 1999; Collins *et al.*, 2002). Such studies provide evidence that generalist predators can have an important controlling effect on pest populations in arable land. Unlike specialists and their prey, rarely has there been found examples of generalists forming long-term coupled relationships with their prey. However, this can situation can arise where one particular prey item forms a substantial portion of the generalists' diet, for example, in subarctic mammal predators (Turchin & Hanski, 1997; Angerbjorn *et al.*, 1999). Symondson *et al.* (2002) showed this to be the case for *P. melanarius* and slug prey when they found that beetle numbers had a significant effect on slug population growth for at least five years. The work in this thesis has therefore added to the evidence provided by other studies that some generalist predators can have significant effects on the dynamics of pest populations and therefore may become useful biocontrol agents for pests if effectively managed.

Carabid beetles, and *P. melanarius* in particular, need to be considered when developing integrated control management for slugs. *P. melanarius*, which is principally found in the middle of crop areas rather the field margins, may not benefit from the development of beetle banks as other carabids do, but they can be detrimentally affected by farming practices such as ploughing (Symondson *et al.*, 1996), applying insecticides (Buchs *et al.*, 1986; Purvis & Bannon, 1992; Holland *et al.*, 2000) and spring-cultivating rather than autumn-cultivating. Further work is needed to investigate how carabid management affects the temporal and spatial dynamics of slugs, and whether carabid management should be adopted more widely for controlling slugs and other pests.

7.4. Further research

In this section is a broad description of experiments and ideas that would have been interesting to investigate as part of this project, but could be not due to time and practicality constraints. I will also describe potential areas for further research in the field of slug-carabid dynamics.

Much of the work in this project focussed on *D. reticulatum*, but since *D. reticulatum* shows contrasting spatial arrangements to *A. intermedius*, and they show different behaviour towards *P. melanarius* (Pakarinen, 1994a; Chapter 3), it would have been interesting to have been able to do more work with *A. intermedius*. However, *A. intermedius* was rarely found under baited cover traps, because they are primarily subterranean. Collecting enough *A. intermedius* for experiments was always a problem as taking soil samples for flooding is very time consuming and there is no guarantee that any *A. intermedius* will be found. Experiments to examine the preferences of *P. melanarius* for *A. intermedius* and *D. reticulatum*, and how this co-varies with their species-specific defence mechanisms would have been interesting. It is not really known to what degree *P. melanarius* prey upon *A. intermedius* in the field, but some mini-plot work may have shown whether *P. melanarius* encounter and eat *A. intermedius*, particularly since *A. intermedius* spends most of it's time under the soil. Different predation rates on the two species might help to explain some of the differences in their spatial dynamics observed by Bohan *et al.* (2000b).

The work in Chapter 5 needs to be completed as it would be interesting to identify the chemical in fraction D that was shown to have antifeedant effects on *D. reticulatum*, and was potentially responsible for slugs avoiding areas previously exposed to *P. melanarius*. In addition to analysis of the NMR output, a sample from fraction D is going to be analysed using spray ionisation mass spectrometry, and this will hopefully provide more information about the chemical to aid its identification. One experiment that was considered but could not be carried out due to time constraints was to isolate the chemicals from the exterior of *P. madidus* and *P. cupreus* and test these in leaf disc choice bioassays against slugs. In lab experiments *D. reticulatum* avoided paper exposed to *P. madidus* but not *P. cupreus* (Chapter 3), and chemical analysis of these beetles would provide information about whether *P. madidus* possesses a similar chemical profile to *P. melanarius*. It might also be interesting to test whether beetle faeces are also avoided by slugs since in the field these could provide a cue to indicate the recent presence of the beetle.

A larger scale study, repeating some of the fieldwork by Bohan et al. (2000a) but with some modifications to the methods would be beneficial. Some sampling for other prey could be undertaken, especially since recent work has shown that *P. melanarius* have spatial associations with aphids (Winder *et al.*, 2001; 2004). Given recent advances in techniques that allow identification of prey DNA in predator guts (Zaidi *et al.*, 1999; Symondson, 2002b; Harper *et al.*, in press), it would be possible to identify the range of prey in guts of *P. melanarius*, and make trophic and spatial links between the beetles and their specific prey. Some modifications to the spatial sampling would also be needed. Winder *et al.* (2001) found a lagged positive spatial association between beetles and aphids, with the beetles aggregating in areas where patches of aphids had been recorded one week before. This lagged response could not be picked up by the data in the Bohan *et al.* (2000a) study because beetles were sampled one week before the slugs rather than the other way around. Modifying the times at which prey and predators are sampled may allow lagged spatial responses to be found. Finally, sampling the carabid population density could be done using pitfall traps combined with mark-recapture work, which would provide a better estimate of absolute densities than if pitfall trapping were used alone.

An individual-based model being developed by Dr Yoon Choi at Rothamsted Research aims to look at the effects of carabid beetles on the spatial distribution of slugs. He is currently attempting to incorporate the beetle avoidance behaviour into the model to look at how this might affect the variance to mean ratio for slugs. It will be interesting to see the results from this analysis and whether the beetle avoidance behaviour may be able to help explain some of the spatial patterns observed by Bohan *et al.* (2000a; 2000b).

The next important step in this field of research is to set up field trials to see how effective some of the carabid management strategies are at reducing slug populations. Several studies have found that some farming practices increase numbers of carabid beetles (Powell *et al.*, 1985; Chiverton & Sotherton, 1991; Thomas *et al.*, 1991; Lys & Nentwig, 1992; Pickett & Bugg, 1998; Landis *et al.*, 2000), but few studies to date have examined the influence of such measures on prey removal rates from the field, and the long term effects of such management on pest populations. We have seen that the common carabid beetle *P. melanarius* has a strong influence on the spatial and temporal dynamics of slugs, so the next step is to examine the effects of *P. melanarius* management on slugs as part of an integrated control program.

Chapter 8

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Appendices

APPENDIX A - **ARMSWORTH, C. G., BOHAN, D. A., POWERS, S. J., SYMONDSON, W. O. C. & GLEN, D. M.** 2005. BEHAVIOURAL RESPONSES BY SLUGS TO CHEMICALS FROM A GENERALIST PREDATOR. *ANIMAL BEHAVIOUR*, IN PRESS.

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Behavioural responses by slugs to chemicals from a generalist predator

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Antipredator behaviour is not expected to evolve in response to generalist predators unless the prey forms a substantial proportion of the total diet of the predator and this predation is a major mortality factor. Since the generalist predator *Pterostichus melanarius* (Coleoptera: Carabidae) can affect the temporal and spatial dynamics of slugs, we investigated whether the slug *Deroceras reticulatum* responds to chemical cues from these beetles. We recorded the movements of slugs in arenas incorporating both a zone containing paper upon which the predatory beetles had been maintained and a control zone. Significantly more slugs accumulated on the control half of arenas after 24 h, with small slugs being quickest to respond. Slugs avoided paper exposed to both male and female beetles. They did not respond to paper that had been exposed to beetles and then stored for 5 days before the test. Slugs moved faster, turned less, spent proportionately more time moving and spent less time overall on paper exposed to male beetles than on control paper. This is consistent with a kinesis that would enable the slugs to escape rapidly from areas where predators were present. We conclude that slugs have evolved behavioural responses to chemical cues from either this generalist carabid predator in particular, or carabid beetles generally, many species of which include molluscs in their diets.

The detection and avoidance of predators is important to prey because predation has severe and immediate consequences on fitness (Dicke & Grostal 2001). Visual and physical detection of predators can be risky for prey but chemical detection may allow areas of high predation risk to be avoided without risking contact with the predator. Predators produce a variety of volatile and nonvolatile chemicals, such as those for communicating with conspecifics, or from excreta and faeces, and prey can use these cues to avoid the predator (Dicke & Grostal 2001). For organisms such as slugs, chemical cues may be important in antipredator behaviour because visual input is limited and escape-driven dispersion is slow relative to the speed of their predators. Although there are reports in the literature of marine gastropod antipredator behaviour (reviewed by Kats & Dill 1998), it is not yet known how terrestrial gastropods detect and behave in response to their predators. In this study, we tested the response of the major slug pest *Deroceras reticulatum* (Müller) to chemical cues from a generalist predator, the carabid beetle *Pterostichus melanarius* (Illiger).

Pterostichus melanarius is one of the most abundant carabids in arable land and an important predator of slugs (Symondson et al. 1996, 2002a; Bohan et al. 2000a). The detection of slugs by *P. melanarius* has been investigated under laboratory conditions using classical behavioural and electrophysiological approaches (McKemey et al. 2004). However, one might expect generalist predators, such as *P. melanarius*, to exert insufficient selection pressure on a single prey class (molluscs) for antipredator responses to evolve in their prey. Evolution of behaviour might be expected if the prey is the preferred food for that species or forms a major constituent of the total available prey (Symondson et al. 2002b). *Pterostichus melanarius* can affect the temporal and spatial dynamics of slugs (Symondson et al. 1996, 2002a; Bohan et al. 2000a). For example, changes in the spatial dynamics of the slug and

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beetle populations in the summer of 1997 were thought to depend on both attraction of *P. melanarius* to locales of high slug abundance and their predation of those slugs (Bohan et al. 2000a). The observed variance to mean ratio for slug counts in the field decreased in the summer, when *P. melanarius* were present, and Bohan et al. 2000b argued that this effect might stem from slugs adopting predation avoidance behaviour such as seeking refuge or moving to areas of lower beetle density.

Avoidance/escape is the most commonly reported type of antipredator behaviour (Kats & Dill 1998). Escape usually involves an increase in speed or overall activity to leave a high-risk environment. For nonvolatile chemical cues, only the intensity of the stimulation can be measured and there is no directional information. Changes in kinesis behaviour are therefore the most likely response to this type of chemical stimulus (Fraenkal & Gunn 1940). Kinesis is defined as movement that is proportional to the intensity and is independent of the spatial properties of the stimulus (Gunn et al. 1937). Responses usually take the form of a change in velocity (orthokinesis) or a change in the rate of turning (klinokinesis).

We investigated whether *D. reticulatum* adopts antipredator behaviours towards a generalist predator, by testing the null hypothesis that the slugs do not respond to residual *P. melanarius* chemicals. We conducted choice experiments to measure the effects of slug size, beetle sex and chemical persistence (longevity) on avoidance by slugs of paper that had been exposed to the predator. Slug size is important in prey choice by *P. melanarius* (McKemey et al. 2000), and both male and female beetles prey upon slugs in the field (Symondson et al. 1996; Bohan et al. 2000a). To measure the responses of slugs to beetle chemicals, we used a video-tracking program to record velocity and turning rates so that kinesis could be measured accurately. These experiments are part of a larger research programme investigating the use by slugs of chemical cues to detect and respond to their predators, and to identify the chemicals involved.

METHODS

We collected *D. reticulatum* and *P. melanarius* from grassland and wheat fields around Rothamsted Research, Harpenden, U.K. Slugs were stored in groups of 20 in clear Perspex boxes (26 × 14 cm and 9 cm high), lined with moistened cotton wool. The slugs were fed on Chinese cabbage and maintained in the dark in an incubator at 10 ± 2°C for up to a week before each experiment. This low temperature inhibits the spread of disease among individuals and reduces the mortality rate of stored slugs (personal observation). We used a higher temperature of 15°C for running the experiments, because this is closer to the optimum level for growth and activity (South 1992) and is more typical of temperatures at the time of year (June–September) when *P. melanarius* adults prey on slugs. We used pitfall traps to collect *P. melanarius* during July and August. The beetles were sexed, stored in groups of 20 and placed in Perspex boxes (as described above), containing 5 cm of moist peat. They were fed on Hilife Complete Moist Menus dog food. The boxes were stored in the dark at 10 ± 2°C for up to a month before the behavioural choice experiments, and up to 3 months before the slug video-tracking experiment.

Behavioural Choice Experiments

We did three experiments to test the responses of slugs to beetle residual chemicals on test papers within arenas that were the same Perspex boxes (26 × 14 × 9 cm) as used above for maintaining slugs and beetles. Each arena wall was painted with Fluon to ensure all slug movements were confined to the arena floor (Symondson 1993a). The bases of the arenas were lined with a thin layer of cotton wool moistened with tap water. Test and control paper could then be laid over the cotton wool and be kept uniformly moist throughout the experiments. We created beetle-exposed test papers by covering the bases of similar Perspex boxes with two sheets of moistened filter paper (14 × 13 cm) placed side-by-side. Six *P. melanarius* were then placed in these boxes, the lids closed, and the beetles allowed to walk over the paper for 24 h in the dark at 15 ± 2°C. After this period, we removed the beetles and, for testing, we randomly assigned each sheet of beetle-exposed paper to one half of a test arena. The other half of each arena was then covered with a control of plain moistened filter paper that had not been exposed to beetles. In each choice experiment we added six slugs to each arena, three on each piece of paper, and then closed the lids. We numbered the arenas and then stored them at 15 ± 2°C in the dark. Half of the arenas were oriented with control paper on the left and the others were oriented with control paper on the right. We recorded the number of slugs on the test and control paper at 1, 6 and 24 h. This basic design was used for all three of the choice experiments described below.

Choice experiment 1: slug size bioassay

We stored three male and three female *P. melanarius* in each box containing test papers for 24 h before the experiment. Sixty slugs were selected, 20 from each of three size categories: small (30–100 mg), intermediate (100–200 mg) and large (200–500 mg). The small class represents presexual slugs, the intermediate class represents slugs reaching sexual maturity and the large slugs represent sexually mature adults. We used 10 replicate arenas each containing two slugs from each size category. For each arena, one slug of each size group was placed in the centre of the control paper and the other in the centre of beetle-exposed paper.

Choice experiment 2: beetle sex bioassay

To test the effect of beetle sex on slug behaviour, we set up 16 arenas with eight replicates for each beetle sex. We stored pairs of test papers with either six female beetles or six male beetles for 24 h before the choice experiment. Slugs collected for this experiment weighed 200–800 mg and we randomly assigned six slugs to each arena, three in the centre of each piece of paper.

Choice experiment 3: chemical persistence bioassay

We stored pairs of test papers with either six female or six male beetles for 24 h. We removed the beetles and the exposed test papers were then stored for a further 5 days before being placed in the arenas for the start of the choice experiment. Slugs collected for this experiment weighed 200–800 mg and we placed six slugs, randomly selected, in each arena, three in the centre of each piece of paper. There were eight replicate arenas for each beetle sex.

Statistical analysis of choice experiments

For analysis we modelled the proportion of slugs on the control side, separately for each time interval and for each choice bioassay, using a generalized linear model assuming a binomial distribution (McCullagh & Nelder 1989). Replication was given by the number of arenas. We fitted a constant term for the mean proportion of slugs on the control side. The standard error for this constant term was then used to test whether the mean proportion was greater than 0.5 using a one-tailed t test. We used a one-tailed test because a preliminary experiment had shown avoidance of beetle-exposed paper. To check whether slugs were orienting to a particular side (left or right) regardless of treatment, we tested a factor for arena orientation in the model. In choice experiment 1 there were three size groups per arena, so we included the effect of arena as a blocking factor. In choice experiments 2 and 3, we fitted beetle sex as a factor to see if there was a significant effect. Overdispersion (see, for example, Collett 1991) of the data, given that expected for a proposed binomial distribution, was accounted for where necessary by setting the dispersion parameter to the residual mean deviance rather than assuming unity in the calculation of standard errors. In these analyses, given equal binomial totals, the result of this is to scale the standard errors of estimated parameters (proportions) by the square root of the residual mean deviance. We ran all analyses using the GenStat version 7 (Lawes Agricultural Trust, Rothamsted Research, Harpenden, U.K.) statistical package.

Video Tracking of Slug Movements

The beetle-exposed papers were stored in the dark with either six male or six female beetles for 24 h at 15 ± 2°C before each recording. We arranged the arenas on a bench in a controlled environment room (18 ± 1°C), and for each recording a pair of arenas were positioned under a camera so that one arena had the control paper on the left and the other arena had the control paper on the right. The room was illuminated with a red strip light and an additional desk lamp fitted with a red bulb was mounted directly overhead to provide uniform lighting over the arena floors. We suspended a monochrome camera overhead and this was connected to a VCR set in lapse mode to record 12 h of footage on 30 min of a 3-h tape when played back in normal mode.

Using a paintbrush we transferred one slug (400–800 mg) to the centre of each arena, along the divide between the two pieces of paper. We videoed pairs of arenas simultaneously over 12 h and made 10 recordings (of pairs of arenas), five for male beetles and five for females, giving 10 replicates for each sex of beetle. We used each slug and each piece of control paper and beetle-exposed paper only once and washed arenas thoroughly after each use.

We analysed the video data using a video-tracking system, Noldus EthoVision Pro (Noldus et al. 2002) to calculate parameters for the movement and activity of the slugs. First, a frame grabber digitized the video image and each frame was converted to a grid of pixels. The slug pixels were identified against the white contrasting background and tracked. The digitized images were sampled by the program at a rate of five per time-lapse second (this is equivalent to a real-time sampling rate of once every 4.8 s over the 12-h period of filming). In each sample image, the coordinates of the slug were recorded and each position was then marked on the screen with lines connecting successive points to produce the track to be analysed. For each recording we defined both the two test arenas and 'zones' for the control and beetle-exposed papers, within each arena, on the computer screen.

The movement parameters that we selected for analysis were the time spent in the zone, distance moved, mean velocity, mean absolute angular velocity, mean absolute meander and time spent moving. By nesting the data by zones we could compare the values of each parameter on control and beetle-exposed paper. Time spent in each zone was calculated as the total time (s) that a slug spent in each zone. Distance moved was the total length of the tracks (cm) within each zone. Mean velocity was the average speed of slugs in each zone (cm/s). Mean angular velocity (°/s) was the average change in direction of the slug in each zone. Mean meander (°/cm) was the average change in direction of slugs in each zone relative to the distance moved. The time spent moving included any time when the slug moved more than 0.2 cm between image samples. We set this threshold to avoid slight movements caused by noise or body wobble being interpreted by the program as movement. We divided the time spent moving by slugs in each zone by the time spent in each zone to get the proportion of time in each zone that was spent moving.

We checked whether orientation was having an effect on each movement parameter using one-way analyses of variance (ANOVA) applied to the data calculated as for the differences between control paper and beetle-exposed paper for each slug. Paired one-tailed t tests were calculated to assess whether these differences were significantly different from zero, with separate tests being performed for data deriving from male and female beetles. As before, we used one-tailed tests because a preliminary experiment had shown avoidance of beetle-exposed paper.

RESULTS

Behavioural Bioassays

The proportional data for the effect of arena orientation from each choice experiment after any interval were nonsignificant for all experiments.

Choice experiment 1: slug size bioassay

When arena was fitted as a factor in the model, it was significant after 1 h and 6 h (chi-square test: 1 h: $\chi^2_9 = 19.74$, $P = 0.020$; 6 h: $\chi^2_9 = 17.80$, $P = 0.038$) and nonsignificant after 24 h ($\chi^2_9 = 14.59$, $P = 0.103$). Arena was therefore maintained as a blocking factor in each model. When fitting this model to the data we found evidence of overdispersion after 1 and 6 h, so we adjusted standard errors accordingly. Although size was not a significant factor for each model overall, with this term included only the slugs in the smallest class were in significantly greater numbers on the control paper after every time interval (1 h: $t_{18} = 3.16$, $P = 0.003$; 6 h: $t_{18} = 4.39$, $P < 0.001$; 24 h: $t_{18} = 3.72$, $P < 0.001$). There were significantly more intermediate-sized slugs on the control paper than on beetle-exposed paper only after 24 h (1 h: $t_{18} = 0.94$, $P = 0.180$; 6 h: $t_{18} = 1.56$, $P = 0.068$; 24 h: $t_{18} = 3.72$, $P < 0.001$) and significantly more large slugs on the control paper at 6 and 24 h but not at 1 h (1 h: $t_{18} = 0.94$, $P = 0.180$; 6 h: $t_{18} = 2.72$, $P = 0.007$; 24 h: $t_{18} = 2.94$, $P = 0.004$). Thus, *D. reticulatum* of all sizes moved away from the beetle-exposed paper and accumulated on the control paper.

Choice experiment 2: beetle sex bioassay

When beetle sex was fitted as a factor to each model it proved to be nonsignificant at each time interval ($P > 0.368$). The model fit showed that there were significantly more slugs on the control paper than on the beetle-exposed paper at each time interval (1 h: $t_{15} = 3.92$, $P < 0.001$; 6 h: $t_{15} = 4.30$, $P < 0.001$; 24 h: $t_{15} = 2.35$, $P = 0.016$). Thus, paper exposed to either male or female beetles was avoided by slugs, and the effect persisted for at least 24 h.

Choice experiment 3: chemical persistence bioassay

As in the previous experiment, the effect of beetle sex was nonsignificant at each time interval ($P > 0.213$). There was no significant difference in the number of slugs on the control paper or the beetle-exposed paper at any of the time intervals (1 h: $t_{15} = 0.96$, $P = 0.176$; 6 h: $t_{15} = 0.69$, $P = 0.252$; 24 h: $t_{15} = 1.23$, $P = 0.119$). Thus, any chemical cues left on the paper had faded after 5 days so that slugs no longer detected or responded to them.

Video Tracking of Slug Movements

Video-tracking analyses indicated that arena orientation had no effect on any of the movement parameters. There were significant differences in slug movement parameters in the presence of male, but not female, beetle-exposed paper (Fig. 1). As none of the parameters measured were affected by papers exposed to female beetles, the results below are (unless stated otherwise) for males only.

Slugs spent significantly more time on control paper than on male beetle-exposed paper (*t* test: $t_9 = 2.20$, $P = 0.027$; Fig. 1a). There was no significant difference between the distance moved by slugs on control and beetle-exposed paper when the paper had been exposed to either males (*t* test: $t_9 = 0.10$, $P = 0.462$; Fig. 1b) or females (*t* test: $t_9 = 0.11$, $P = 0.542$). Slugs moved significantly faster (*t* test: $t_9 = 3.34$, $P = 0.004$; Fig. 1c), and spent more time moving ($t_9 = 2.08$ $P = 0.034$; Fig. 1d) on male beetle-exposed paper than on control paper. Angular velocity of slugs was significantly greater ($t_9 = 2.73$, $P = 0.012$; Fig. 1e) and slugs meandered significantly more ($t_9 = 2.23$, $P = 0.026$; Fig. 1f) on control paper than on male beetle-exposed paper.

DISCUSSION

The results clearly show that *D. reticulatum* avoided crawling over paper that had been exposed to *P. melanarius*. This work represents the first detailed study of predator avoidance behaviour by a terrestrial mollusc and is important in that it shows that slugs respond to a predator that is a generalist and not a mollusc specialist.

In choice experiments, slugs preferred to accumulate on control paper that had not been exposed to the predator. This is consistent with the hypothesis that residual kairomones left behind by the carabids crawling over the test paper were detected and avoided by the slugs. In choice experiment 1, slugs from all size classes avoided crawling on beetle-exposed paper. Although only small slugs (30–100 mg) were recorded in significantly greater numbers on the control paper at every time interval, size was not significant when fitted as a factor in the model. In choice experiment 2, paper exposed to either male or female beetles elicited equally strong avoidance responses in slugs, and consequently it would seem likely that slugs avoid both sexes. Tests of the guts of field-caught beetles for slug protein using antisera have shown that both males and females prey upon slugs to a similar extent (Symondson et al. 1996; Bohan et al. 2000a). In choice experiment 3, 5-day-old beetle-exposed paper was not avoided by the slugs. This could be the result of the slugs either not detecting chemicals from the beetles after this period, or assessing how long ago the beetles were in an area by the concentration of residual chemicals present on the surface and hence the current risk of predation. It would not be beneficial to initiate potentially costly predator avoidance behaviour or forgo exploiting areas where predation risk is no longer high, since this can interfere with reproduction and foraging (Lima & Dill 1990).

In contrast to the findings of choice experiment 2, slugs responded in the video-tracking study only to male beetle-exposed paper. The beetles had been stored for 1 month before the choice experiments but up to 3 months before the video-tracking experiments and this may have affected the chemicals they deposited on the paper in some way. During these 3 months beetles in the field would normally be either dying off or overwintering. Females are also known to be less active than males (Thomas et al. 1998), whereas males remain active in their search for females. Consequently, after an extended period in which females are well fed, they might become progressively less active in the laboratory and leave less chemical residue on test papers.

Results from the video-tracking study showed that the accumulation of slugs on the control paper observed in

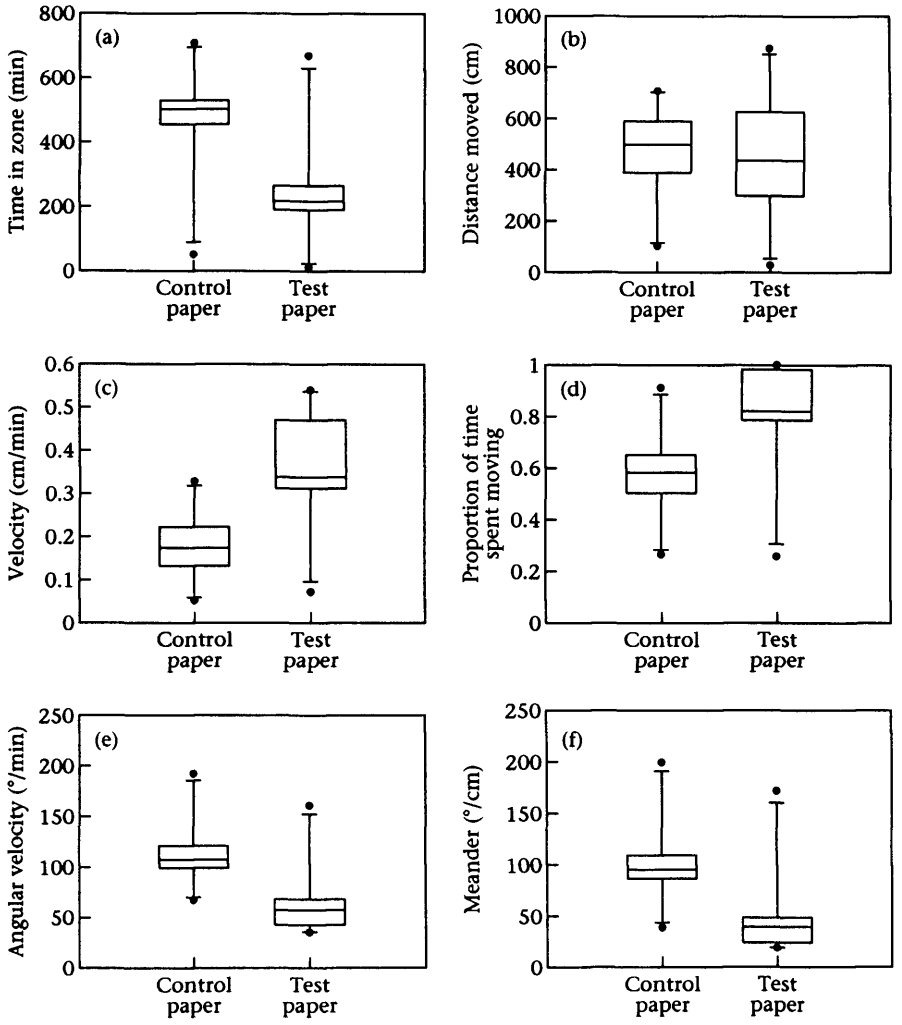

Figure 1. Box plots of (a) time in zone, (b) distance moved, (c) mean velocity, (d) time spent moving, (e) mean absolute angular velocity and (f) mean absolute meander for *Deroceras reticulatum* in arenas with male *Pterostichus melanarius*-exposed paper and control paper. The median is indicated by the horizontal bar inside the box, the 1st and 3rd quartile by the box itself and 1.5 times the interquartile range by the horizontal bars outside the box. Individual outlines are shown by closed circles.

choice experiments 1 and 2 was a result of slugs increasing their velocity and time spent moving and reducing their rate of turning (meander and angular velocity) on the male beetle-exposed side. This behaviour shows that the slugs were responding to the predator cue using klinokinesis and orthokinesis simultaneously (Fraenkal & Gunn 1940). These responses would enable the slugs to leave an area where predators were present more rapidly. Some marine molluscs also alter their velocity in the presence of predator chemical cues. Marsh periwinkles, *Littoraria irrorata* (Say), crawled faster when they detected mucus from the predatory conch *Melongena corona* (Gmelin) (Dix & Hamilton 1993); direction of travel was random and the increase in speed appeared to be for the purpose of escaping by finding a suitable plant stem to crawl up. Limpets, *Acmaea (Collisella) limatula* (Say) and *Acmaea (Notoacmea) scutum* (Rathke), moving upward on a vertical surface increased their speed but maintained the same direction of movement when odour from a predatory starfish *Pisaster* spp. flowed over them (Phillips 1975). As in the present study, these studies of marine molluscs showed that individuals did not respond to the direction of the chemical cue but altered the rate of movement in response to an increased intensity of the stimulus. To the best of our knowledge, our study is one of the first to measure kinesis quantitatively as a method of avoiding predation and the first to examine the behavioural responses of a terrestrial mollusc to risk associated with predation.

What kairomones the carabids produce and whether they are volatile or nonvolatile are not known; work is currently underway to identify them. Preliminary evidence from biochemical work suggests that the kairomones are likely to be found on the beetle's exterior and not in the excreta. From our results we cannot tell whether the slugs responded to a kairomone produced by *P. melanarius* specifically or to one produced by all carabids. However, work is underway to test the specificity

of the responses by repeating all the experiments reported here with other carabid species. Electrical activity in the olfactory nerve of *D. reticulatum* has been induced by blowing volatile odours from *P. melanarius* over tentacular preparations (Dodds et al. 1997). Although this did not, by itself, indicate how *D. reticulatum* might have responded to the odour of this predator, similar nerve firing was not induced by the herbivorous carabid, *Zabrus tenebrioides* (Goeze). Should the same chemical(s) prove to be present in all carabids it may still be selectively advantageous for slugs to avoid areas of high carabid beetle density since most large and medium-sized carabids prey upon slugs in the field (Tod 1973; Ayre & Port 1996) and few are purely phytophagous.

As with this study, most research that has investigated antipredator behaviour has reported changes in movement of prey, specifically avoidance/escape responses (Kats & Dill 1998). Further work is planned to examine the influence of *P. melanarius* on feeding, sheltering and egg-laying behaviour of *D. reticulatum* in semifield conditions. In a complex field environment, slugs may not respond to the chemical cues from *P. melanarius* by moving away across the surface of the soil, but they may choose to take refuge under the soil or among plants. In a laboratory study (McKemey 2000), *D. reticulatum* responded to the presence of *P. melanarius* by aggregating out of reach of the beetles on the control side of arenas. Symondson (1993b) found that slugs were susceptible to attack by the carabid *Abax parallelepipedus* (Piller & Mitterpacher), another generalist predator that includes slugs in its diet, only in immature lettuce plants, because in mature plants they responded to beetle presence by climbing up into the leaves out of reach of attack. Our results may also help explain patterns of spatial dissociation between beetles and slugs observed by Bohan et al. (2000a), as both direct attacks by beetles and the repellent effects of the presence of residual chemicals on the soil surface may result in slug mortality and movement away to areas of lower beetle density, respectively.

Despite *P. melanarius* being a generalist predator, our results suggest that *D. reticulatum* might have been exposed to sufficient selection pressure by this beetle for this slug to have evolved mechanisms for detecting and identifying predator kairomones, and taking avoidance action. Previous work, showing coupled dynamics between *P. melanarius* and *D. reticulatum* (Symondson et al. 2002a), suggested that slugs comprise a substantial proportion of the total prey available to the beetles, driving changes in the slug population. Understanding the mechanics behind exactly what cues slugs use, and how they detect these cues, is the next step to understanding the interaction between these two species in the field.

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APPENDIX B - SUPPLIERS CITED

Gemini Dataloggers (UK) Ltd

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Internet: http://www.geminidataloggers.com

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Golden

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804401-1866

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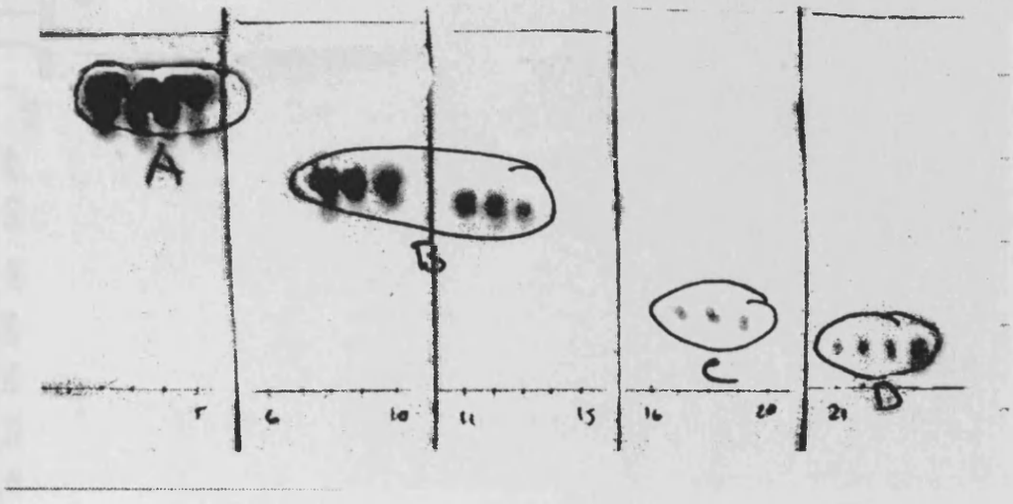
APPENDIX C - FITTED DISTRIBUTIONS (CHAPTER 2) - The fits of the distributions for descriptions involving the factors

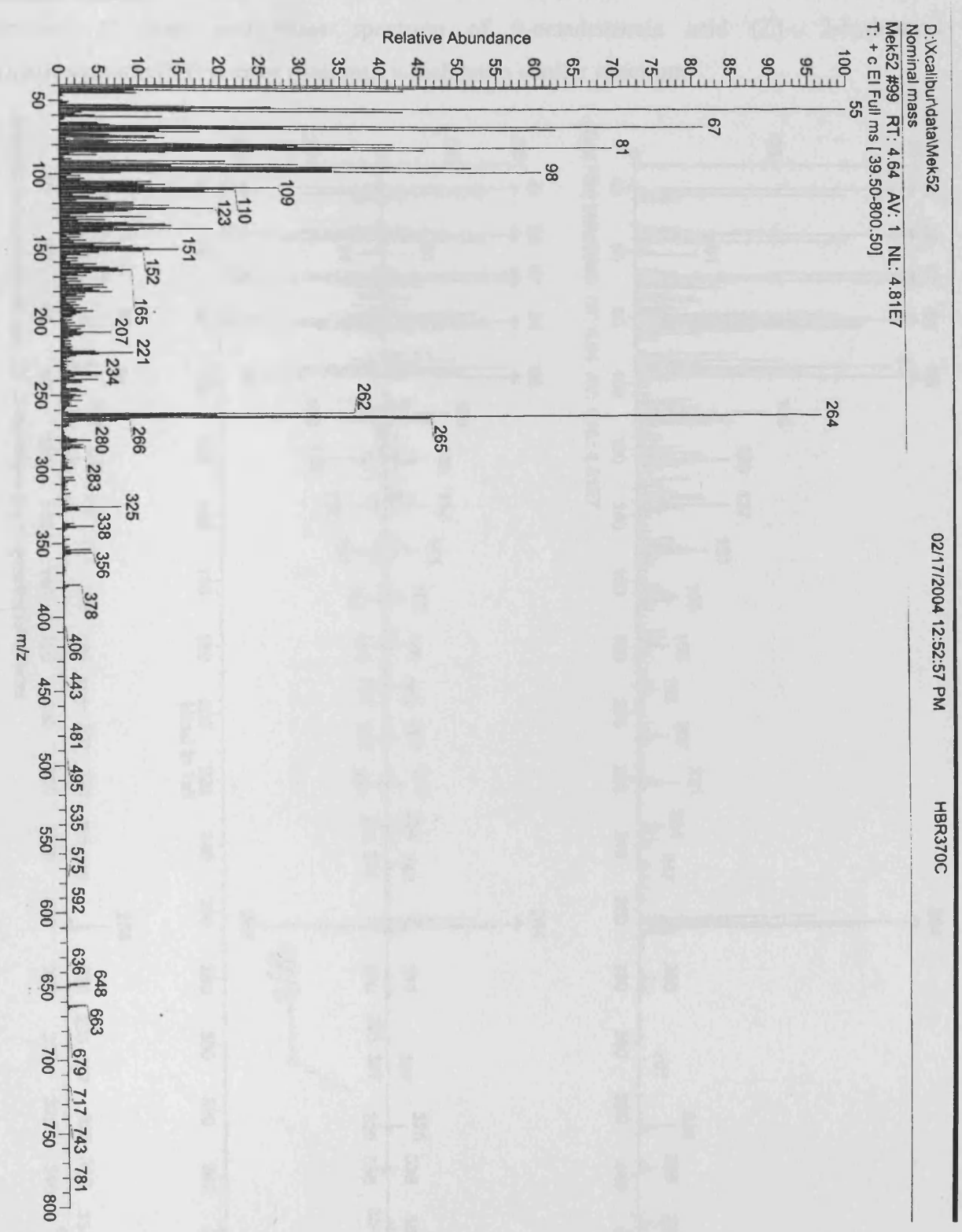
Model Fitted	Factor Level(s)			Deviance	d.f.	p-value
	Date	Slug	Sex			
Date	2			27.63	21	0.151
	3			20.07	21	0.517
	4			23.03	21	0.342
	5			24.53	21	0.225
Sex	M			27.46	22	0.194
	F			32.61	22	0.068
Slug	N			25.74	25	0.422
	Y			46.83	25	0.005
Date × Slug	**2**	**N**		**20.87**	**17**	**0.232**
	2	**Y**		**23.69**	**17**	**0.128**
	3	**N**		**14.81**	**17**	**0.609**
	3	**Y**		**24.53**	**17**	**0.106**
	4	**N**		**17.41**	**17**	**0.427**
	4	**Y**		**14.41**	**17**	**0.638**
	5	**N**		**19.06**	**17**	**0.325**
	5	**Y**		**15.30**	**17**	**0.574**
Date × Sex	2		M	11.97	14	0.609
	2		F	12.70	14	0.550
	3		M	19.69	14	0.140
	3		F	20.41	14	0.118
	4		M	17.55	14	0.228
	4		F	14.57	14	0.415
	5		M	7.96	14	0.891
	5		F	23.05	14	0.059
Sex × Slug		N	M	27.77	17	0.048
		N	F	14.04	17	0.664
		Y	M	27.51	17	0.051
		Y	F	44.84	17	<0.001
Date × Slug × Sex	2	N	M	17.28	11	0.100
	2	Y	M	17.31	11	0.099
	2	N	F	13.91	11	0.238
	2	Y	F	18.13	11	0.079
	3	N	M	10.46	11	0.490
	3	Y	M	15.30	11	0.169
	3	N	F	17.42	11	0.096
	3	Y	F	13.59	11	0.257
	4	N	M	Insufficient Data		

4	Y	M		Insufficient Data	
4	N	F	10.51	11	0.485
4	Y	F	9.97	11	0.533
5	N	M		Insufficient Data	
5	Y	M		Insufficient Data	
5	N	F	13.00	11	0.293
5	Y	F	9.30	11	0.594

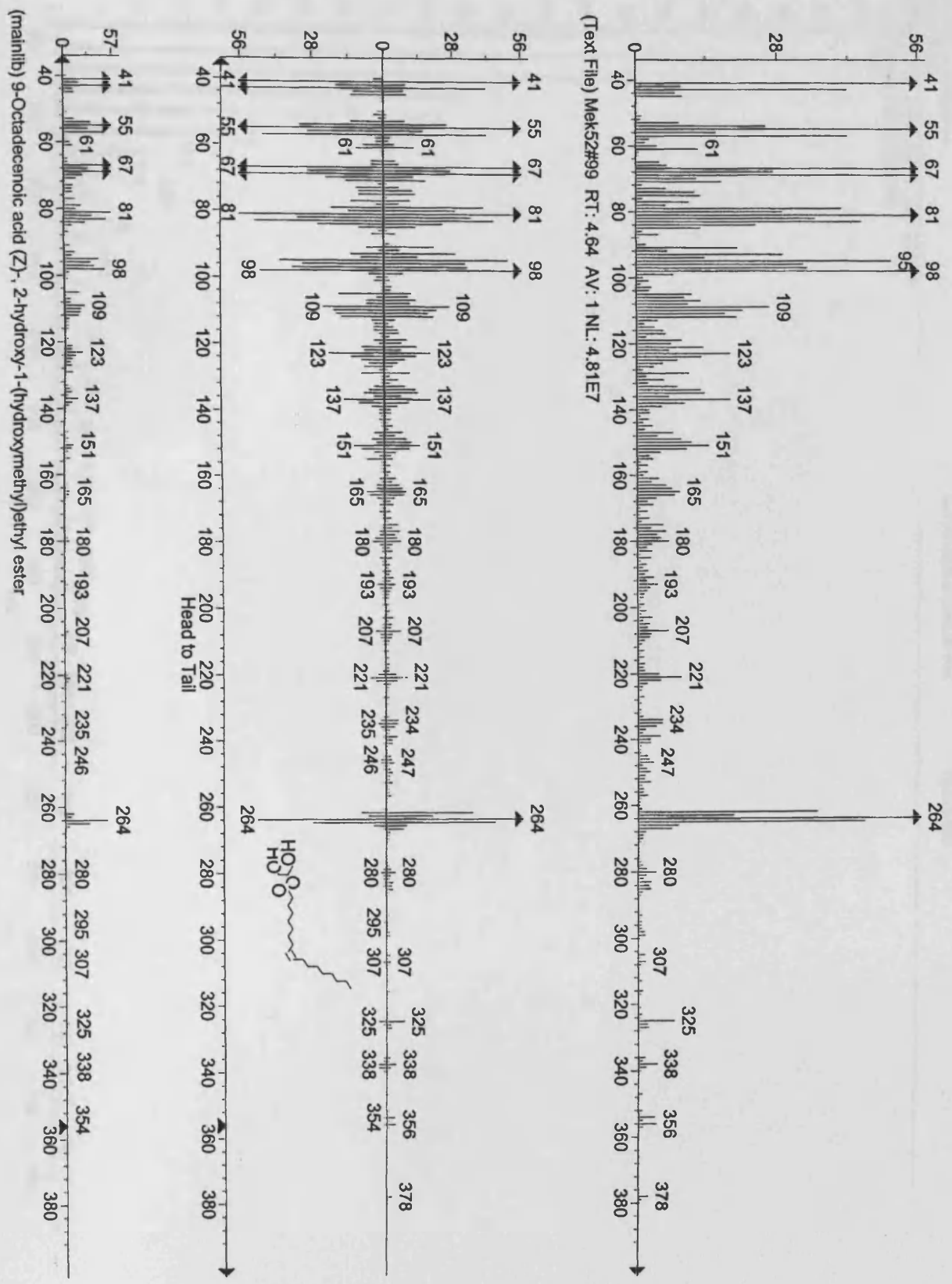
APPENDIX D - THIN LAYER CHROMATOGRAPHY (CHAPTER 5) - Thin layer chromatography (TLC) revealed that the fractions obtained from HPLC of the beetle extract contained four main compounds. Fractions containing the same chemicals were combined to produce four fractions (A-D). A very small quantity of a fifth compound (E) was also obtained.



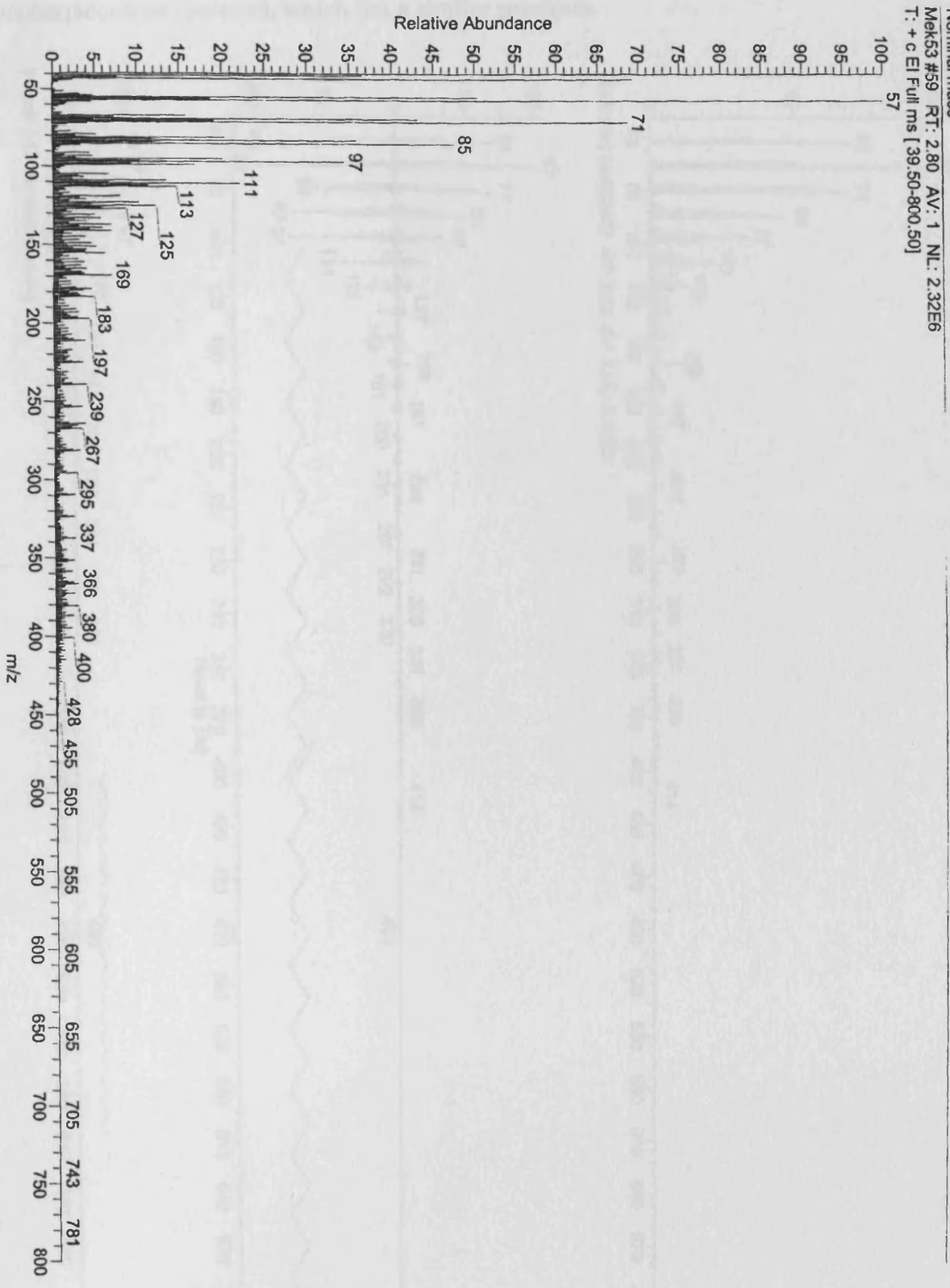
APPENDIX E - MASS SPECTRUM (CHAPTER 5) - Fraction C



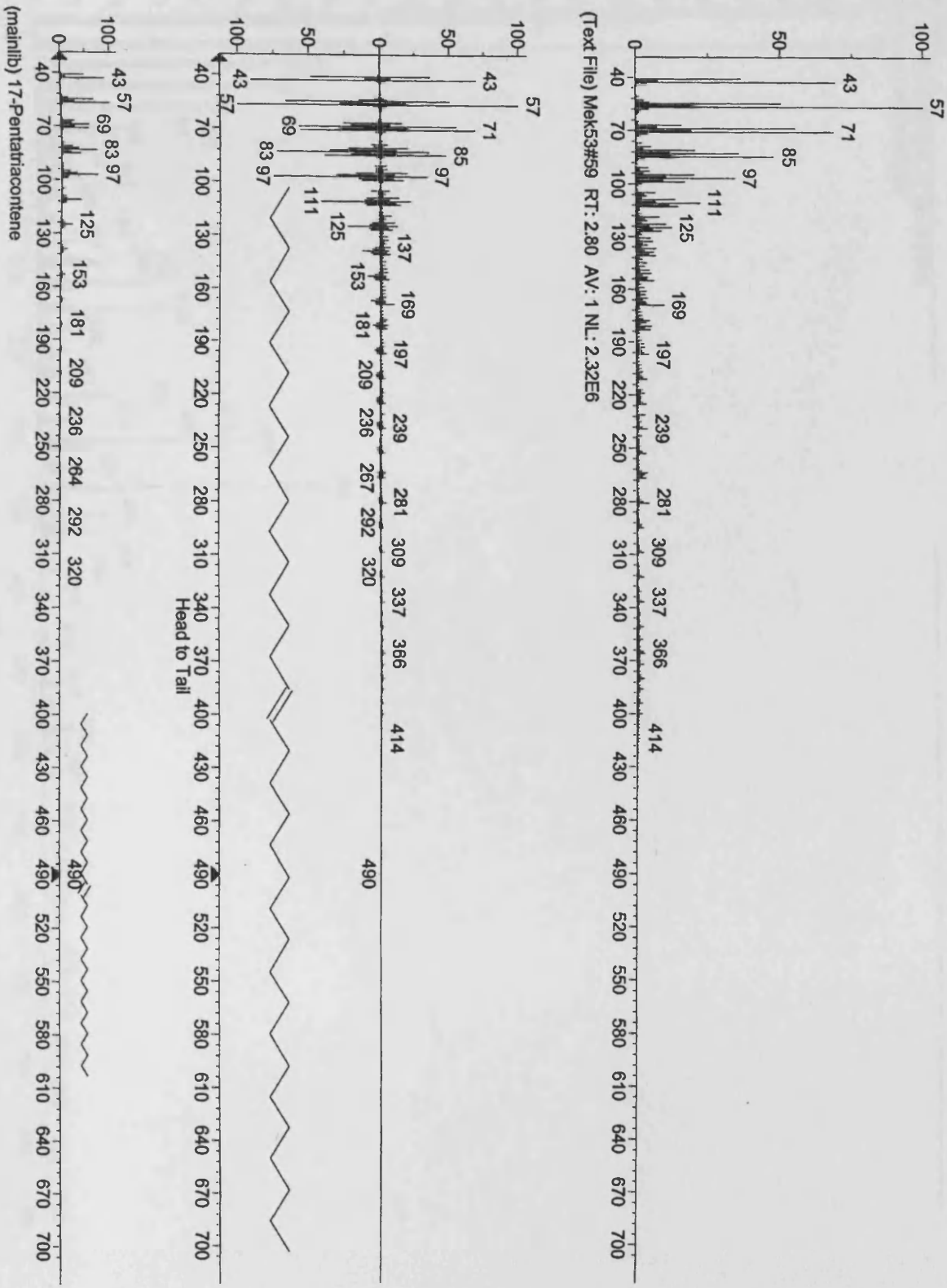
APPENDIX F - MASS SPECTRUM (CHAPTER 5) - Mass spectrum of compound in fraction C (top) and mass spectrum of 9-octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester (bottom), which has a similar spectrum.



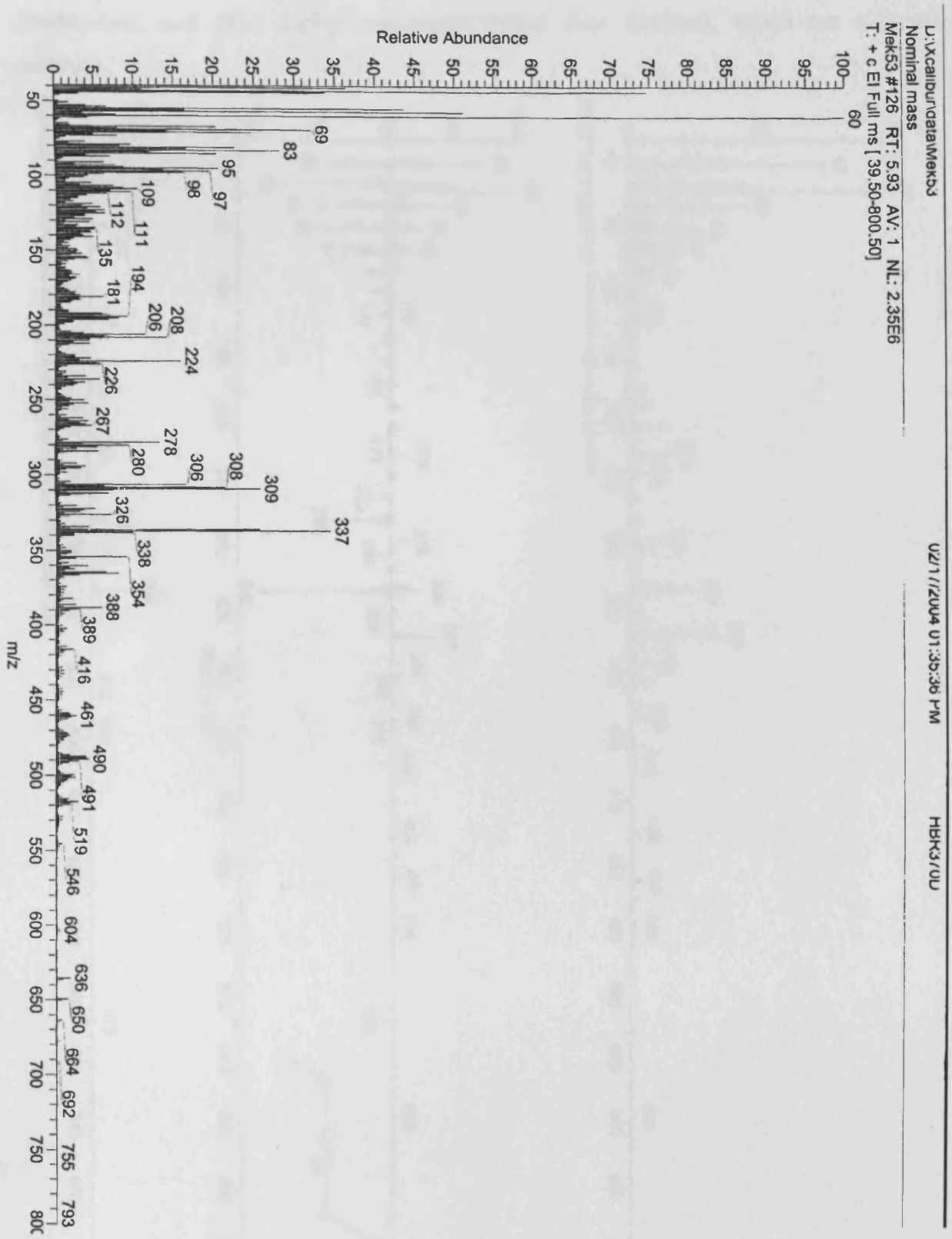
APPENDIX G - MASS SPECTRUM (CHAPTER 5) - Mass spectrum of the compound in fraction D that produced the first peak in the spectrum.



APPENDIX H - MASS SPECTRUM (CHAPTER 5) - Mass spectrum of compound in fraction D that produced the first peak in the spectrum (top) and mass spectrum of 17-pentatriacontene (bottom), which has a similar spectrum.



APPENDIX I - MASS SPECTRUM (CHAPTER 5) - Mass spectrum of the compound in fraction D that produced the second peak in the spectrum.



APPENDIX J - MASS SPECTRUM (CHAPTER 5) - Mass spectrum of compound fraction D that produced the second peak in the spectrum (top) and mass spectrum of octadecenoic acid (Z)-, 2-(9-octadecenyloxy)ethyl ester (bottom), which has a simil spectrum.