Predation and scavenging by the generalist predator *Pterostichus melanarius*

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Adam Powell

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ProQuest LLC
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106-1346

Abstract

The carabid beetle *Pterostichus melanarius* is a generalist that may be a significant predator of pest slugs. Generalists tend to be opportunists that feed on food according to its availability, but may not possess the necessary behavioural or physiological characteristics to effectively prey upon some species, or certain groups of individuals within species. Consequently, their utility in pest control may be limited.

The research reported in this thesis investigated the ability of *P. melanarius* to control slug populations, and the impacts that alternative prey, particularly carrion, has on the efficacy of this predator as an agent of slug pest control. A suite of laboratory- and field-based experiments were conducted to achieve those ends. The main findings were: (1) Prey vital status was significant in determining the feeding preference hierarchy of *P. melanarius*. The mucus defence of live slugs (*Deroceras reticulatum*) deterred attacks by beetles, but feeding on dead *D. reticulatum* emphasized a preference for this prey type by *P. melanarius*. (2) The survival rate of *D. reticulatum* bitten by *P. melanarius* was not different to that of non-attacked control slugs. Attacking bites by *P. melanarius*, visited upon live slugs, did not yield slug DNA-positive results during molecular analysis of beetle foregut contents. (3) *Pterostichus melanarius* was not able to detect by olfaction the presence of live or 12 h-decayed dead *D. reticulatum.* (4) The feeding history of *P. melanarius* had a significant influence on subsequent prey selection. However, the effect interacted with an innate, overarching prey preference hierarchy. (5) A large-scale semi-field experiment identified that *P. melanarius* fed upon slugs, but the effect of predation pressure was not sufficient to induce negative growth in slug population density. The presence of alternative prey, and the increasing mass of individual slugs exerted rate-limiting effects on slug-predation by *P. melanarius.*

Declaration

The work contained within this thesis has not previously been accepted in substance for any degree and is not concurrently submitted in candidature for any degree.

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This thesis is submitted in partial fulfilment of the requirements for the degree of Ph.D.

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This thesis is the result of my own independent work and investigation, except where otherwise stated. Other sources are acknowledged by explicit reference.

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Table of Contents

Chapter 1 -

General Introduction

1.1 Background

Slugs are significant agricultural pests, whose activity can cause severe reductions in crop yields (Port and Port, 1986; Glen, 1989; Glen and Moens, 2002). In attempts to control slug populations and mitigate crop-yield losses, agriculturalists may employ an integrated approach to pest management, through a combination of cultural control (Stephenson and Bardner, 1977; Glen *et al.*, 1984, 1988, 1989; Glen, 1989; Moens, 1989; George *et al.*, 1995; Symondson *et al.*, 1996), and chemical control in the form of molluscicide application (Godan, 1983; Port and Port, 1986; Kelly and Martin, 1989; Bailey, 2002). Natural enemies can also have effects on pest populations, either through predation (South, 1989b, 1992; Peng *et al.*, 1995; Symondson *et al.*, 1996, 2002a; Lang *et al.*, 1999; Bohan *et al.*, 2000a) or parasitism (Wilson *et al.*, 1994; Georgis *et al.*, 2006; Rae *et al.*, 2007). In order to maximize slug pest control, incorporation of natural enemies into the integrated approach may be desirable.

Numerous species of animals are reported to feed on slugs (Tod, 1973; South, 1989b, 1992), but carabid beetles are considered to be the main predators (Symondson *et al.*, 1996, 2002a; Symondson, 2004). *Pterostichus melanarius* (Illiger) (Coleoptera: Carabidae) is one of the carabid species that has received attention. Analyses of gut contents have confirmed that this beetle consumes slug tissue (Ayre, 1995; Ayre and Port, 1996; Symondson *et al.*, 1996; Bohan *et al.*, 2000a; Dodd, 2004; Bell *et al.*, 2010), but importantly, the interaction between *P. melanarius* and slugs was reported to be significant enough that the two exhibited coupled population cycles over a five year period (Symondson *et al.*, 2002a).

Pterostichus melanarius is, however, a generalist predator. As such, slugs may be just one of a multitude of prey upon which beetles feed (Pollet and Desender, 1985, 1986; Symondson *et al.*, 2002b). Generalist predators have been shown to exert significant control on prey populations (Symondson, 2002b), but their generalist habit may make them unreliable as agents of pest control (Symondson *et al.*, 2006). Additionally, the opportunistic nature of generalists may predispose them to making use of any available resources, including carrion. Scavenging on carrion may reduce the efficacy of an agent of

pest control, provide misleading data on predation rates (Sunderland, 1996), and have detrimental effects on carabid populations, through indirect poisoning after feeding on cadavers killed by pesticides (Kennedy, 1990; Purvis and Bannon, 1992; Gyldenkaerne *et al.*, 2000; Mauchline *et al.*, 2004; Navntoft *et al.*, 2006).

1.2 Slugs as crop pests

Slugs are pests of arable, fodder, vegetable and soft fruit crops (Garthwaite and Thomas, 1996), but in monetary terms, they are most damaging to winter wheat and potatoes (Port and Port, 1986). Agriculturalists considered slugs to be the most significant pest in the first wheat crop following oilseed rape (Glen, 1989). Cereal crops sustain greatest damage during establishment, when slugs may damage or kill seeds and seedlings (Moens, 1989; Glen, 1989; Glen and Moens, 2002). In this way, an entire crop may be destroyed (Gould, 1961). Crops sown in early autumn grow rapidly, and so quickly pass through the stage at which they are vulnerable to destruction by slug attack. However, winter wheat crops sown in late autumn remain at the vulnerable stage for longer, at a time when slug population density is higher (Port and Port, 1986), and may therefore sustain severe damage.

Recent data show that the quantity of molluscicide applied to all crop groups increased considerably during the period 1990 to 2006 (Central Science Laboratory, 2008). In 2006, the total weight of molluscicide applied in Great Britain was 315,688 kg, which represents a 471 % increase over the quantity used in 1990. These data may be interpreted such that agriculturalists perceive the threat posed by slugs to have greatly increased during this period. The same data show large between year fluctuations in the quantities of molluscicides being applied. Since agriculturalists are unable to accurately predict when slug damage will occur (Glen, 1989), coupled with the financial cost of application, and the finite duration over which molluscicide is viable, it is likely that most application will be reactionary rather than precautionary. Therefore, the data may represent a good indicator of relative slug abundance.

1.3 Slugs and their ecology

1.3.1 Slug species present on arable land

Slugs from four families are present in the British Isles: Arionidae, Limacidae, Milacidae and Testacellidae (Cameron *et al.*, 1983). *Deroceras reticulatum* (Müller) (Pulmonata: Limacidae) is regarded as the main pest species (Glen and Moens, 2002): it may be highly abundant in arable fields, often in conjunction with one or more *Arion* (Pulmonata: Arionidae) species (Glen, 1989), and is widely distributed throughout Britain (South, 1992; Glen and Moens, 2002). The *Arion hortensis* (Férussac) aggregate and *Milax budapestensis* (Hazay) (Pulmonata: Milacidae) are also important pest species (Port and Port, 1986). An indication of the slug densities observed on arable land in the UK is presented in Table 1.1. These data suggest that slug densities can vary considerably between crops and among sites.

Table 1.1 Estimates of slug densities that may be present under different crop types on arable land in the UK (after South, 1992; Symondson *et al.*, 1996).

Habitat	Species	Density (m^{-2})
Arable	*A. fasciatus*	2
	A. hortensis	18 – 39
	A. intermedius	25
	D. reticulatum	18 – 245
	M. budapestensis	37
Potatoes	*A. fasciatus*	8 – 18
	A. hortensis	36 – 21
	D. reticulatum	21 – 60
	M. budapestensis	66
Winter barley	Total slugs	200
Winter wheat	*D. reticulatum*	54
	Total slugs	57 – 180

1.3.2 Biology and ecology of *Deroceras reticulatum* and *Arion* spp.

Weather and microclimate conditions are of critical importance for slugs (South, 1989b; Young and Port, 1989). This was emphasized in a deterministic model of *D. reticulatum* population dynamics based on daily average air

temperature and daily average rainfall. Using just these two variables, the model was able to explain 81 % of the variance in field data (Choi *et al.*, 2004). A moist environment is critical for slugs because they consist of 80 – 90 % water (Lyth, 1982; Godan, 1983), and water is necessary for the production of pedal and defence mucus. South (1992) highlighted the importance of temperature: he observed that most life history traits in *D. reticulatum* are temperature sensitive.

Response to temperature appears to be species specific. For example, *D. reticulatum* grows and reproduces over a wider temperature range than *Arion intermedius* (Normand) (South, 1982, 1992). South (1989b) reported that a population of *A. intermedius* aestivated during a long summer drought, and their abundance was unaffected. During the same period, *D. reticulatum* remained active, and there was a reduction in their abundance. The behaviours of homing and huddling exhibited by slugs are also likely to be responses to the need to minimize water loss (South, 1992).

Slugs are hermaphrodites and may reproduce by self-fertilization. However, in some species the temporal difference between maturation of spermatozoa and oocytes makes self-fertilization unlikely (Port and Port, 1986; South, 1992). *Milax budapestensis* have a biennial life cycle. Mature slugs breed from autumn until spring. The eggs then generally hatch between May and August (Hunter, 1966; Port and Port, 1986).

In *D. reticulatum*, the majority of breeding occurs when conditions are most suitable, but this species may breed continuously throughout the year (South, 1989a). Development of eggs is also temperature dependent (South, 1982). Given the propensity to breed continuously, and the temperature-dependence of life history events, the generation pattern is complex (Port and Port, 1986), but in general, *D. reticulatum* produces two generations per year (Hunter, 1966). Most *D. reticulatum* eggs hatch in May, but there may be a second hatching peak during September (Hunter, 1966; South, 1992). *Deroceras reticulatum* lays eggs in batches of 22 on average, and can lay 200 – 300 eggs (South, 1982; Godan, 1983). Exceptional individuals may lay up to 500 eggs (Carrick 1938). Individuals may live for approximately nine – 13 months (Godan, 1983), although South (1982) reported a maximum age of 906 days for *D. reticulatum* under laboratory conditions.

Slugs belonging to the genus *Arion* have an annual life cycle (Hunter, 1966; Godan, 1983). *Arion hortensis* agg. breeds from autumn into the spring (Godan, 1983; Port and Port, 1986). Individuals lay eggs in batches of up to 30 (Quick, 1960), and may lay over 200 eggs in total (South, 1982). Eggs may hatch in late spring (Port and Port, 1986), or into July or even later (Hunter, 1966). *Arion intermedius* lays a similar number of eggs (South, 1982) in the autumn, which then hatch by early spring (South, 1992). *Arion circumscriptus* produces fewer eggs (approximately 100), but *Arion subfuscus* produces more (approximately 300) (Godan, 1983). Godan (1983) reported that arionid slugs generally live for 12 months, but South (1982) reported individuals living up to 938 days under controlled conditions.

1.3.3 Spatial distribution: horizontal and vertical

The distribution of slugs is usually aggregated (South, 1965; Hunter, 1966; Bohan *et al.*, 2000a, b). Aggregations may be resolved at multiple scales, which may differ according to species (Bohan *et al.*, 2000b).

Egg laying behaviour and dispersal ability may go some way to explain aggregated distributions. Slugs lay eggs in batches, which are aggregated (South, 1965). Therefore, when the juvenile slugs hatch, they are aggregated. Armsworth (2005) showed that, immediately after hatching, juveniles of the species *D. reticulatum* and *A. intermedius* rapidly dispersed away from the location of the egg batch. However, the distance of dispersal was limited: after 72 h, none of the slugs tested reached the perimeter of test arenas (600 mm x 800 mm). In the field, South (1965) observed similar results: released *D. reticulatum* dispersed rapidly over the first two or three days, but showed little further dispersion thereafter. After initial dispersion has occurred, many species appear to exhibit a homing behaviour, whereby individuals return to a home site after nocturnal activity (South, 1992).

Although the vertical distribution of slugs in soil is partly determined by species preferences, it is largely dependent upon the soil moisture content and the weather conditions. Hunter (1966) reported that 83 % of *D. reticulatum,* 62 % of *A. hortensis*, and 50 % of *M. budapestensis* were found in the top 7.5 cm of soil. During prolonged periods without rainfall, which result in low soil

moisture content, slugs migrate downwards through the soil, to regions where moisture content is higher (Hunter, 1966; Glen, 1989).

1.3.4 Population dynamics

Numbers of slugs present in soil varies greatly within and between years (Glen, 1989; Symondson *et al.*, 2002a). Estimated annual molluscicide usage data (Garthwaite and Thomas, 1996; Central Science Laboratory, 2008) show large fluctuations, highlighting between year variations in slug numbers perceived by agriculturalists.

The size of a population can be described fundamentally by the function:

$$N_{n+1} = N_n + B - D + I - E$$

where: N_{n+1} is the current population size, N_n was the population size at the end of the previous time period, B is the number of births, D the number of deaths, I the number of immigrants, and E the number of emigrants (Begon *et al.*, 2006). Based on the observations of significant genetic variation among *D. reticulatum* within fields, Fleming (1989) contended that within-field populations of *D. reticulatum* are collections of sub-populations. Thus, Fleming (1989) argued for the low dispersive power of slugs, and that recruitment within sub-populations occurs *in situ* rather than through immigration. Other authors have also reported low dispersion in slugs (South, 1965; Armsworth, 2005). It follows logically therefore, that emigration from a population must also be insignificant. Consequently, for a given population of slugs, the current population size might be considered mainly dependent upon the previous population size, births (hatchings) and deaths.

Biotic and abiotic factors may affect the birth and death processes. The principal biotic factors are predators, parasites and pathogens (Port and Port, 1986). The most important abiotic factor is the weather (South, 1989b; Glen and Moens, 2002; Choi *et al.*, 2004, 2006). On agricultural land, cultural activity may also have a large impact on slugs; it may injure them, alter their exposure to biotic and abiotic factors, provide or deprive them of food and shelter, and expose them to harsh microclimates and predators.

1.3.5 Control of slug populations

1.3.5.1 Cultural control

The first line of defence which agriculturalists can employ against slugs is that of cultural control. This involves using farming methods and timing of activities that are detrimental to slugs. Crop history may be the cultural practice to have the most significant effect on slug population size. According to Moens (1989), it is second only in importance to summer wetness. One of the reasons suggested to explain why winter wheat is usually severely damaged by slugs, is that it is often sown in rotation after oilseed rape (Glen, 1989). Oilseed rape has a dense canopy that provides good moisture and temperature conditions for slugs, and thus provides habitat in which slug population density can increase.

Disposal of crop residue may be important, otherwise it can provide food and shelter for slugs. Two options exist for agriculturalists in the treatment of crop residue: baling and removal, or chopping and spreading for later incorporation. Glen *et al.* (1984, 1988) found there to be no overall effect on slug population size between plots treated with these methods, although Symondson *et al.* (1996) found that the chopping and spreading method was more favourable to slug populations.

The tillage regime is important, since the greater its intensity, the greater the negative effect on the slug population (Glen, 1989; Symondson *et al.*, 1996; Symondson *et al.*, 2002a). Ploughing may cause slug mortality through direct physical damage. Additionally, slugs may be subjected to increased risk from predation and changes in microclimate.

Good drainage may reduce slug survival by lowering soil moisture. Conversely, good drainage may reduce water logging which can increase slug survival (Stephenson and Bardner, 1977), and fecundity (Carrick, 1942). Other cultural methods that might mitigate slug damage include: intercropping to divert slug feeding away from crop plants (George *et al.*, 1995), soil consolidation to increase compaction and thus reduce the availability of resting places within the soil matrix (Glen, 1989), managing the drilling date so that the time at which seeds and establishing plants are vulnerable does not coincide with the time at

which the slug population is at its highest (Department for Environment Food and Rural Affairs, 2001), and increasing seed drilling depth (Glen *et al.*, 1989).

1.3.5.2 Chemical control

An alternative method is to treat fields with molluscicide. The two main types of molluscicide used, and the relative proportions in which they are applied in Great Britain, are: metaldehyde (55 %) and carbamates (45 %) (Garthwaite and Thomas, 1996). Metaldehyde attacks, and may destroy, the mucus producing cells of slugs (Kelly and Martin, 1989; De Sangosse, 2008). In an attempt to detoxify, slugs produce excess mucus, but the toxic effect is irreversible. Thus, the production of mucus ceases after consumption of a lethal dose. Unable to produce mucus for locomotion or defence, slugs die from starvation, dehydration or predation. However, if a lethal dose is not consumed, slugs may become rehydrated and recover in the presence of environmental moisture (Godan, 1983). Carbamate-based molluscicides are broad-spectrum neurotoxins which, when ingested, destroy the nervous systems of slugs (Kelly and Martin, 1989; De Sangosse, 2008). Carbamates are less affected than metaldehyde by environmental moisture (Godan, 1983).

In a review of experiments that compared the efficacy of metaldehyde and methiocarb, Port and Port (1986) reported there to be generally no difference between the two molluscicides in terms of the amount of damage done to crops. Both may fail to give adequate control, because often slugs may not consume a lethal dose (Bailey, 2002). Multiple applications of molluscicide may be required, since degradation occurs, and efficacy is reduced relatively rapidly (Bailey, 2002). However, the financial cost of multiple applications may be greater than the loss which would be incurred through slug damage were the crop left untreated (Symondson *et al.*, 2002b).

Recently, metaldehyde has been detected in drinking water by some water companies in England and Wales (Environment Agency, 2010). In some instances levels of contamination were close to, or above the limits (0.1 $\mu g.L^{-1}$) set by the Drinking Water Directive (98/83/EC). In response, the Environment Agency, which is responsible for implementing the Water Framework Directive (2000/60/EC), is working with stakeholders to identify threats posed by contamination, and ways to mitigate those threats. Clarke *et al.* (2009) identified

the potential loss of metaldehyde as a threat to slug pest control. However, to date this molluscicide has not been banned. Instead, mitigation of contamination has concentrated on educating users on best practice, restricting the permissible application dosage, and recommending buffer zones around water courses. Monitoring of the efficacy of these measures is ongoing.

1.3.5.3 Natural enemies

Slug populations have been observed to decline and remain at low levels even when moisture conditions were suitable and there were no apparent changes in agricultural practices (Glen *et al.*, 1988). Predation was the mechanism suggested to be controlling the slug population in these cases. Birds, slow-worms, lizards, hedgehogs, shrews, moles, badgers, and arthropods have all been reported to eat slugs (Tod, 1973; South, 1989b, 1992; Symondson *et al.*, 1996; Nyffeler and Symondson, 2001; Symondson, 2004), but of all potential natural enemies, carabid beetles are likely to have the greatest effect on slug populations (Symondson *et al.*, 2002a).

Many carabid species are reported to prey on slugs (Tod, 1973; Godan, 1983; Symondson, 2004), but evidence for the potential of carabid beetles in controlling slug populations is mixed. Mini-plot experiments have reported that carabids reduce slug numbers under these field-simulated conditions (McKemey *et al.*, 2003; Symondson *et al.*, 2006). Field-scale studies have shown apparently coupled population dynamics (Symondson *et al.*, 2002a), and coincident spatial aggregations (Symondson *et al.*, 1996; Bohan *et al.*, 2000a; Bell *et al.*, 2010) between slugs and carabids. Most significantly, carabids have been reported to exert control on slug populations in lettuce (Symondson, 1989), clover (Asteraki, 1993), and canola (Nash *et al.*, 2008). Conversely, the mini-plot experiments of Symondson *et al.* (2006) reported that, in the presence of an abundant prey community, slug numbers and biomass were not affected by the presence of carabids.

The parasitic nematode *Phasmarhabditis hermaphrodita* is commercially available for use in slug pest control (Georgis *et al.*, 2006). Efficacy of *P. hermaphrodita* compared to methiocarb was tested on a field sown with winter wheat: application of nematodes did not reduce the numbers or biomass of slugs, but did result in significantly greater numbers of undamaged plants

(Wilson *et al.*, 1994). The use of *P. hermaphrodita* as a pest control agent of slugs is not widespread. This may be due to a lack of understanding of some aspects of the nematode's ecology, high unit cost, and short shelf life of the product (Georgis *et al.*, 2006; Rae *et al.*, 2007).

1.4 Generalist predators as agents of pest control

In the science of biological control, it is generally thought that for agents to be successful at controlling target pest populations, without having significant negative effects on general biodiversity, the agents should have high specificity to the pest species (Hassell and May, 1986). This specificity refers to: the pest constituting a large proportion of the predator's total prey intake; the predator should be efficient at locating the pest; the predator should be able to survive in the same set of environmental conditions as the pest; and the predator should have a similar reproductive capacity to that of the pest. The obvious potential failing in reliance upon generalist predators for pest control purposes is their polyphagous habit, which allows them to feed on alternatives to the target pest species.

The polyphagous nature of generalist predators may be a 'double-edged sword': in addition to providing the potential for predators to be diverted away from feeding on the target species, it may buffer their populations against low population density, and thus low prey density, of the target species. Symondson *et al.* (2000) suggested that for *P. melanarius*, earthworms were a non-preferred prey type that were eaten when preferred prey were scarce, and their availability helped sustain the beetle population. This ability to switch among prey gives generalist predators a potential advantage over specialist predators as agents of pest control: generalists are able to maintain high population densities before the pest population is established. An established generalist predator population may then be better able to exert control on an establishing pest population, whereas a specialist predator population requires the existence of an established pest population in order to become established itself.

In a review of manipulative field experiments that tested the ability of generalist predators to control pest species, Symondson *et al.* (2002b) reported

that generalist predators can indeed be highly effective at prey population control. Pest abundance was significantly reduced in 74 % of experiments when generalists were present as single species, 79 % of experiments when they were present in assemblages, and 89 % of experiments when they were present in assemblages that included specialist predators. Despite having the ability to switch among prey types, generalists may still favour certain prey, and feed primarily on those prey. Indeed, the population dynamics of some generalist predators have been reported to vary in response to perturbations in populations of their main prey species, indicating a strong trophic linkage between generalist predator and prey (Villafuerte *et al.*, 1996; Angerbjorn *et al.*, 1999).

In the UK, many carabid species inhabit arable fields, but those that might be most important in terms of slug predation include: *Abax parallelepipedus* (Piller & Mitterpacher), *Amara similata* (Gyllenhal), *Carabus violaceus* (Linnaeus), *Cychrus caraboides* (Linnaeus), *Harpalus rufipes* (Degeer), *Nebria brevicollis* (Fabricius), *Pterostichus madidus* (Fabricius), *P. melanarius*, and *Pterostichus niger* (Schaller) (Symondson, 1989, 1994; Asteraki, 1993; Pakarinen, 1994a; Ayre, 1995; Symondson *et al.*, 1996, 2002a; Kromp, 1999; Langan *et al.*, 2001; Mair and Port, 2001). Species such as *C. violaceus* and *C. caraboides* have developed slug-attacking techniques that make them more efficient slug predators than other species such as *P. niger* (Pakarinen, 1994a), *N. brevicollis* and *P. madidus* (Mair and Port, 2001). Population density of a carabid species may need to be high in order for it to have a chance of exerting control on a slug population. Presence and density of species may vary greatly among sites (Ayre, 1995; Ayre and Port, 1996), and some species may not be present in sufficient numbers to produce a significant effect. *Pterostichus melanarius* is considered as potentially the main candidate to elicit the most significant effects on slug populations (Symondson *et al.*, 1996, 2002a; Bohan *et al.*, 2000a), although in some studies, its slug-consumption rate was reported to be low (Ayre, 1995; Ayre and Port, 1996; Dodd, 2004).

1.5 Biology and ecology of *Pterostichus melanarius*

1.5.1 Life history and general attributes

Pterostichus melanarius is one of the largest carabid beetles (12 – 18 mm in length) to be found on arable land in the UK. Individuals may have a biennial life cycle, and both larvae and adults may overwinter in the soil (Wallin, 1989; Matalin, 2006); adults that overwinter may be immature or those that have reproduced. Adults tend to emerge in May or June (Thomas *et al.*, 2002a), although emergence may be protracted (Ericson, 1977; Matalin, 2006), even extending into the autumn (Wallin, 1989). The main period of adult activity may be through June to August (Ericson, 1977; Luff, 1982; Symondson *et al.*, 1996), but continues into the autumn. Due to the existence of overlapping breeding cohorts, oviposition may occur throughout the summer and into the autumn (Wallin, 1989; Matalin, 2006). Egg load in females may be up to 26 eggs (Luff, 1982), with a mean of < 10 eggs per female (Wallin, 1989).

Pterostichus melanarius is described as an 'open-field' species (Lee and Landis, 2002), in that most individuals overwinter in the field rather than in the field-boundary. In common with other carabid species, *P. melanarius* orientate by microhabitat cues of temperature, humidity, light and other abiotic factors (Thomas *et al.*, 2002a). *Pterostichus melanarius* is mainly night active, and is a generalist, opportunist predator.

1.5.2 Density and activity

Pterostichus melanarius is one of the most abundant of the larger carabid species, although density can vary considerably among sites. Population size within sites may remain relatively stable among years (Luff, 1982) or change over time (Symondson *et al.*, 2002a).

Carabid beetles are typically caught using pitfall traps. This relies on beetles walking into the traps. Therefore, it is considered to be a combined measure of density and activity (Mitchell, 1963), and is usually referred to as 'activity-density'. Beetle activity may be dependent upon a number of factors, such as sex, time of day, microclimate, soil moisture, vegetation density (Mitchell, 1963;

Chiverton, 1984; Wallin and Ekbom, 1994; Thomas *et al.*, 2006), although the most important factor may be the level of satiation (Mols, 1993; Fournier and Loreau, 2001, 2002). This, in turn, depends upon prey availability (Chiverton, 1984; Fournier and Loreau, 2001, 2002), and temperature (Luff, 1982; Ayre, 2001). *Pterostichus melanarius* may be highly mobile; Thomas *et al.* (1998) reported a mean displacement of 5.3 $m.day^{-1}$ during August.

Reports on the activity-density of *P. melanarius* range between 0.01 m^{-2} and 11 m^{-2} (Frank, 1967; Ericson, 1977; Thiele, 1977; Scheller, 1984; Thomas *et al.*, 1998; Ayre, 1995), with most estimates being in the lower half of the range. Differences in environment, prey diversity, and prey biomass on a gradient from hedge rows to field centres, may lead to differential habitat use and activity of *P. melanarius* (Kennedy, 1990; Thomas *et al.*, 1998; Fournier and Loreau, 2001, 2002; Anjum-Zubair *et al.*, 2010). The propensity of *P. melanarius* to aggregate (Symondson *et al.*, 1996, 2002a; Bohan *et al.*, 2000a) may also affect measures of activity-density.

1.5.3 Trophic activity

1.5.3.1 Prey taxa consumed

Pterostichus melanarius is an opportunistic, generalist predator, taking almost any prey item that it encounters (Symondson *et al.*, 2002b). The wide range of prey items that may be consumed by this species was demonstrated by Pollet and Desender (1985, 1986), who identified that *P. melanarius* consumed prey from 49 families. Previous studies have shown *P. melanarius* to feed on Annelida, Arachnida, Diptera, Hemiptera, Hymenoptera, Homoptera, Lepidoptera, Coleoptera and Mollusca (Pollet and Desender, 1986; Symondson *et al.*, 1996, 2000, 2002b; Kromp, 1999; Sunderland, 2002). *Pterostichus melanarius* is not confined to feeding on animals; it will also feed on seeds (Honek *et al.*, 2003; Koprdova *et al.*, 2008). It has been suggested that food availability for arthropods in arable fields may be scarce (Sunderland, 1975; Van Dijk, 1982, 1996). The ultra-generalist habit of *P. melanarius* would be advantageous in an environment of prey scarcity, because most invertebrate organisms encountered might then represent potential food. Indeed, prey

scarcity may provide an explanation of why *P. melanarius* has been recorded to consume such a wide ranging diet.

Evidence from previous studies suggests that certain prey types may be more important for *P. melanarius*. During the period from April to October, 36 % of *P. melanarius* collected had consumed earthworm protein (Symondson *et al.*, 2000). Between July and September, 84 % of *P. melanarius* tested had consumed slug tissue (Symondson *et al.*, 1996). In the first of these two studies, there was a negative relationship between the quantity of earthworm protein present in the foreguts of *P. melanarius* and the foregut biomass. The authors interpreted this to indicate that the carabids ate earthworms when there was little or no alternative prey available. An alternative explanation is that earthworms represent high quality food for *P. melanarius*, and thus those individuals that had consumed earthworms were satiated in terms of their dietary requirements (protein, fat and carbohydrate). Those individuals that had not consumed earthworms had fed on lower quality food, and needed to consume more of it to fulfil their dietary requirements. However, Symondson *et al.* (2000) reported that *P. melanarius* consumed proportionately more earthworms when alternative prey was scarce, which supports the first hypothesis.

The importance of slugs as a food for *P. melanarius* was reported by Symondson *et al.* (2002a), who established that growth of a *P. melanarius* population was strongly related to slug density in the previous year. From their observations, Symondson *et al.* concluded: slugs were such an important prey for the *P. melanarius* population that it was the numbers of slugs present in one year which determined the population size of *P. melanarius* in the following year. However, conflicting evidence reported by Symondson *et al.* (2006) showed that in mini-plots manipulated with *P. melanarius* and prey items, the feeding behaviour of *P. melanarius* differed according to the diversity of prey items present. Alternative prey diverted *P. melanarius* away from eating slugs. These studies indicate that there may be strong temporal variation in the food intake of *P. melanarius* as species composition and relative abundances change within prey communities.

The mechanisms governing selection of prey may be quality of prey items, encounter rate, ability to locate prey, and ability to overpower and kill prey. In

turn, these mechanisms may be related to factors such as the mode of locomotion, size, and defence mechanisms of prey organisms.

1.5.3.2 Nutritional quality of prey

Prey differ in their nutritional quality for arthropod generalist predators (Toft, 1996; Toft and Wise, 1999a; Bilde *et al.*, 2000; Fagan *et al.*, 2002). Differential quality foods, and dietary mixing, may lead to differential growth, development, fecundity, and survival (Van Dijk, 1982, 1994; Wallin *et al.*, 1992; Toft, 1995; Toft and Wise, 1999a, b; Harwood *et al.*, 2009). It has been suggested by a number of authors that generalist arthropod predators may select among potential prey in response to the differential quality of prey items. Toft and Wise (1999a, b) reported that in laboratory bioassays, wolf spiders (*Schizocosa* spp.) selected to avoid low quality food. Greenstone (1979) asserted that, based on gut content analysis of free-living wolf spiders (*Pardosa ramulosa*), 80 % of whose diet is made up by three prey species, spiders selected among prey in order to optimize their intake of essential amino acids. Mayntz *et al.* (2005) contended that generalist predators may select among prey in order to redress imbalances in protein and lipid. Through the use of laboratory bioassays, Mayntz *et al.* reported that generalists may employ different mechanisms to achieve selective feeding. A highly mobile carabid beetle (*Agonum dorsale*) selected among food items that had been provided to emulate different species of prey. An intermediately mobile wolf spider (*Pardosa prativaga*) fed selectively on prey of the same species according to the protein:lipid ratio of individual prey items. The least mobile of the tested species, the spider *Stegodyphus lineatus,* differentially extracted nutrients from prey. Raubenheimer *et al.* (2007) reported that *A. dorsale* fed selectively in order to redress imbalances in protein:lipid ratio after emerging from diapause.

Analysis of gut contents (Sunderland, 1996; Symondson, 2002a; 2002b; King *et al.*, 2008) can assist greatly in identifying the organisms that have been eaten by generalist predators. However, when gut content analysis is performed on field-caught carabids, it is not possible to know whether prey were selected because they constituted an optimal diet. It simply indicates the prey that were available to, and eaten by the carabid. Knowledge of the relative abundance of the organisms consumed can, to some extent, help inform whether prey were

preferred, or eaten only because preferred alternatives were absent. For example, Symondson *et al.* (2000) identified that *P. melanarius* ate proportionately more earthworms when alternative prey was scarce. This may imply that earthworms were acceptable food, but only in the absence of preferred alternatives. According to Toft and Bilde (2002), for a given species of consumer, the value attributed to a particular food will be dependent upon the relative proportions of energy, nutrients, toxins and indigestibles contained within. The same authors suggested that for a given species of consumer, the value of a particular food will vary according to developmental phase and on food previously consumed. Knowledge of what constitutes high and low quality food may be gained through experience of trial and error predation. Therefore, feeding history may be an important factor in immediate and future prey preference.

The absolute quality of slugs as a food for *P. melanarius* is unknown, although Harwood *et al.* (2009) reported that *P. melanarius* reared on *D. reticulatum* had greater mass than those reared on aphids. Toft and Bilde (2002) argued that, since some carabid species have evolved as specialized snail and slug feeders, these must be high quality foods. This could be correct, but species may be adapted to poor quality resources. For instance, many vertebrate and invertebrate species have evolved to feed on grass, which is nutritionally low quality (Hartley and Jones, 1997). Modification of behaviour and life history traits allows such species to cope with the low quality of their diet. Inside mini-plots manipulated with different combinations of prey taxa, *P. melanarius* readily consumed and significantly reduced the population of slugs when there was no alternative prey (Symondson *et al.*, 2006). However, in the presence of certain alternative prey taxa, slug populations were not significantly reduced. Therefore, either the alternative prey were more easily captured and killed, or they were preferred. To date the work done on the prey preferences of *P. melanarius* has not established a clear prey preference hierarchy. Such data is important for future research into the potential of *P. melanarius* as an agent of slug pest control.

1.5.3.3 Prey searching

The diet of generalist arthropod predators might reflect the proportional abundance of prey in the environment, because generalist arthropods may consume prey as it is encountered (Lang and Gsodl, 2001). Alternatively, carabids may search for aggregations of prey (Den Boer, 1982). After finding an aggregation, generalists may then remain in the area searching for more of the same prey (Mols, 1993).

Generalist predators locate prey by sight or chemical cues (Wheater, 1989; Lovei and Sunderland, 1996). *Pterostichus melanarius* is a nocturnal hunter, as such, prey finding by sight is unlikely. Indeed, Wheater (1989) observed no response to visual cues by *P. melanarius*. Studies suggest that chemoreception may be used to locate prey. In a continuous-airflow olfactometer, *P. melanarius* responded to aphid alarm pheromone (Kielty *et al.*, 1996). Kirkland *et al.* (1998) caught more carabids in field plots in which aphid alarm pheromone was released than in control plots where there was no release of pheromone. Electroantennograms showed that *P. melanarius* responded strongly to decaying, dead slugs (McKemey *et al.*, 2004). Although the behavioural response to the stimuli detected by the electroantennograms remains unknown, the hypothesis may be formed that the antennal stimulus would act as a cue for food finding. In the same electroantennogram tests, there was no response to live, nematode-infected, injured or freshly killed slugs. This may suggest that the volatile chemicals emitted as products of the decomposition process were the catalyst for the antennal response (Houston, 1986; DeVault *et al.*, 2003). Results from field trials appear to confirm these observations: pitfall traps baited with dead slugs trapped greater numbers of *P. melanarius* than unbaited traps or traps baited with dead aphids (Foltan *et al.*, 2005). McKemey *et al.* (2004) also suggested that *P. melanarius* responded to slug mucus trails, since carabids spent more time, moved greater distances, and increased their turning rates in areas containing mucus trails.

1.5.3.4 Characteristics of preferred prey

Laboratory feeding tests suggest that *P. melanarius* prefer to prey upon smaller sized slugs (McKemey *et al.*, 2001). However, laboratory tests have

been criticized (Sunderland, 2002; Symondson, 2002a) for lacking realism in replicating the interactions between predator and prey under natural conditions, due to the confined area available in arenas, the lack of environmental heterogeneity, and the limited choice of available prey. The potential problems of extrapolating from a small scale to a larger scale were highlighted by McKemey *et al.* (2003) (*c.f.* McKemey *et al.*, 2001): in the laboratory *P. melanarius* preferentially consumed small slugs, but in mini-plots, *P. melanarius* reduced the abundance of slugs from all size classes. However, in a subsequent experiment using the same sized mini-plots, the mean size of slugs remaining at the end of the experiment was that of small neonates (Symondson *et al.*, 2006). Evidence from other studies of carabids feeding on molluscs suggests that neonates are often overlooked (Symondson, 2004).

At the field-scale, Bohan *et al.* (2000a) observed that slug-positive *P. melanarius* were not spatially associated with small slugs (< 25 mg). They were, however, positively associated with larger slugs (> 25 mg) in June, although, in July, the relationship was one of dissociation. The conclusion drawn by Bohan *et al.* was that predation on larger slugs, in those areas where *P. melanarius* were aggregated, reduced slug density to the extent that slug aggregations were no-longer resolved. Bohan *et al.* (2000a) stratified slug size, and classified large slugs as those > 25 mg. The stratified group classified as 'large slugs' was broad, and would have included slugs that were of a size that could be attacked and subdued by *P. melanarius* (McKemey *et al.*, 2001) as well as those that were too large for *P. melanarius* to kill.

Laboratory tests suggest that, given the choice, carabids will choose to eat dead slugs in preference to live ones (Langan *et al.*, 2001; Foltan *et al.*, 2005). Foltan *et al.* (2005) observed that there was no significant difference between live or dead slugs being attacked first, but there was significantly greater consumption of dead slugs. The increased feeding on dead slugs may be explained by difficulties associated with overcoming the defence mechanisms of live slugs (Pakarinen, 1994; Mair and Port, 2001, 2002; Foltan, 2004). Defences include movement away (including climbing) from a predator, contraction of body, tough skin, tail wagging, and production of mucus (Rollo and Wellington, 1979; South, 1992). Defence mechanisms are species specific, and during laboratory-based feeding experiments they were the main factor influencing

prey preference (Foltan, 2004). Lang and Gsodl (2001) reported that the prey selection of a generalist carabid predator, *Poecilus cupreus*, changed according to whether prey were dead or alive. When prey were alive, the hierarchy of preference was in the order: *Rhopalosiphum padi* (aphid) > *Drosophila melanogaster* (fruit fly) > *Acheta domestica* (house cricket). The order of preference was the same as that of the searching and handling time for the respective species. When prey were presented dead, the preference hierarchy was reversed. This illustrates the importance of prey vulnerability on influencing the diet of carabids, but also, that carabids may be able to obtain a higher quality diet through scavenging than through predation.

1.6 *Pterostichus melanarius* as an agent of slug pest control

Pterostichus melanarius possesses some of the characteristics described in section 1.4 that are desirable for an agent of slug pest control. *Pterostichus melanarius* shares the same arable habitat as those slugs that are crop pests, and beetles do feed on slug tissue (Ayre, 1995; Ayre and Port, 1996; Symondson *et al.,* 1996; Bohan *et al.*, 2000a; Dodd, 2004; Bell *et al.*, 2010). Adult *P. melanarius* are, however, active only from late May to September, whereas slugs are active all year round. Although, the larvae of *P. melanarius*, which also feed on slug tissue (Thomas *et al.*, 2009), are present in the soil during the remainder of the year. The reproductive capacity of *P. melanarius* (Thiele, 1977; Luff, 1982; Wallin, 1989) is likely to be lower than that of slugs (Carrick 1938; Quick, 1960; South, 1982; Godan, 1983), but *P. melanarius* may be present in relatively high densities (Scheller, 1984; Kromp, 1999), and individuals may be capable of consuming large quantities of prey (Thiele, 1977; Kromp, 1999). *Pterostichus melanarius* appears able to detect slug mucus trails, and responds by increasing searching activity (McKemey *et al.*, 2004).

A synthesis of the research done to date on the ecology of *P. melanarius* does not provide a clear indication of whether predation by *P. melanarius* on slugs is important enough to result in significant slug-population controlling effects. Laboratory-based work has reported that: slug size is a critical factor in

determining whether predation can occur (McKemey *et al.*, 2001); slug mucus defence may be effective in resisting attacks (Foltan, 2004); dead slugs are consumed in preference to live slugs within certain periods of decomposition (Foltan *et al.*, 2005); dead slugs and slug mucus trails can be detected, but live slugs cannot (McKemey *et al.*, 2004); and *D. reticulatum* tentacles respond to volatile chemicals from *P. melanarius* (Dodds, 1997), and *D. reticulatum* avoid areas in which *P. melanarius* have previously walked (Armsworth, 2005; Armsworth *et al.*, 2005). Mini-plot experiments have reported that: *P. melanarius* will consume slugs from all size classes in the range 2 – 100 mg (McKemey *et al.*, 2003); they generally do not consume neonate slugs (Symondson *et al.*, 2006); and that slugs are consumed only when there is low density of preferred alternative prey (Symondson *et al.*, 2006). Perhaps the most important tests are those done in-field, because all relevant environmental factors are acting on the interspecific interaction. However, due to the complexity inherent in-field, greater levels of human interpretation are required to draw conclusions from data. Two of the most important studies claiming a significant role for *P. melanarius* in slug population control through predation are those by Bohan *et al.* (2000a) and Symondson *et al.* (2002a). Respectively, they reported apparent relationships in spatial and population dynamics between *P. melanarius* and slugs. The relationships indicated that the population dynamics and distribution of *P. melanarius* affected, and were affected by, the population dynamics and distribution of slugs. These observations suggest that *P. melanarius* may have significant effects on slug population size.

Bohan *et al.* (2000a) reported that predation on slugs by *P. melanarius* is direct and dynamic rather than opportunistic. Bohan *et al.* identified an apparently dynamic relationship between the distributions of *P. melanarius* and slugs at the largest scale that was sampled (16 m between adjacent points). In June, both *P. melanarius* and slugs were aggregated. Additionally, there were associations between the spatial distributions of the two taxa, i.e., where there were more slugs there were more carabids. In July, both slugs and *P. melanarius* were randomly distributed. At that time, there were dissociations between the two taxa, i.e., where there were greater numbers of carabids there were fewer slugs. The distributions of neither carabids nor slugs were

considered to be associated with soil or crop factors. The data can be interpreted such that after emergence in May or June, *P. melanarius* adults aggregated to aggregations of slugs, for the purpose of feeding upon the latter. By July, the effects of *P. melanarius* predation on slugs had reduced the densities of slugs in aggregations to the extent that their distribution became random rather than clumped. The subsequent low densities of slug prey forced *P. melanarius* to disperse to find food, and their distribution also became random. Data also suggested there was no association between slug-positive *P. melanarius* and small slugs (< 25 mg) in either month. In June, slug-positive *P. melanarius* were spatially associated with large slugs (> 25 mg), but in July there was spatial dissociation.

Mair *et al.* (2001) challenged some of the conclusions made by Bohan *et al.* (2000a). Principally, Mair *et al.* contended that: reduction in slug density between months was implicit; the *per capita* rate of predation required to reduce slug density would be unfeasibly high; and the influence of environmental factors on distributions could not be accurately tested, because data were collected at inappropriate spatial and temporal scales. Mair *et al.* hypothesized that aggregated egg laying by slugs might be important, and was not sufficiently considered by Bohan *et al.* Aggregations of eggs should lead to aggregations of juveniles, but subsequently, as slug size increases, slugs might disperse more widely.

Symondson *et al.* (2002a) reported a coupled relationship between the sizes of slug and *P. melanarius* populations over a five-year study period. *Pterostichus melanarius* foregut mass was positively related to abundance of slugs, and between year population growth of *P. melanarius* was positively related to slug abundance. However, the abundances of other organisms, representing alternative prey for *P. melanarius*, were not monitored. Therefore, any effects that might have been caused by the dynamics in their densities could not be commented upon.

A similar relationship to that identified by Bohan *et al.* (2000a) was identified between *P. melanarius* and two species of aphid (Winder *et al.*, 2001). Sunderland (2002) speculated that these relationships might be observed because: in the first case, slugs may represent the majority of prey biomass in an area, and thus attract *P. melanarius;* in the second case, honeydew-feeding

insects and other predators will be attracted to aphid aggregations, thus, there will be a community of insect prey available which might attract *P. melanarius*.

The potential for complementarity between metaldehyde and *P. melanarius* exists when used together in an integrated pest management programme. Not all slugs that come into contact with the molluscicide consume a lethal dose (Godan, 1983). Those that consume a sub-lethal dose produce excessive amounts of mucus in an attempt to detoxify, after which slugs may be rendered immobile and relatively defenceless until sufficient environmental moisture can be absorbed to produce more mucus. During this period slugs may be highly susceptible to predation by *P. melanarius* (Langan *et al.*, 2004).

A possible negative effect attributable to large generalist predators such as *P. melanarius* is that they may feed on other beneficial arthropods such as spiders and other carabids, which in turn feed on other pest species (Symondson *et al.*, 2002b). Although, in the case of slug pest control, it is mainly medium to large predators that have the potential to prey upon slugs (Ayre, 1995), therefore, predation on other slug predators is likely to be less of an issue.

In addition to the somewhat unresolved issues on the extent of predation on slugs in-field by *P. melanarius*, there is also the confounding factor of whether slug tissue consumed in-field was actually predated or merely scavenged.

1.7 The ecology of scavenging

Studies of feeding behaviour in wild populations benefit greatly from the use of molecular techniques to identify the origin of foregut contents. Currently, the most effective methods employ PCR techniques. Species specific and generic molecular markers have been developed which allow the user to confirm that a predator consumed DNA from a particular species or genus of prey (Harper *et al.*, 2005; King *et al.*, 2008), within a certain period of time before the predator itself died (Dodd, 2004; Foltan *et al.*, 2005). Unfortunately, molecular methods cannot distinguish between prey that was killed by the predator, and that which was secondarily predated or scavenged (Sunderland, 1996; Harwood *et al.*, 2001a; Sheppard *et al.*, 2005). Wilson *et al.* (2010) recently suggested a novel

approach to estimating whether cause of death was due to predation or not. The technique measures the pH and water content of thoracic muscle in dead animals, and compares the values against standard values obtained under controlled conditions. The overriding assumption behind the technique is that death occurring through predation results in higher levels of stress than are experienced by animals dying by all other means. The pH of thoracic muscle is sensitive to stress, and it is this effect that the technique seeks to detect. During laboratory- and field-based trials, correct identification of predation or scavenging was achieved on 88 % of occasions. However, the principal limiting factor for the utility of this technique is that it is the prey rather than the predator that is tested. This makes the technique only really of use when studying central-place foragers, i.e. those that return to a specific location, usually a nest or colony, with their prey (Bell, 1991). Feeding events performed by itinerant animals, such as carabids, may be ephemeral, and occur *in situ*; thus, the predator will likely be stationary during the feeding event, and therefore difficult to catch in the act of feeding, when sampling is reliant upon activity. Thus, in non-central-place foragers, a method to distinguish between predated and scavenged food remains elusive. The ecology of scavenging has not received a great amount of attention, and much of the work that has been done has focused on mammals and birds. Instead, scavenging is often viewed as a "behavioural curiosity" (DeVault *et al.*, 2003).

Death is the fate of all living organisms, and may come about in many ways, for example: predation, parasitism, disease, starvation, dehydration, injury, or exposure to adverse climatic conditions. Predation and parasitism may not always leave carrion, but the other modes of death would leave a corpse. The proportions of organisms killed by predation may depend upon both the ecosystem type and the relative size of the organisms. Among different ecosystems, predation (where predator populations are present) may account for 5 % to 75 % of large mammal deaths (DeVault *et al.*, 2003). In small mammal populations, predation may account for the majority of deaths (Korpimaki and Krebs, 1996; Wirsing *et al.*, 2002). The large proportions of mammal deaths caused by predation means that only small proportions of the animals die from the other natural causes previously mentioned. However, it may be that animals that are moribund due to disease, injury, hunger, or other

factors are more susceptible to predation, either because of their weakened state, or modifications in their behaviour make them easier to catch and kill (Pole *et al.*, 2004). In such cases, those animals may become food for the predator guild rather than for the scavenger guild, although the distinction between these two trophic categories is often blurred. The effect of predators killing and consuming moribund animals may provide a significant limiting factor on the availability of carrion for the scavenger guild. However, predators may not always consume the entire prey carcass, therefore, a proportion may be available for scavengers. Predatory organisms that seek out moribund individuals upon which to feed may have limited value in pest control. Predators that kill and consume moribund prey organisms potentially do so in place of killing and consuming fitter pest organisms. Even after causes of death other than predation, carcasses of dead animals may not be available for scavengers; the locations and accessibility of microhabitats in which organisms die will determine whether carrion is available for use by scavengers (Pechova and Foltan, 2008).

Behaviour and use of habitat by species are key factors influencing the microhabitat in which individuals die. Large mammals that have died in open habitats will usually be readily scavengable. Carcasses of these animals have high apparency for scavengers, because they are visible and any volatile chemicals emitted from the decomposing tissues are more freely dispersed. Detection of volatile chemicals from decomposing carcasses may be a significant means of detection by scavengers (Houston, 1986; DeVault *et al.*, 2003; McKemey *et al.*, 2004). Turkey vultures were more successful at locating one-day old carcasses than fresh ones (Houston, 1986), and electroantennograms of *P. melanarius* antennae showed no response to freshly killed slugs, but responses were elicited by slugs killed up to 48 hours prior to testing (McKemey *et al.*, 2004). In contrast to surface dwelling organisms, soil dwelling invertebrates that die in the soil matrix may be difficult for scavengers to locate. Although, Thomas *et al.* (2008) reported that *P. melanarius* larvae were able to detect slugs under soil.

The distribution of individuals within populations, and the mobility of those populations are likely to influence the spatial distribution of carrion. Slugs are known to aggregate (Hunter 1966; Bohan *et al.*, 2000a, b), have low rates of

dispersal (South, 1965; Fleming, 1989; Armsworth, 2005), and establish home ranges (South, 1965, 1992). Thus, it may be hypothesized that slug carrion should be aggregated and spatially coincident with live slugs. However, since slugs often reside subterraneanly a proportion of any slug carrion may be subterranean (Pechova and Foltan, 2008).

Carrion availability may vary temporally: under conditions of increased mortality during winter, a disease outbreak, or a peak in a parasite population cycle. During periods of increased carrion availability, facultative scavengers may obtain a greater proportion of their food intake from carrion (Houston, 1979; Wilton, 1986). On arable land, cultivation, and application of insecticides and molluscicides should produce pulses of invertebrate carrion. The duration of these pulses of increased carrion availability may be relatively brief, and depending upon the organisms killed by the pesticide, carrion distribution may be aggregated (Bryan and Wratten, 1984; Harwood *et al.*, 2001b; Winder *et al.*, 2001; Bell *et al.*, 2010).

The availability of carrion in some ecosystems is sufficient that species have evolved as obligate scavengers, examples of these are: vultures, carrion beetles, and carrion flies. However, there are few data available on the amount of carrion available in any given habitat. This is perhaps due to the inherent difficulties in gathering such data. The problems relate to: microhabitats in which carcasses reside may make finding them difficult; the potential for a large degree of spatial and temporal variation in the amount of carrion; and carcasses may be quickly consumed or removed by scavengers, leaving no visible trace of existence of the carrion.

Scavenger efficiency, in terms of the proportion of carcasses discovered, can be high. In a review of vertebrate scavenging, DeVault *et al.* (2003) reported that in studies using experimentally placed carcasses, the median percentage of carrion baits that were consumed, totally or in part, by vertebrate scavengers was 87.5 %, and six out of the 22 studies reported 100 % scavenger efficiency. Additionally, carrion may be discovered quickly, and thus remain *in situ* for only a short period of time. In a deciduous forest, dead insects were quickly removed from the forest floor (Fellers and Fellers, 1982). The median time for which carrion carcasses were available was only 3.75 min, before they were taken by ants. Similarly, insect carcasses placed on top of

forest litter were quickly discovered and removed by vespid wasps and ants (Seastedt *et al.*, 1981). However, in the same study, insect carcasses placed in the forest litter remained *in situ* for longer. This illustrates the importance of carcass apparency on the likely duration between death and discovery of the carcass, and also to the overall probability of scavenging.

There may be a high degree of competition for carrion. Competition may be manifest at many levels: intra- and inter-specific among invertebrates (Seastedt *et al.*, 1981), intra- and inter-specific among vertebrates (Houston, 1979), and among vertebrates and invertebrates (DeVault *et al.*, 2003). Additionally, there is competition among animals, prokaryotes, and fungi. Janzen (1977) suggested that strong selection pressure acts on microbes to make carrion unacceptable to larger organisms in the shortest time possible. In choice experiments, dead prey, whilst in the early stages of decay, were preferentially attacked and consumed by *P. melanarius* (Foltan *et al.*, 2005). After 96 hours of aphid decomposition and 168 hours of slug decomposition, preference switched to live prey. However, since the decomposition process results in emissions of volatile chemicals that may be detectable by predators (McKemey *et al.*, 2004), in many instances it may be the microorganisms which alert the larger organisms to the presence of carcasses.

The costs involved in scavenging on carrion are largely incurred in overcoming the chemicals associated with the microbial decomposers, and the products of the decomposition process (Janzen, 1977). After a certain period of decay, carrion may become no-longer attractive to scavengers (Foltan *et al.*, 2005).

The major benefit to consumers, accrued through scavenging, is the reduction in handling costs involved in attaining food (Bilde *et al.*, 2000). Lang and Gsodl (2001) demonstrated that *P. cupreus* selected among live prey according to the handling costs involved: *R. padi* was selected over *D. melanogaster* which was in turn selected over *A. domestica*. However, when the same prey were presented as carrion, the preference hierarchy was reversed. From these results, it may be hypothesized that *A. domestica* is the highest quality food, but due to its size and mobility the handling costs, when alive, are too high for *P. cupreus*. Therefore, beetles must trade-off food quality for ease of handling, and choose *R. padi* or *D. melanogaster*. Slugs possess defence

mechanisms that include production of mucus and tail wagging (Rollo and Wellington, 1979). When slugs are dead these defence mechanisms are neutralized, therefore slug carrion incurs lower handling costs for *P. melanarius*. This may make slug carrion preferable to live slugs for *P. melanarius* (Langan *et al.*, 2001; Foltan *et al.*, 2005).

1.8 Scavenging by *Pterostichus melanarius*

Electroantennogram tests demonstrated a response by the antennae of *P. melanarius* to slug carrion (McKemey *et al.*, 2004), and laboratory experiments suggested that beetles feed on carrion when available (Langan *et al.*, 2004; Foltan *et al.*, 2005). Evidence from the field suggested that carabids scavenge aphids (Halsall and Wratten, 1988), onion fly pupae (Menalled *et al.*, 1999), and slugs (Foltan *et al.*, 2005). That *P. melanarius* might scavenge potentially reduces its efficacy as an agent of slug pest control, whether the scavenging is on slug cadavers or cadavers of other animals. Every cadaver eaten is potentially one fewer live slug killed, and each live slug killed potentially reduces the future population size through the loss of the reproductive potential of that slug. Additionally, a history of feeding on slug tissue may affect future prey preferences (Greenstone, 1979; Toft, 1996; Mayntz *et al.*, 2005; Raubenheimer *et al.*, 2007). A history of feeding on slug tissue gained through feeding on slug carrion might further reduce the utility of beetles as agents of slug pest control. However, since the volatile chemicals emitted from slug carcasses during the decomposition process might be used as a cue during prey searching (McKemey *et al.*, 2004; Foltan *et al.*, 2005), and the location of slug carrion may be spatially coincident with that of slug aggregations, the presence of carrion might be useful in directing *P. melanarius* to aggregations of live slugs. In this sense, the presence of carrion may be advantageous for the purposes of slug pest control.

Slug carrion may be abundant soon after the application of molluscicide. Application of methiocarb has been reported to have negative effects on carabid populations (Kennedy, 1990; Purvis and Bannon, 1992). The longevity of *P. melanarius* after consumption of a single slug killed by methiocarb was greatly

reduced, whereas the longevity of *P. melanarius* after consumption of slugs killed by metaldehyde was similar to that of control beetles (Langan *et al.*, 2004). Buchs *et al.* (1989) found that *P. melanarius* was less sensitive to methiocarb than some other carabid species. Nevertheless, 25 % mortality was still observed in beetles when in the presence of methiocarb. Thus, scavenging on methiocarb-killed slugs may reduce the population size of *P. melanarius*, and therefore its efficacy at slug population control.

1.9 Summary and project outline

Slugs are serious pests to arable agriculture, and over the last 40 years the quantity of molluscicide used to mitigate the damage they cause has increased many times over. Reliance solely on application of molluscicide may be imprudent due to the limitations of this mode of control, the financial cost involved, and the potential for wider environmental contamination. Carabid beetles are believed to be one of the main natural enemies of slugs, and have potential to act alongside cultural and chemical controls as part of integrated pest management programmes. To date, the work done on understanding the interspecific interaction between *P. melanarius* and slugs has provided interesting insights, and suggests that predation by *P. melanarius* might have significant negative effects on slug populations. Research has also produced some apparently contradictory findings. Therefore, the status of *P. melanarius* as a pest-controller of slugs remains unresolved.

One of the aims of this project was to provide further in-field evidence on the trophic activity of *P. melanarius*, and its effects on slug population dynamics. To that end, an experiment was undertaken to test whether adult populations of *P. melanarius* are capable of reducing slug population density whilst in the presence of naturally occurring alternative prey taxa.

The presence of carrion and certain alternative prey may divert *P. melanarius* away from consuming live slugs. These observations pose further questions, such as: does prey vital status affect the relative feeding preference for slugs by *P. melanarius*? Is the prey preference hierarchy dependent upon

the feeding history of beetles? Can *P. melanarius* detect live and dead slugs by olfaction?

The following chapters report on the field- and laboratory-based experiments that were conducted to address these questions. The main hypotheses being tested were:

- Prey vital status is an important factor in determining prey preference by *P. melanarius*. (Chapter 2)
- *Pterostichus melanarius* is able to detect dead, but not live *D. reticulatum* by olfaction. (Chapter 4)
- *Pterostichus melanarius* select against prey which individuals have recently fed upon. (Chapter 5)
- Predation pressure exerted by *P. melanarius* on field-populations of slugs is insufficient to have a significant controlling effect on slug population size. (Chapter 6)

Additionally, in response to observations made during encounters between *P. melanarius* and *D. reticulatum* potential-prey during experimentation conducted to test one of the aforementioned hypotheses (Chapter 2), a further pair of linked hypotheses was tested:

- *Pterostichus melanarius* that have attacked *D. reticulatum* test positive for the presence of slug DNA during molecular analysis of beetle foregut contents, but slug survival rate is not diminished by aborted attacks. (Chapter 3)

Chapter 2 -

Effect of prey vital status on the feeding preferences of *Pterostichus melanarius*

2.1 Introduction

The literature surrounding studies on *P. melanarius* have variously reported that *P. melanarius* prey upon slugs in a direct and dynamic, rather than opportunistic manner (Symondson *et al.*, 1996; Bohan *et al.*, 2000a), and to such an extent that they can control the between year dynamics of slug populations (Symondson *et al.*, 2002a). The notion that *P. melanarius* consumes slug tissue is not disputed; numerous field-based studies have reported that the guts of *P. melanarius* often contain slug tissue (Ayre, 1995; Ayre and Port, 1996; Symondson *et al.*, 1996; Bohan *et al.*, 2000a; Dodd, 2004; Bell *et al.*, 2010). However, secondary predation and scavenging both represent potential sources of error in concluding that the presence of slug tissue in predator gut contents is the result of predation.

Secondary predation occurs when one predator consumes another predator, which itself had already consumed the prey type of interest. Secondary predation was reported to be an unlikely source of error when using ELISA techniques for detection of prey in guts (Harwood *et al.*, 2001a), but the advent of PCR-based gut content analysis, with the increased sensitivity these techniques provide, has increased the likelihood of this source of error occurring (Sheppard *et al.*, 2005). However, it might still be of low importance, because detection periods for secondarily predated prey DNA are considerably shorter than those for direct consumption of prey. Conversely, scavenging may be a significant factor in the trophic interactions between *P. melanarius* and slugs, and has the potential to be a significant source of error in estimating predation rates. The probability of DNA amplification by PCR, for decaying slug tissue, decreases as the period of decay increases (Foltan *et al.*, 2005). Therefore, the detection rate for scavenged tissue in gut content analysis will be lower than that for tissue that was live immediately before feeding occurred. However, the decay rate is relatively slow; the decay half-life for detection (the time at which positive samples yielding a positive result is reduced to 50 % of the total number of samples) is approximately 172 h, which is significantly longer than the reported mean duration (11.1 h) that slug cadavers remain *in situ* in arable

fields (Foltan *et al.*, 2005). Thus, the effect of decreased detection rate may be negated, and differentiating between scavenged and predated tissue might be impossible. Older studies that utilized ELISA to establish trophic links were subject to similar problems of being able to detect decaying tissue, but unable to differentiate it from freshly killed tissue (Calder *et al.*, 2005).

Scavenging may be a common event; many animals will scavenge when presented with the opportunity (Houston, 1979; Seastedt *et al.*, 1981; Fellers and Fellers, 1982; Wilton, 1986; DeVault *et al.*, 2003), and may preferentially feed on carrion (Langan *et al.*, 2001; Foltan *et al.*, 2005). *Pterostichus melanarius* has been reported to feed on slug cadavers (Calder *et al.*, 2005), and aggregate to slug carrion (Foltan *et al.*, 2005).

The possibility that *P. melanarius* may scavenge slug cadavers increases the uncertainty in predictions of slug-predation rates. Additionally, scavenging of slugs might reduce the efficacy of these beetles as agents of slug pest control, because feeding on cadavers would result in fewer live slugs being killed. Scavenging may also have toxic effects on the predator, if the reason for slug mortality was the result of chemical control (Langan *et al.*, 2004; Mauchline *et al.*, 2004). This would impact negatively on the population size of pest species' natural enemies (Kennedy, 1990; Purvis and Bannon, 1992; Gyldenkaerne *et al.*, 2000; Navntoft *et al.*, 2006).

An animal may choose to eat carrion because the handling costs involved are lower than those incurred during the act of predation. Indeed, Lang and Gsodl (2001) reported how prey selection in the carabid *Poecilus cupreus* changed in response to the vital status of prey. When prey were alive, predation rates on three prey types were positively correlated to ease of handling, but when prey were dead, and the constraint of prey handling was reduced, rates of predation on the various prey types changed significantly. A similar response might exist in the interaction between *P. melanarius* and slugs, particularly since the mucus defence of live slugs is reported to be effective against some carabid species (Pakarinen, 1994a, Mair and Port, 2002).

The aim of this study was to test whether *P. melanarius* exhibits a feeding preference among an array of prey that includes slug, and whether feeding preference is dependent upon the vital status of the prey. It might be expected that *P. melanarius* would favour certain prey types over others due to differential

nutrient and energy constitution of prey (Greenstone, 1979; Mayntz *et al.*, 2005; Raubenheimer *et al.*, 2007), but that translation of preference into feeding may be constrained by prey defence mechanisms (Pakarinen, 1994a; Mair and Port, 2002). Therefore, higher handling costs incurred in overcoming live-prey defences might result in higher frequencies of behavioural activities, and shorter feeding duration overall. These expectations were tested using laboratory based feeding trials, under the following null hypotheses: (1) when presented with an array of prey taxa, all with the same vital status, *P. melanarius* does not exhibit a preference in terms of first contact; and (2) *P. melanarius* does not exhibit a preference in terms of feeding duration. (3) The relative duration *P. melanarius* spends feeding on different prey taxa does not change in response to prey vital status. (4) Prey vital status does not have an effect on the frequency of behavioural activities performed by *P. melanarius*; (5) prey vital status does not affect the behaviour profile; and (6) prey vital status does not affect the overall feeding duration of *P. melanarius*.

2.2 Methods

2.2.1 Collection, maintenance and preparation of prey

The prey chosen for use in bioassays were *Deroceras reticulatum*, *Aporrectodea caliginosa* (Savigny) (Oligochaeta: Lumbricidae), *Calliphora vomitoria* Linnaeus (Diptera: Calliphoridae) larvae, and conspecific beetles. The first three are all prey for *P. melanarius* under natural conditions (Symondson *et al.*, 1996; Menalled *et al.*, 1999; King *et al.*, 2010). Feeding on conspecifics has been observed under controlled conditions (personal observations).

Deroceras reticulatum to be used as dead-prey were collected on 8 August 2008, from fields planted with winter wheat, on Rothamsted Farm. Slugs were rapidly killed by freezing at -43 °C, and then maintained at that temperature until required for bioassay. *Deroceras reticulatum* to be used as live-prey were collected on 25 September 2008. Those slugs were maintained in large plastic boxes containing moist cotton wool substrates. They were kept in a controlled environment room at 11 ± 1 °C on a light regime of 16 h:8 h (light:dark). Food was provided *ab libitum* in the form of *Brassica chinensis*.

Aporrectodea caliginosa were collected on 25 September 2008, from fields planted with winter wheat, on Rothamsted Farm. Earthworms to be used as dead-prey were killed by freezing at -43 °C, and maintained at that temperature until required. Earthworms to be used as live-prey were housed in a plastic box, with a substrate of moist soil taken from the field in which the earthworms were collected, and maintained under the same environmental conditions as *D. reticulatum*.

Calliphora vomitoria larvae were purchased from a local fishing tackle supplier on 26 September 2008. Those to be used as dead-prey were killed immediately by freezing at -43 °C and maintained at that temperature until required. The remainder, to be used as live-prey, were housed in a plastic box, and maintained under the same environmental conditions as *D. reticulatum*.

Pterostichus melanarius were caught by dry pitfall trapping. Traps were set-out on 8 August 2008, and emptied four days later. Captured *P. melanarius*

were housed in large plastic boxes (approximately 55 cm x 35 cm x 16 cm) containing a 5 cm deep layer of moist, peat-based substrate. The beetles were kept in a controlled environment room at 17 ± 1 °C, 60 – 75 % humidity, and on a reversed light regime (darkness commenced at 9 am) of 16 h:8 h (light:dark). Food was provided in the form of tinned cat food (Whiskas Supermeat), initially provided *ab libitum*. Eight days before bioassays commenced, potential live-prey beetles were sexed and placed individually into plastic containers. The containers used were clean pitfall trap cups (diameter = 6 cm, depth = 7.5 cm) that contained 3 cm of moist peat-based substrate. Each beetle was provided with cat food and allowed to feed *ab libitum* for a period of 24 h after which the food was removed and a period of starvation commenced. The starvation period lasted six days; on the seventh day beetles were provided with cat food and allowed to feed *ab libitum*. The purpose of the starving and feeding process was to ensure that the potential-prey beetles fed to satiation on the day before the bioassays, such that during bioassays they would be less likely to interact with the other potential-prey items. *Pterostichus melanarius* to be used as dead-prey were starved for six days, then provided with cat food and allowed to feed *ab libitum* for 24 h. The beetles were then killed by freezing at -43 °C, and maintained at that temperature until required. The purpose of the starving then feeding process was to yield live- and dead-prey beetles of equal satiation status, and therefore homogeneity, as a potential food for the predator beetles. Additionally, they would smell equally of cat food.

2.2.2 Collection, maintenance and preparation of predators

Capture and containment of the predator beetles were the same as those for the potential-prey beetles. Similarly, eight days before bioassays commenced, predator beetles were sexed, placed individually into plastic containers, and allowed to feed on cat food *ab libitum* for a period of 24 h, after which the food was removed and a period of starvation commenced. The period of starvation for the predator *P. melanarius* lasted for the seven days prior to the bioassays.

2.2.3 Execution of bioassays

Bioassays were conducted during the first week of October 2008, under red light, in a controlled environment room at 17 °C. Test arenas were 9 cm-diameter Petri dishes (base and lid), the inside walls of which had been painted with polytetrafluoroethylene (Whitford Plastics Ltd.) to inhibit *D. reticulatum* climbing off the arena floors (Symondson, 1993). A filter paper, moistened with a few drops of water, was used as a substrate. *Pterostichus melanarius* (mean mass ± SEM: male = 142 ± 3.48 mg, female = 172 ± 5.58 mg) were presented with the four prey items (Plate 2.1): *D. reticulatum* (157 ± 8.89 mg), *A. caliginosa* (150 ± 6.55 mg), *C. vomitoria* (79.1 ± 3.27 mg), and *P. melanarius* (male = 174 ± 6.38 mg, female = 212 ± 5.42 mg). Bioassays were performed using either all live- or all dead-prey.

Before commencement of bioassays, predator *P. melanarius* were placed individually into Petri dish arenas to allow them to become accustomed to their new environment. Live potential-prey *P. melanarius* were retained in their individual pitfall trap cups, but the soil substrate was removed. *Deroceras reticulatum*, *A. caliginosa*, *C. vomitoria* and dead potential-prey *P. melanarius* were retained in a cool-box containing one ice pack that was periodically removed and replaced in order to maintain a temperature of approximately 10 °C inside the cool-box. Approximately 30 min before they were used in a bioassay, the antennae, labial palps, maxillary palps and mandibles of the potential-prey beetles were removed using a sharp pair of scissors. This was done to reduce the likelihood of live potential-prey beetles interacting with the other prey items, and the predator beetles. The potential-prey *P. melanarius* used in any particular bioassay was the same sex as that of the predator *P. melanarius*. The predator beetle was temporarily removed from the test arena whilst the four prey items were transferred into the arena. Prey were set out at the four cardinal points, near the perimeter of the arena, and in a random arrangement. The arena was placed directly under a video camera (Sanyo B/W CCD camera, model number: VCB-3412P). The predator *P. melanarius* was re-introduced into the centre of the arena, which marked the commencement of the bioassay. Digital video recordings of all bioassays were made using an

Archos 604 Portable Multimedia Player and DVR Station. Each bioassay (n_L = 25, n_D = 30) was allowed to run for a duration of 10 min.

Although filming of the bioassay ceased after the 10 min period, the arenas containing predators and prey were maintained under the same environmental conditions for 24 h. The vital status of the predator beetles was assessed after that 24 h period.

Plate 2.1 Experimental arena for a dead-prey bioassay. The arena, a 9 cm-diameter Petri dish with a moist filter paper substrate, contains a live *Pterostichus melanarius* (nearest the centre of the arena) and the four prey around the perimeter: (clock-wise from top centre) *Calliphora vomitoria*, *P. melanarius*, *Aporrectodea caliginosa*, and *Deroceras reticulatum*.

2.2.4 Data collection from digital videos

Data recording of the types and durations of behaviours exhibited was done by watching the video clips recorded during bioassays. The behaviours exhibited by the predator beetles were classified into 10 categories: feeding (one category for each of the four prey types), prey-interaction excluding feeding (PIEF) (one category for each of the four prey types), cleaning, or non-prey-interaction (NPI). The category of NPI consisted of all behaviours that were not encompassed by the feeding, PIEF, or cleaning behaviour categories.

When scoring the behaviours of an individual beetle, the type and duration of each individual behaviour was recorded. Later, the duration of all individual behaviour events performed, over the 10 min bioassays, were summed to provide cumulative durations for each of the 10 behaviour categories. Therefore, the data used in the statistical analyses were, on a per beetle basis, the total duration spent performing each of the 10 behaviour types.

2.2.5 Statistical analysis

In this and all subsequent chapters, statistical analyses were conducted using GenStat v.12 software (VSN international Ltd.), and probability values quoted are for two-tailed tests unless otherwise stated.

2.2.5.1 Overall feeding preferences

The data obtained from experimentation were compositional, because the sum of the cumulative durations for all behaviour types equalled 10 min. Therefore, in order to facilitate comparative analyses among the behaviour categories, a log-ratio analysis approach was employed, similar to those described in Pell *et al.* (1997), and Roy *et al.* (1998). For each behaviour category in turn, the cumulative duration spent performing that behaviour type was expressed as a proportion of the cumulative duration spent on NPI. Non-prey-interaction was selected to be the denominator in ratios for three reasons: it was less important than the prey-interaction categories, it was the category with the fewest zero values, and across all bioassays the distribution of data within this category was fairly homogeneous. A ratio for the category of feeding

on beetles was not calculated because all values were zero, since none of the predator beetles fed on the potential-prey beetles. The formula:

$$R_{i,j} = \log_{10}(\Sigma d_{\theta i}/\Sigma d_{\gamma})$$

was used when calculating the ratio, R, for the cumulative duration (d) spent performing behaviour type θ_i expressed as a proportion of the cumulative duration spent performing behaviour type γ, for the j^{th} beetle, where j lies inside the range 1 to 47 (n_L = 22, n_D = 25), and where i is one of eight behaviour type categories. The $\log_{10}$ factor was applied to stabilize variance. Before calculating ratios, it was necessary to make adjustments to the cumulative duration data in order to eliminate any zero values present. The replacement method described by Aitchison (1986) was used for this operation. Applying the replacement method to data has the effect of adding the quantity x to all zero value categories, where:

$$x = [\delta(\alpha+1)(\beta-\alpha)]/\beta^2$$

and subtracting the quantity y from each of the non-zero value categories, where:

$$y = [\delta\alpha(\alpha+1)]/\beta^2$$

and where: δ is a correction factor, α is the number of zero value categories, and β is the number of categories. The replacement method correction was applied to data on an individual bioassay basis, i.e. for a given beetle, where the cumulative duration for any one of the nine behaviour categories was zero, the correction was applied to the data for that beetle. The value used for δ was 0.5. The resulting eight corrected ratios for each bioassay were then used in a CVA and a MANOVA. Canonical variates analysis was used because it can discriminate relationships and find group structure within a set of data that are all described by the same variables (Gardner *et al.*, 2006). It is mathematically equivalent to MANOVA (Beaghen, 1997). Two categories for each of prey status and beetle sex meant that there were four possible grouping factors in the CVA. The treatment structure in the MANOVA was: prey status x sex of *P. melanarius*, where prey status was either alive or dead. Terms in the

multivariate linear model produced by the MANOVA were tested by Roa's F-approximation for Wilk's Lambda (GenStat Committee, 2009).

2.2.5.2 Primary interaction with prey

Preference among prey items, in terms of primary interaction (where primary interaction was either feeding or PIEF) by *P. melanarius*, was tested by chi-squared goodness-of-fit calculated using the maximum likelihood method. The observed frequencies of bioassays in which each prey type was the subject of primary interaction, were compared against expected frequencies in which primary interaction was a random event, and thus each of the four prey types would be the subject of primary interaction during 25 % of bioassays.

2.2.5.3 Cleaning behaviour

The effects of prey vital status on the duration beetles spent engaged in cleaning behaviour was assessed by reclassifying the behaviours of *P. melanarius* that had in any way interacted with *D. reticulatum* (n_L = 12, n_D = 17), as being one of three behaviour types, either: Interacting with *D. reticulatum*, Cleaning, or Other. The same methodology as that described in section 2.2.5.1 was applied to data from the three reclassified behaviour categories, on a per beetle basis, in order to eliminate zero values by the replacement method (δ = 0.5), and to then calculate ratios based on the corrected data. The category of 'Other' was used as the denominator in the calculation of ratios. The resulting two corrected ratios were then input into a MANOVA, in which the treatment structure was: prey status x sex of *P. melanarius*.

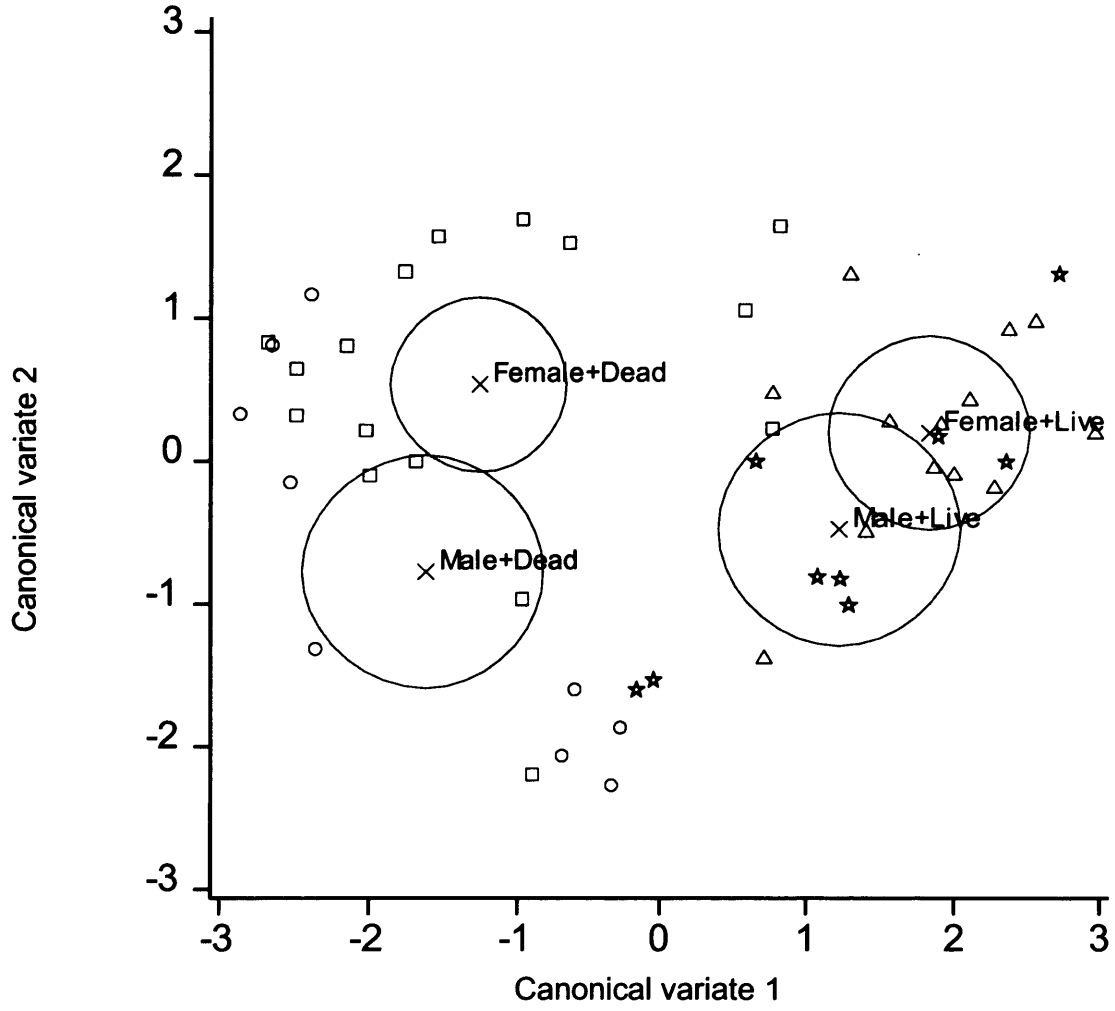
2.3 Results

2.3.1 Data excluded from analyses

Data for predators that did not move during their bioassay, and those that died within 24 h following their bioassay, were excluded from the analysis (n_L = 3, n_D = 5). This decision was made on the grounds that those beetles were probably moribund during their bioassays, therefore their behaviours were probably different to those of healthy beetles.

2.3.2 Overall feeding preferences

Prey vital status was identified as canonical variate 1 in a CVA (Figure 2.1). Among all bioassays, 85.6 % of the variation in data was accounted for by the vital status of prey. The second and third canonical variates were beetle sex, and the interaction between prey vital status and beetle sex respectively. The corresponding values for the percentage of variation in the data, accounted for by these two variates, were 10.2 % and 4.20 % respectively. The use of MANOVA identified that prey vital status was significant ($F_{8,36}$ = 10.8, $P < 0.001$), but neither beetle sex ($F_{8,36}$ = 1.49, P = 0.195), nor the interaction ($F_{8,36}$ = 0.62, P = 0.756) were significant. Since there was no significant difference between the behaviours of male and female *P. melanarius*, beetle sex was ignored and data from male and female beetles were pooled within each of the live-prey and dead-prey sets for the remainder of the analysis.



Data points: ○ Male+Dead △ Male+Live □ Female+Dead ✡ Female+Live

x mean canonical variate for each group

○ 95 % confidence interval for means

Figure 2.1 Canonical variates analysis two-dimension plot for all live- and dead-prey bioassays. The data input into the CVA were, for each beetle, log-ratios expressing cumulative duration spent performing each of eight behaviour types, expressed as proportions of the cumulative duration spent performing non-prey-interaction. Four grouping factors were applied to the data: male & live-prey, male & dead-prey, female & live-prey, and female & dead-prey, where the sex referred to that of the predator beetle.

The duration spent feeding by *P. melanarius*, as a proportion of total bioassay duration, was not significantly different among live- and dead-prey bioassays: 67.5 % and 72.2 % respectively ($U = 222$, $P = 0.263$, $n_L = 22$, $n_D = 25$). However, the proportions of time spent feeding on the different prey types

differed significantly among live- (H_2 = 20.9, P < 0.001, n = 22) and dead-prey (H_2 = 14.6, P < 0.001, n = 25) bioassays. The mean duration spent feeding on each of the prey types, as a proportion of total duration spent feeding, was 0%, 67.6 %, 32.4 %, and 0 % during live-prey bioassays, and 61.6 %, 26.6 %, 11.8 %, and 0 % during dead-prey bioassays (Figure 2.2), for *D. reticulatum*, *A. caliginosa*, *C. vomitoria* and *P. melanarius* respectively (feeding on beetles was excluded from the Kruskal-Wallis tests since all values were zero and therefore did not warrant testing for difference). Therefore, the food preference hierarchy of *P. melanarius* changed according to the vital status of the prey. When prey were alive, the preference hierarchy was *A. caliginosa* followed by *C. vomitoria*; *D. reticulatum* and *P. melanarius* were not eaten in any live-prey bioassays. When prey were dead, the preference hierarchy was *D. reticulatum* followed by *A. caliginosa* followed by *C. vomitoria*; again *P. melanarius* were not eaten.

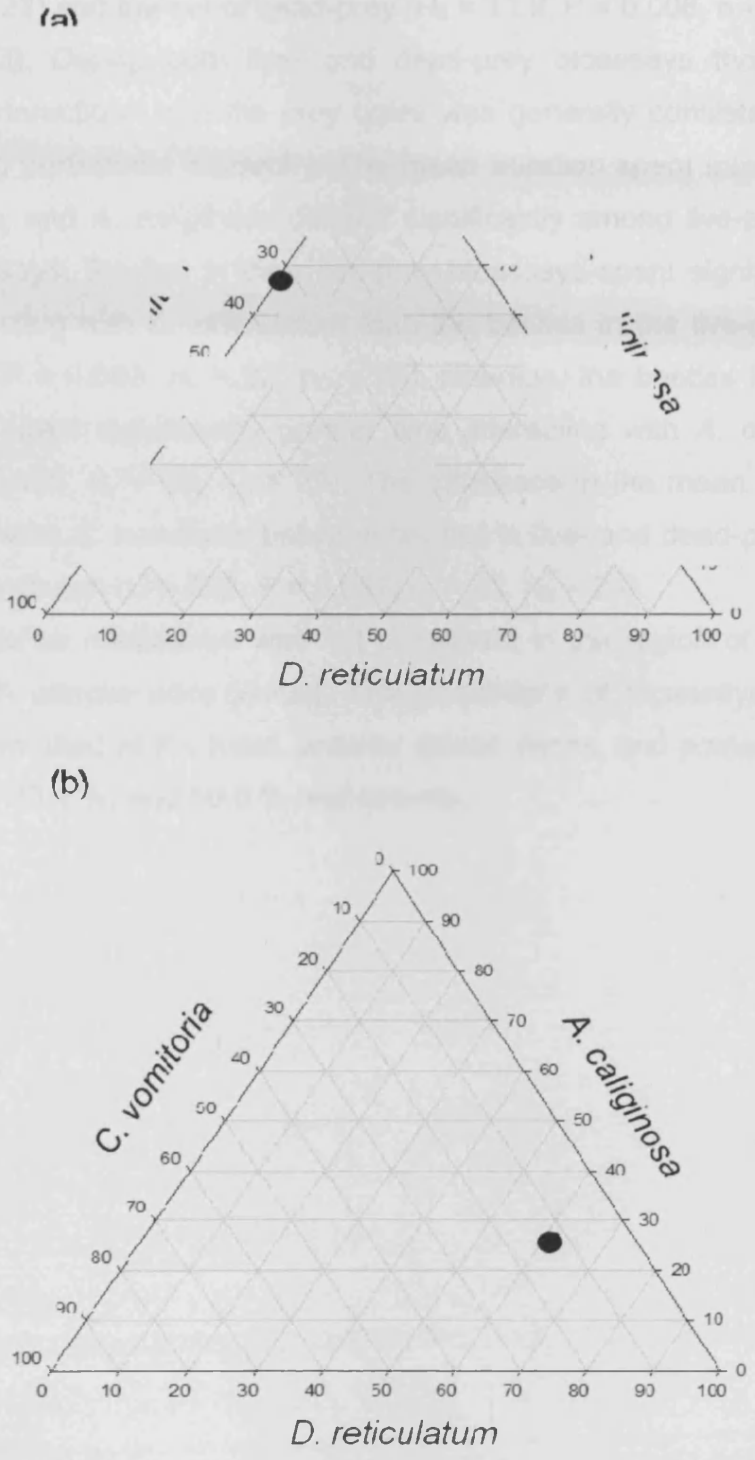


Figure 2.2 Mean duration spent feeding on each of *Deroceras reticulatum*, *Aporrectodea caliginosa* and *Calliphora vomitoria* prey types, as a proportion of the total duration spent feeding on all prey types, for (a) live-prey, and (b) dead-prey bioassays. Pooled data for male and female *Pterostichus melanarius*.

The profile of mean durations spent interacting (feeding plus PIEF) with each prey type differed considerably within the set of live-prey (H_3 = 24.3, P <

0.001, n = 22) and the set of dead-prey (H_3 = 11.9, P = 0.008, n = 25) bioassays (Figure 2.3). During both live- and dead-prey bioassays the trend among predator interactions with the prey types was generally consistent with that of the feeding preference hierarchy. The mean duration spent interacting with *D. reticulatum*, and *A. caliginosa* differed significantly among live-prey and dead-prey bioassays. Beetles in the dead-prey bioassays spent significantly greater time interacting with *D. reticulatum* than the beetles in the live-prey bioassays (U = 139, P = 0.002, n_L = 22, n_D = 25), whereas, the beetles in the live-prey bioassays spent significantly greater time interacting with *A. caliginosa* (U = 147, P = 0.005, n_L = 22, n_D = 25). The difference in the mean duration spent interacting with *C. vomitoria*, between beetles in live- and dead-prey bioassays, was not significant (U = 202, P = 0.087, n_L = 22, n_D = 25).

Pterostichus melanarius was not consistent in the region of the slug body upon which attacks were visited. The proportions of bioassays in which the attacks were sited at the head, anterior dorsal, flanks, and posterior were: 19.2 %, 7.70 %, 23.1 %, and 50.0 % respectively.

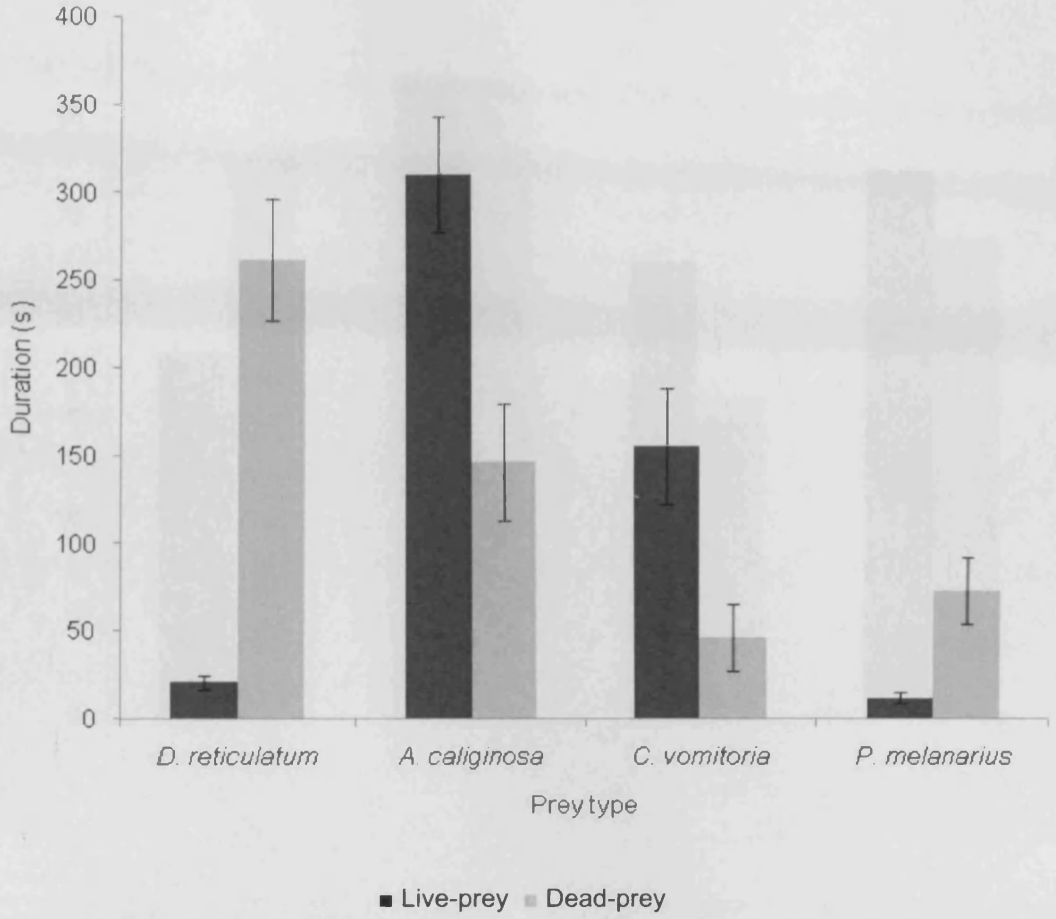


Figure 2.3 Mean (± SEM) duration spent interacting (feeding plus prey-interaction excluding feeding) with each prey type, for all live- and dead-prey bioassays. Pooled data for male and female *Pterostichus melanarius*.

2.3.3 Primary interaction with prey

Pterostichus melanarius did not exhibit a significant preference for any particular prey item, in terms of first contact with prey, in either live-prey (χ^2_3 = 0.92, P = 0.821), or dead-prey (χ^2_3 = 1.48, P = 0.688) bioassays (Figure 2.4). In 18.2 % of live-prey bioassays, the *D. reticulatum* was the first prey to be investigated, but this did not translate to feeding during any of the bioassays (0 %). Indeed, the mean duration *P. melanarius* spent interacting with live *D. reticulatum* was only 20.8 s, indicating that beetles did not persevere for long in their investigations of live *D. reticulatum* as a potential prey, but rather were fairly quickly put-off. In contrast, during the dead-prey bioassays, *D. reticulatum* was the first prey to be contacted in 28.0 % of bioassays, with a mean duration of interactions of 262 s, and 68.0 % of beetles fed on the slugs.

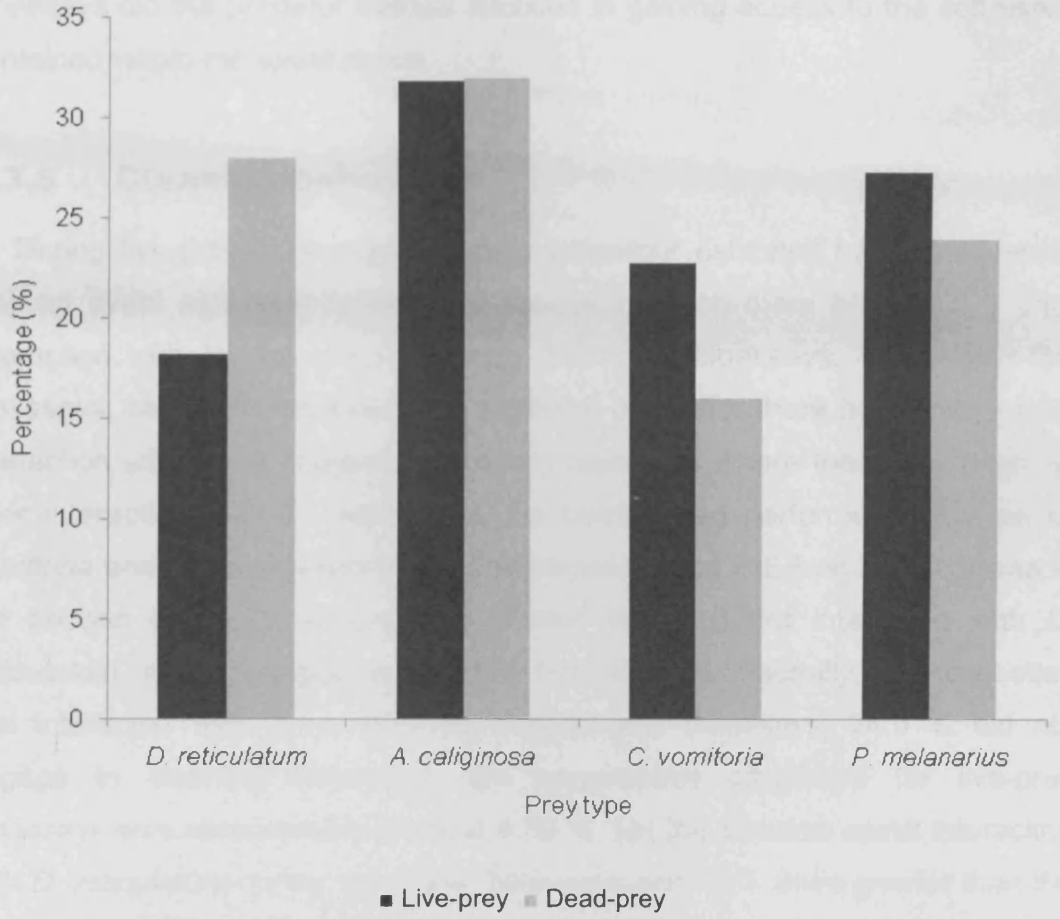


Figure 2.4 The percentage of bioassays in which each of the prey types were the first contacted by *Pterostichus melanarius*; where prey contact was either feeding or prey-interaction excluding feeding. Pooled data for male and female *P. melanarius*.

2.3.4 Intraspecific predation and scavenging

During 54.6 % of live-prey, and 56.0 % of dead-prey bioassays, the predator beetles engaged in PIEF with the potential-prey beetles. The mean duration of PIEF during dead-prey bioassays was greater (mean ± SEM: 73.1 ± 26.0 s) than during live-prey bioassays (11.7 ± 4.07 s), however, the difference was not significantly different ($U = 225$, $P = 0.271$, $n_L = 22$, $n_D = 25$). Some of the predator beetles in the dead-prey bioassays showed considerable interest in the potential-prey beetles, and spent up to 73.3 % of their 10 min bioassay time interacting with them. During these prolonged interactions, the predator beetles persisted in biting the hard exoskeleton of the dead-prey beetles. In none of the

bioassays did the predator beetles succeed in gaining access to the soft tissue contained within the exoskeleton.

2.3.5 Cleaning behaviour

During live-prey bioassays, cleaning behaviour exhibited by *P. melanarius* was an event exclusive to those bioassays in which there had been a prior interaction with *D. reticulatum.* During dead-prey bioassays, in all save two bioassays, cleaning behaviour was exhibited only after there had been a prior interaction with *D. reticulatum.* In the two bioassays where there had been no prior interaction with *D. reticulatum*, the beetles had performed: PIEF on *C. vomitoria* and *P. melanarius* in the first bioassay, and PIEF on *A. caliginosa* in the second bioassay. Among the beetles that had not interacted with *D. reticulatum*, none engaged in cleaning behaviour. Additionally, of the beetles that interacted with *D. reticulatum* in dead-prey bioassays, 24.0 % did not engage in cleaning behaviour, the comparative proportion for live-prey bioassays was considerably lower at 4.55 %, yet the duration spent interacting with *D. reticulatum* during dead-prey bioassays was 12.6 times greater than the duration spent interacting with *D. reticulatum* during live-prey bioassays.

It is evident from MANOVA that the behaviour profiles within this group of beetles were differentiated by the vital status of their prey (prey vital status: $F_{2,24}$ = 6.27, P = 0.006; beetle sex: $F_{2,24}$ = 0.48, P = 0.627; prey vital status x beetle sex: $F_{2,24}$ = 2.54, P = 0.100). When prey were dead, the behaviours of *P. melanarius* were generally: 'Interacting with *D. reticulatum'* or 'Other', indicated by the clustering of points on the 'Interacting with *D. reticulatum'* – 'Other' axis (Figure 2.5). However, when prey were alive, the duration spent cleaning was significantly (U = 29.5, P < 0.001, n_L = 12, n_D = 17) greater (mean ± SEM: live-prey: 74.8 ± 14.6 s; dead-prey: 9.35 ± 3.11 s), and the behaviour profiles of those beetles that had been presented with live-prey did not lie on the 'Interacting with *D. reticulatum'* – 'Other' axis.

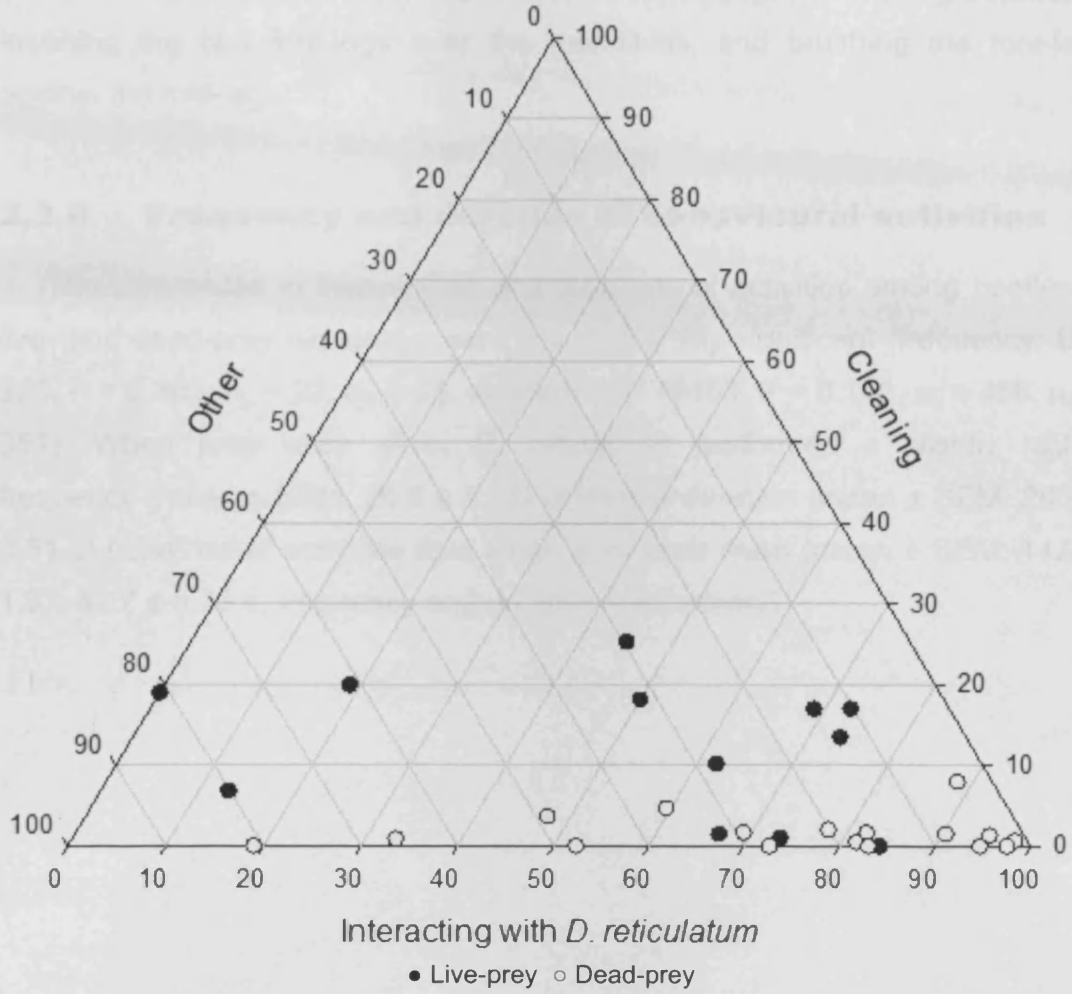


Figure 2.5 Behaviour profiles for all *Pterostichus melanarius* that had interacted in any way with *Deroceras reticulatum*, where all behaviours were categorized as either: Interacting with *D. reticulatum*, Cleaning, or Other. The duration spent performing each of three behaviours was expressed as a proportion of total bioassay duration.

Investigation of prey by *P. melanarius* was performed with any combination of antennae, palps and mandibles. Investigation with antennae and palps often led to cleaning behaviour. Cleaning of the antennae and palps was achieved by wiping with the fore-legs. On occasions when *P. melanarius* did bite *D. reticulatum* (mandibles going from wide open position, to closed position and in the process piercing the body of the slug, then back to the wide open position), mandibles were coated in mucus upon release of the bites. Beetles then engaged in cleaning behaviour in order to rid themselves of the mucus. Cleaning behaviour typically involved: repeated and simultaneous movements of the mouth parts (mandibles, palps, maxilla, labrum and labium) in a manner

similar to that observed when the animals were engaged in feeding behaviour, brushing the two fore-legs over the mandibles, and brushing the fore-legs against the mid-legs.

2.3.6 Frequency and duration of behavioural activities

The differences in frequencies and durations of activities among beetles in live- and dead-prey bioassays were not statistically significant (frequency: U = 226, P = 0.301, n_L = 22, n_D = 25; duration: U = 75155, P = 0.112, n_L = 458, n_D = 351). When prey were alive, *P. melanarius* performed a slightly higher frequency (mean ± SEM: 20.8 ± 3.73) of shorter duration (mean ± SEM: 28.8 ± 3.81 s) behavioural activities than when prey were dead (mean ± SEM: 14.0 ± 1.87; 42.7 ± 5.73 s, frequency and duration respectively).

2.4 Discussion

The behaviour of *P. melanarius* strongly suggests that prey selection changed according to, and was thus dependent upon, the vital status of prey. This observation in *P. melanarius* is similar to that observed in *P. cupreus* by Lang and Gsodl (2001). In *P. melanarius* the prey preference hierarchy changed from being first *D. reticulatum*, second *A. caliginosa* and third *C. vomitoria*, when prey were dead, to being first *A. caliginosa* and second *C. vomitoria*, when prey were alive. *Deroceras reticulatum* went from being the favoured prey when dead, to not being eaten at all when alive. The relative preference hierarchy among the other three prey types remained constant under both live and dead states. The extrapolation of these observations to arable ecosystems potentially undermines the concept of *P. melanarius* as important predator of *D. reticulatum*, and its subsequent efficacy as a part of integrated pest management programmes against slugs. Additionally, since molecular analysis cannot distinguish between predated and scavenged DNA (Foltan *et al.*, 2005), the high propensity of *P. melanarius* to scavenge dead *D. reticulatum* adds weight to the concerns that molecular analysis of beetle foregut contents may overestimate the predatory aspect of the interaction between *P. melanarius* and *D. reticulatum*.

The apparent reason for the low preference rank of *D. reticulatum* when alive, seems to be that the mucus defence caused problems for *P. melanarius*. It was evident from live-prey bioassays that *P. melanarius* were interested in the slugs, because in many of those bioassays the *P. melanarius* interacted with, and indeed attacked the slugs. However, for *P. melanarius*, the outcomes of interactions with live *D. reticulatum* were almost always the transfer of mucus from the slugs to the beetles. Exudation of mucus occurs quickly; Pakarinen (1994a) reported that *D. reticulatum* could produce mucus rapidly in response to stimulation, a copious flow of mucus could be achieved in 4 ± 1.07 s (mean ± SEM). When investigation did not include attack, the transfer of mucus appeared to be in low volume, but when *P. melanarius* attacked *D. reticulatum*, by biting with its mandibles, the quantity of mucus transfer was significantly

greater. The mean duration of *P. melanarius* interactions with live *D. reticulatum* was 20.8 s, but Mair and Port (2002) reported that *D. reticulatum* could produce defence mucus for a duration of three minutes whilst being stimulated at 1 stroke s^{-1}. Although the volume of mucus exuded when under attack may be greater than that when being stroked, this nevertheless indicates that *D. reticulatum* is capable of producing significant quantities of defence mucus, sufficient to repel most attacks by *P. melanarius*.

The transfer of large quantities of mucus on to the mandibles was a significant problem for *P. melanarius*, and required a major cleaning effort by beetles to rid themselves of the mucus. The same phenomenon of post-attack cleaning was reported in *Pterostichus madidus* and *Nebria brevicollis* by Mair and Port (2001), although, the cleaning duration reported in *P. madidus* and *N. brevicollis* after attacking live slugs (10 – 15 s) was shorter than that observed in *P. melanarius* (mean = 74.8 s). The requirement to clean away mucus seemingly existed because the mucus was very sticky and inhibited movement of the mandibles. Thus, for *D. reticulatum*, the mucus defence was effective. Although it did not prevent attack, and hence some slugs sustained bites, it did lead to cessation of all attacks, deterred further attacks, led to prey switching, and facilitated slug survival (at least for the 24 h following bioassays, over which time slug survival was monitored).

The location on the bodies of slugs, at which carabids direct their attacks, is important in determining the ability of slugs to respond to attacks with production of defence mucus, and the consequent success or failure of beetles to kill slugs. Pakarinen (1994a) asserted that the success of the slug specialist carabids, *Cychrus caraboides* and *Carabus violaceus*, lay in their attack *modi operandi*; *C. caraboides* attacked the head, and *C. violaceus* the posterior region of the mantle. Generalist predators may be often unsuccessful in their attacks on slugs because they do not attack the vulnerable regions of slugs (Pakarinen, 1994a; Mair and Port, 2002). *Pterostichus melanarius* were similarly unspecific in the locating of their attacks.

Despite the effectiveness of the mucus defence in the Petri dish environment, it might be argued that in a field environment where predators may be in a high state of starvation (Sunderland, 1975), *P. melanarius* might be more motivated to press home an attack on *D. reticulatum*. The *P. melanarius*

used in this experiment were seven day starved, but prior to that period had received food *ab libitum*, so their general state of nutrition may not have been as low as may be the average for field beetles. It is conceivable that beetles in such a weakened state might find it even more difficult to overpower a difficult prey such as *D. reticulatum*. Additionally, the Petri dish environment did not allow *D. reticulatum* to utilize its full set of anti-predator responses. In the field, a healthy *D. reticulatum* would respond to an attack from *P. melanarius* with mucus exudation, tail wagging and lifting, and rapid movement away from the scene (Rollo and Wellington, 1979); the mucus would foul the beetle's mandibles, the tail wag and lift would unseat the beetle if it were engaged, or otherwise make it difficult for the beetle to engage with its mandibles, then whilst the beetle cleans to rid itself of mucus, the slug would rapidly move away from the location to escape further attack. Future work could investigate the interactions between *P. melanarius* and *D. reticulatum* at differing levels of predator nutritional status, in order to understand whether there is a differential interaction according to the nutritional status of the beetles.

The volume of defence mucus exuded is likely to be proportional to slug size, and were smaller slugs to have been used in the experiment, *P. melanarius* may not have been deterred by significantly lower volumes of defence mucus, and thus may have been able to kill and eat those slugs. Indeed, McKemey *et al.* (2001) demonstrated that in the laboratory, *P. melanarius* preferentially preyed upon smaller sized *D. reticulatum*; frequently killing and consuming slugs < 40 mg, but only killing and consuming slugs > 40 mg after prolonged exposure. Conversely, results from mini-plot experiments (Symondson *et al.*, 2006) suggested that in environments more akin to an arable field environment, *P. melanarius* did not feed to a greater extent on small *D. reticulatum*. Almost no adult slugs survived; generally, only the very smallest slugs, neonates and juveniles, survived. In another mini-plot experiment, again simulating a field environment, *P. melanarius* significantly reduced the frequencies of *D. reticulatum* in all mass-classes inside the range 2 – 100 mg (McKemey *et al.*, 2003). The likely explanation for the inconsistency in this interspecific interaction is that small slugs are indeed easier to handle and kill, indicated by the laboratory experiment result (McKemey *et al.*, 2001), but smaller slugs are more difficult to locate in complex environments, indicated by

the field simulation experiment (Symondson *et al.*, 2006). However, it is more difficult to explain the inconsistency between the results of the two mini-plot experiments, but it may be an artefact of the mini-plot environment. The difference in size between large and small slugs means that a given section of arable habitat will contain greater heterogeneity for small slugs. Higher environmental heterogeneity may provide smaller slugs greater ability to remain hidden from large predators such as *P. melanarius*. Thus, the probability of *P. melanarius* encountering a large slug might be greater than the probability of it encountering a small slug. Coupled to that may be the relative scarcity of food availability for *P. melanarius* in arable fields (Sunderland, 1975; Van Dijk, 1982, 1996). Under such conditions it is probable that *P. melanarius* must attempt to make use of any food resource that it comes across, and as suggested, that may more likely be a large slug rather than a small one. Therefore, it is important to understand the interactions between *P. melanarius* and larger slugs.

Nearly all invertebrates secrete mucus (Denny, 1989, cited in Skingsley *et al.*, 2000), and earthworms are not an exception. They produce mucus primarily for locomotion (Jégou *et al.*, 2001), although, it has been reported to be an effective defence against ants (Gaume *et al.*, 2006). Earthworm mucus is not as viscous or sticky as the defence mucus secreted by slugs. Indeed, it appeared to be only the defence mucus of *D. reticulatum* that was problematic for *P. melanarius*. The cleaning behaviour exhibited by *P. melanarius* in live-prey bioassays was exclusive to those beetles that had engaged in prior interaction with *D. reticulatum*, and none of the beetles that refrained from interacting with *D. reticulatum* engaged in cleaning activity. Therefore, any mucus secretions by *A. caliginosa* or *C. vomitoria* appeared not to cause problems for *P. melanarius* such that cleaning behaviour was necessary, and beetles were not deterred from feeding on those prey.

Under laboratory conditions, when prey are freshly dead, as was the case in the dead-prey bioassays of this experiment, there are apparently no external factors, such as prey defence mechanisms, influencing the prey selection of a predator. Under such conditions, predators may select prey according to the balance between their individual nutritional requirements, and the nutritional value of the prey (Greenstone, 1979; Mayntz *et al.*, 2005; Raubenheimer *et al.*,

2007). Therefore, it may be hypothesized that, for the *P. melanarius* used in this experiment, slugs represented the highest quality food, since, when there was an absence of external factors, such as prey defence, influencing their prey selection, they were motivated to eat *D. reticulatum*. However, this raises two questions: firstly, why did not all *P. melanarius* in dead-prey bioassays eat slug? Secondly, why did not all *P. melanarius* in live-prey bioassays investigate and attack the slug? There might be a number of possible reasons that satisfy these questions. The first is to explain them as a phenomenon induced by the artificial environment of the Petri dish arenas. The arenas were small, such that a short initial movement of just a few centimetres by the beetle would bring it into contact with a prey item. Coupled to this, the *A. caliginosa* and *C. vomitoria* also appeared to be readily acceptable food types. Thus, if after walking just a couple of centimetres, a seven-day starved beetle encountered an *A. caliginosa* or *C. vomitoria* there might be little motivation to reject it, and seek better alternatives. Implicit in that explanation is the assertion that sensory cues were not present, were not important, or could not be detected, but *P. melanarius* has been reported to respond to chemical cues (Kielty *et al.*, 1996; McKemey *et al.*, 2004). An alternative area of explanation is that there were physiological differences among individual *P. melanarius*, perhaps brought about by disease, parasite infection, mating, reproduction, age, or feeding history. Raubenheimer *et al.* (2007) reported that *Agonum dorsale* fed differentially to redress imbalances in its protein:lipid ratio induced by life-history events. Physiological differences in feeding histories have been included as a possible explanation here, despite the attempt to maintain beetles on a diet of cat food only, because beetles were observed to feed on conspecifics whilst in captivity prior to the execution of bioassays. Whether this was predation or scavenging is unknown. Future research in this area should investigate the nutritional composition of the prey types, and determine whether there are significant differences in the quantities and relative proportions of the major nutritional elements: amino acids (Greenstone, 1979), proteins and lipids (Mayntz *et al.*, 2005; Raubenheimer *et al.*, 2007).

Pterostichus melanarius exhibited preference among prey taxa when feeding, but no preference among taxa was exhibited in terms of prey first investigated. This suggests that either: *P. melanarius* did not discriminate

among the prey types as suitable foods, and thus all prey were equally attractive; or first contact was a response made at the level of the individual according to differential motivational states (Mols, 1993), rather than a species level response; or sensory perception did not drive the beetle to contact the prey item that was first contacted, but rather the prey item first investigated was the first one encountered in a random walk (Reynolds and Rhodes, 2009). Since there was a distinct feeding preference hierarchy among prey types, then it may be unlikely that the first scenario was correct. The second scenario may be partly responsible for the effect; during the period before the bioassays were conducted, all beetles were maintained under the same controlled environmental conditions in an attempt to minimize the physiological differences among beetles, but beetles may have been in different physiological states due to age, sex, disease, parasite infection, or mating. The third scenario may also be feasible. It has been demonstrated that the antennae of adult *P. melanarius* respond to odour from decaying *D. reticulatum* (McKemey *et al.*, 2004), but the observations reported by Kielty *et al.* (1996) highlighted the potential problems inherent in predicting behaviour based on extrapolation from electroantennogram results. Further work in the area of *P. melanarius* sensory perception is required.

Predator *P. melanarius* spent considerable time investigating the potential-prey beetles in some of the bioassays. In none of the bioassays were the predators able to feed upon the potential-prey beetles. However, observations of *P. melanarius* feeding on conspecifics, made whilst beetles were being maintained prior to execution of bioassays, suggest that either predation or scavenging could occur. It is likely that 10 min is insufficient time for feeding to occur, due to the high handling costs involved in breaking through the hard exoskeleton, particularly when easier alternatives are present. Cannibalism has been widely reported among arthropods, and as a potential problem in biological control (Symondson *et al.*, 2002b), but among carabids the phenomenon has usually been reported among larvae (*sensu* Frank *et al.*, 2010), and no incidences of adult *P. melanarius* cannibalism in the field environment have been reported in the research literature. At the field scale *P. melanarius* aggregate (Symondson *et al.*, 1996; Bohan *et al.*, 2000a), and therefore intraspecific interactions may be frequent. Whether killing of

conspecifics occurs at higher densities is unknown, however, it seems likely that dead conspecifics might be utilized as a food resource, and aid in sustaining the remaining population. In any case, feeding on conspecifics, were it to occur, would provide a potential route for secondary predation of pest species. That would then have consequent problems for molecular-based gut content analysis (Sheppard *et al.*, 2005).

Since live-prey possess defence mechanisms that are elicited when under attack, but dead-prey do not, it may be expected that *P. melanarius* in the presence of live-prey would incur greater costs during their trophic activity, and that these additional costs would be most evident in terms of increased effort to overpower prey (Lang and Gsodl, 2001). Therefore, under the conditions prevalent during this experiment, it might be expected that the additional costs would be manifest in *P. melanarius* performing higher frequencies of shorter duration behavioural activities as beetles attempted to overcome prey defences before feeding could occur. This hypothesis was rejected in this experiment. There were differences in durations and frequencies of behavioural activities among live- and dead-prey bioassays, but they were not statistically significant. There was a suggestion that when in the presence of live-prey, beetles performed shorter duration activities than did those beetles in the presence of dead-prey. Over fixed duration bioassays this translated to higher frequencies of behavioural activities during live-prey bioassays. It seems that *P. melanarius* was often quickly deterred by defence mechanisms, for example, the mean duration *P. melanarius* spent interacting with live slugs was only 20.8 s. The presence of less well defended alternative prey, *A. caliginosa* and *C. vomitoria*, removed the motivation to persevere with further attacks on *D. reticulatum*, and then long feeding durations could be achieved on the alternative prey types. Ultimately, the proportions of total time spent feeding in both live- and dead-prey bioassays were not statistically different. Therefore, the phenomenon of behaviour activity frequency and duration appears to have not affected overall duration spent feeding. Although, as previously discussed, the factor driving the behavioural activity duration was prey defence, which did influence the type of food consumed. Additionally, inside the Petri dish arena, and in the face of prey defence, *P. melanarius* was able to switch its attention to alternative prey, an

option that might not be available in a field environment (Sunderland, 1975; Van Dijk, 1982, 1996).

The observations made during this experiment, of *P. melanarius* interacting with and attacking slugs, but without killing and feeding on slug tissue, raises an interesting point concerning the use of molecular analysis of foregut contents to determine the recent feeding history of an individual. It is recognized that molecular analysis of foregut contents is unable to distinguish between DNA from prey that was killed by the predator and then eaten, that which was already dead and scavenged by the predator, or that which was secondarily predated (Foltan *et al.*, 2005; Sheppard *et al.*, 2005). The data obtained in this experiment suggest that it may be possible for false positive results to occur when the predator has not fed at all, but only attacked a slug. The observations made of the live-prey bioassays in this experiment showed that *P. melanarius* will attack live slugs, and in so doing will extract material from the slug, with its mouth parts. This material is probably mostly mucus, but may also contain some living tissue. Some of the material extracted from the slug may be cleared away from the mouth with the fore-legs; however, it is likely that some of the material is ingested, because the movements of the mouth parts during the cleaning process are similar to those when feeding. Molecular analysis of foregut contents typically uses PCR and gel electrophoresis to amplify and identify DNA within the foregut contents. If both living tissue and sloughed-off cells suspended in mucus contain DNA, irrespective of the relative proportions of each that is ingested, it is possible that the presence of material in the foregut, resulting from one attacking bite by *P. melanarius*, may elicit a positive result in a test for the presence of slug DNA. This is being termed a false positive result here, because typically the purpose of the foregut content analysis is to determine prey consumption by the predator, and in the context of pest control, the implication that the prey organism was killed, albeit with the *caveat* that prey could have been scavenged or secondarily predated. However, in the scenario being described here, prey has not been consumed. More specifically, *P. melanarius* foregut content analysis for the presence of slug DNA has often been performed in order to suggest predation by *P. melanarius* and thus control of a pest organism (Symondson *et al.*, 1996; Bohan *et al.*, 2000a); however, if false positive results can occur by the means described

here, then the value of *P. melanarius* to slug pest control might be overstated. In this experiment, the fate of slugs that had sustained an attack bite from *P. melanarius* was only observed for 24 h, it is possible that attack bites might not be immediately fatal. However, such bites could be ultimately fatal, either directly through trauma, or indirectly by making slugs more vulnerable to attack by other predators (Pakarinen, 1994a; Mair and Port, 2002) or facilitating infection by parasites or other pathogens. In any case, *P. melanarius* could subsequently feed on the slug carcasses, although, in terms of slug pest control, it is the killing of the slugs that is important. Whether or not *P. melanarius* actually feeds on the carcasses is of less importance, providing *P. melanarius* can obtain sufficient food elsewhere to sustain its population. Additionally, although water is the major component of mucus, it also contains proteins and carbohydrates (Cottrell *et al.*, 1993; Skingsley *et al.*, 2000), which if ingested by *P. melanarius* may provide some nutritional value.

Chapter 3 -

Attacks by *Pterostichus melanarius* on *Deroceras reticulatum*: consequences for slug survival and molecular analysis of beetle foregut contents

3.1 Introduction

Field-based studies estimate rates of predation by *Pterostichus melanarius* on slugs through the use of molecular analysis on beetle gut contents; formerly using ELISA (Ayre, 1995; Ayre and Port, 1996; Symondson *et al.,* 1996; Bohan *et al.*, 2000a), but more recently using PCR (Dodd, 2004; Harper *et al.*, 2005; Bell *et al.*, 2010). Both techniques may over-estimate rates of predation by being unable to distinguish secondary predation and scavenging from true predation (Harwood *et al.,* 2001a; Sheppard *et al.*, 2005). However, there may be an additional source of error, not previously considered in the literature on molecular detection of prey in gut content analysis, namely, tissue ingestion by a predator following an aborted attack on prey.

Sunderland (1996) discussed the potential for fatal wounding of pests to contribute towards pest control, but the absence of ingested tissue in guts would result in underestimation of the predation rates attributed to predators. The error being hypothesized in this Chapter is the reverse of that hypothesized by Sunderland (1996). The data presented in Chapter 2 demonstrated that attacks by *P. melanarius* on live *Deroceras reticulatum* may be terminated soon after initiation, due to the deterrent effects of the slug mucus defence. It was then suggested that this phenomenon has the potential to produce false positive results in PCR-based molecular tests for the presence of slug DNA in beetle foregut contents. The term false positive was used because slugs may not be killed as a result of aborted attacks, and thus, such positive molecular results would not represent contributions by predators to pest control.

Much of the material that is transferred onto the mandibles of *P. melanarius* during attacks on live *D. reticulatum* is likely to be mucus, which consists of water, proteins and carbohydrates (Cottrell *et al.*, 1993; Skingsley *et al.*, 2000), but might also contain sloughed-off cells. Additionally, living tissue might also be extracted. This potential for transfer of cells, coupled with the movements of beetles' mouth parts following attacks resembling those observed when feeding (Chapter 2), means the possibility exists for DNA detection during gut content analysis. The aims of the work reported here were to test whether aborted

attacks by *P. melanarius* represent an additional source of error in estimating predation rates based on the results of gut content analysis, and whether *D. reticulatum* survives aborted attacks by *P. melanarius*. The aims were tested using a laboratory-based experiment, under the null hypotheses: (1) that PCR-based molecular analysis of *P. melanarius* foregut contents, extracted after beetles had made attacking bites, would not detect *D. reticulatum* DNA, and (2) that there would be no significant difference in survival rates among attacked and non-attacked *D. reticulatum*.

3.2 Methods

3.2.1 Collection, maintenance and preparation of predators and prey

Pterostichus melanarius were trapped on Rothamsted Farm between 01 and 08 June 2009. Beetles were maintained under the same conditions as those described in section 2.2. Food was provided in the form of cat food, initially provided *ab libitum*. The final feeding was provided four days before the beetles were to be used in bioassays, and removed after 24 h, so that the beetles received no food during the 72 h before bioassays.

Deroceras reticulatum were collected on Rothamsted Farm on 17 July 2009. They were maintained under the same conditions as those described for live-prey in section 2.2.1. Food was provided *ab libitum*, in the form of *Brassica chinensis*.

3.2.2 Execution of bioassays

Slugs were weighed then placed individually into 9 cm-diameter Petri dishes containing moist filter paper substrates. A beetle was held over a slug and allowed to make a bite in the dorsal, posterior region of the slug. A bite entailed full closure of the mandibles into the body of the slug, then opening of the mandibles and release of the slug. After executing a bite on a slug, the beetle was killed by freezing and kept frozen until required for molecular analysis of foregut contents. Four treatment levels were applied to beetles ($n_m = n_f = 80$); those that had bitten a slug were frozen either: 15 min, 6 h or 12 h post-biting. After biting, beetles were maintained individually in 9 cm-diameter Petri dishes, containing moist filter paper, for the appropriate duration before being frozen. Control beetles that did not bite a slug were frozen in parallel with the attacking beetles frozen after 15 min.

After attacks, slugs were retained in their Petri dishes, into which were placed *B. chinensis* leaves. In addition to the slugs that had been attacked (n =

120), control slugs (n = 40) that had not been attacked were also kept under the same conditions. The vital status of all slugs was assessed at 1 h, 24 h, 48 h and 72 h after attack. The moisture level within the Petri dishes was maintained over the 72 h period by adding a few drops of water to the filter paper. The experimental design was blocked, with each block containing four male and four female *P. melanarius*; one beetle for each treatment level. Attempts were made to ensure that slugs within blocks were of similar mass.

3.2.3 Molecular analysis on the foregut contents of *Pterostichus melanarius*

Molecular analysis of foregut contents was performed on beetles (n_m = n_f = 44) from 11 randomly selected blocks. Beetles were thawed at room temperature, and foreguts dissected-out and weighed. Dissection-out of the foregut followed the procedure described by Symondson *et al.* (2000). DNA was extracted from the foreguts using DNeasy Blood and Tissue Kits (QIAGEN), in accordance with the manufacturer's instructions. Appropriate negative control extractions were also prepared (King *et al.*, 2008). Extracted DNA was suspended in 200 µl AE buffer and stored at -40 °C. Aliquots from each sample were then subjected to two singleplex PCR runs, the first using a DNA primer combination that amplifies *D. reticulatum* (and *Deroceras panormitanum*), and the second using a general invertebrate primer combination for confirmation that the DNA extraction process had been successful. The general invertebrate primer would amplify DNA of *P. melanarius* contained within the sample, and therefore give a positive result, if the DNA extraction process had been successful. The primers used to amplify *D. reticulatum* DNA were: DR11F (5'-CTATACACAATTTTTAAATAAG-3') and DR50R (5'-AAATTTACTTTCAAGTCCAGC-3') which amplify a 294 bp region of the mitochondrial 12S rRNA gene sequence (Dodd, 2004). The general invertebrate primers used were: LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') which amplify a 710 bp region of the mitochondrial cytochrome *c* oxidase subunit I gene (Folmer *et al.*, 1994). PCR amplification of DNA was performed in 10 µl reactions using Multiplex PCR Kits (QIAGEN), in accordance with the manufacturer's

instructions. Each reaction contained 5 µl multiplex master mix, 1 µl primer mix (each primer at 2 µmol), 0.5 µl bovine serum albumin (10 $mg.ml^{-1}$) (BioLabs Inc.), 1 µl (*c.* 0.1 µg) sample template DNA, and 2.5 µl ultrapure H_2O. PCR runs were performed on two machines, a Pelter Thermal Cycler (BIO-RAD Laboratories Inc.), and a Veriti Thermal Cycler (Applied Biosystems). Cycling parameters for the *D. reticulatum* primer combination were: 95 °C for 15 min, then 35 cycles of 94 °C for 30 s, 52 °C for 90 s, 72 °C for 90 s, and then a final extension of 72 °C for 10 min. Cycling parameters for the general invertebrate primer combination were: 95 °C for 15 min, then 45 cycles of 94 °C for 30 s, 47 °C for 90 s, 72 °C for 90 s, and then a final extension of 72 °C for 10 min. Each PCR run contained appropriate positive and negative controls (King *et al.*, 2008). PCR products were separated by electrophoresis on an ethidium bromide-stained, 2 % agarose gel, and visualized by ultraviolet transillumination.

3.2.4 Statistical Analysis

The effect of *P. melanarius* attacks on slug mortality was tested using a chi-square goodness-of-fit test, calculated using the maximum likelihood method. The test compared the frequencies of attacked and control slugs that remained alive at each of the four vital status monitoring time points (1 h, 24 h, 48 h and 72 h post-attack).

3.3 Results

3.3.1 Slug survival after attacks by *Pterostichus melanarius*

Attacks by *P. melanarius* had no effect on slug survival (χ^2_4 = 0.01, P = 1.000) during the 72 h following attacks. After 72 h, 118 attacked-slugs, and 39 control-slugs remained alive. The two attacked-slugs that died were both alive 1 h after attack, but were dead 24 h post-attack.

Attacks by *P. melanarius* involved full closure of mandibles into the body of the slug, followed by opening of the mandibles and release of the slug. Wounds were visible immediately after attack, and in some instances appeared to heal relatively quickly, whilst in other cases wounds appeared to be more serious for the slug and remained highly visible after 72 h (Plate 3.1). In none of the slugs did autotomy occur, although in one individual (Plate 3.2), a failed attempt at autotomy appeared to have occurred.

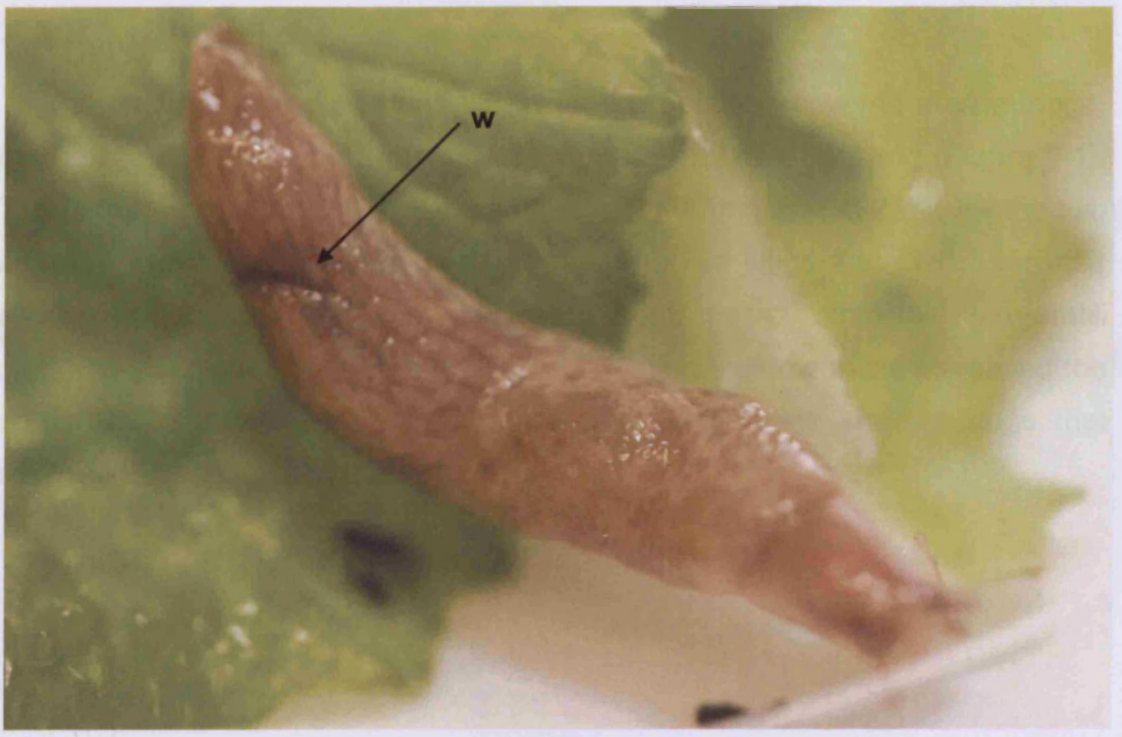


Plate 3.1 *Deroceras reticulatum* 72 h after being attacked by *Pterostichus melanarius*. The bite wound (w) is clearly visible.

Plate 3.2 *Deroceras reticulatum* posterior 72 h after attack by *Pterostichus melanarius*. Autotomy of the tail tip appears to have been attempted.

3.3.2 Slug size

The mass of individual slugs used in the experiment covered a wide range, from 28 to 658 mg, with a mean ± SEM mass of 226 ± 11.4 mg (Figure 3.1). The masses of slugs that died were: 275 mg, 405 mg and 492 mg for the two attacked and the one control respectively. The two slugs that died after their attacks had masses that were above the mean mass. Within this very small proportion of dead slugs there was no suggestion that low mass increased the probability of mortality, because there were very many smaller slugs that survived their attacks.

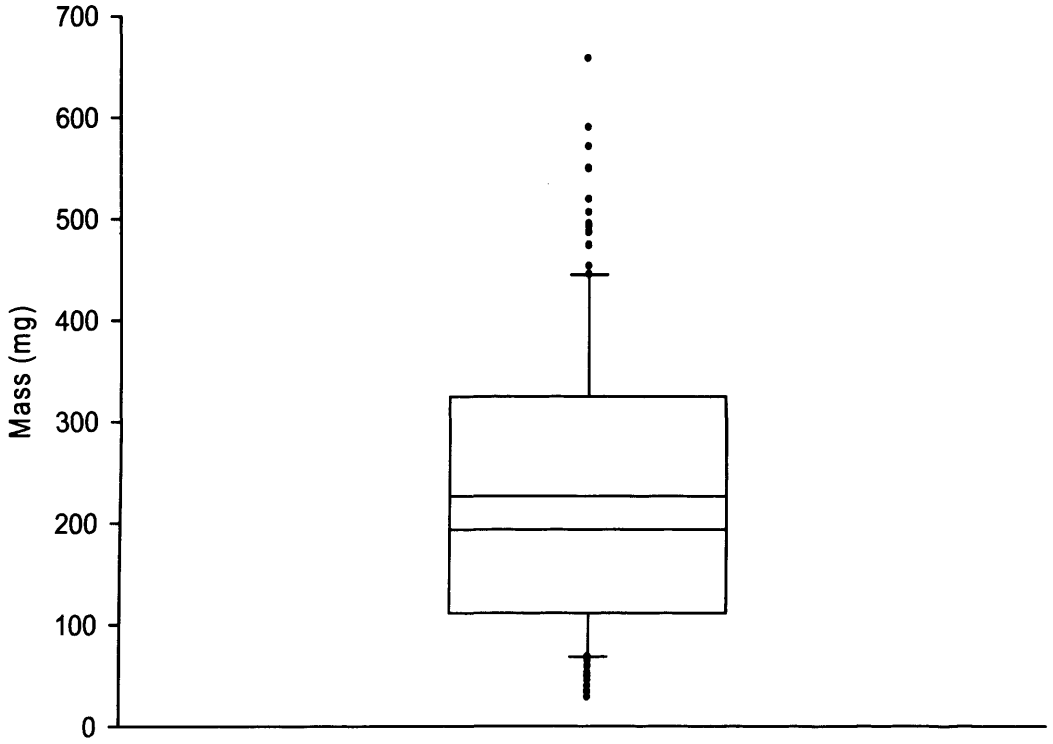


Figure 3.1 Distribution of individual masses of *Deroceras reticulatum* that were attacked by *Pterostichus melanarius*, illustrated in a box plot, where: the lower and upper boundaries of the box indicate the 25th and 75th percentiles respectively, within the box the lower line indicates the median and the upper line indicates the mean, the lower and upper extremities of the whiskers indicate the 10th and 90th percentiles respectively, and the dots indicate the remaining data points.

3.3.3 Molecular analysis on *Pterostichus melanarius* foregut contents

An attacking bite by *P. melanarius* was not sufficient to elicit a positive response in tests for the presence of *D. reticulatum* DNA. All beetle foregut contents subjected to molecular analysis tested negative for *D. reticulatum* DNA. The integrity of the DNA extraction from samples was proven to be good because all samples were positive when tested with the general invertebrate primers.

A bite inflicted upon *D. reticulatum* did not lead to an increase in foregut mass for *P. melanarius*. The mean foregut mass of beetles (Table 3.1) that were frozen 15 min after biting *D. reticulatum* did not differ significantly to those of control beetles (male: U = 50.0, P = 0.509, n = 22; female: U = 48.0, P = 0.438, n = 22).

Table 3.1 Mean (± SEM) foregut mass (mg) of male and female *Pterostichus melanarius* that were killed by freezing either 15 min, 6 h, or 12 h after biting *Deroceras reticulatum*. Control beetles that had not bitten *D. reticulatum* were frozen in parallel with the 15 min post-attack frozen beetles.

	Treatment level			
	Control	15 min	6 h	12 h
Male	11.6 ± 0.870	9.74 ± 1.28	8.33 ± 0.847	10.8 ± 1.35
Female	15.6 ± 1.56	13.6 ± 1.81	11.5 ± 0.835	17.6 ± 1.76

3.4 Discussion

The experiment reported here investigated the potential for aborted attacks on *D. reticulatum*, by *P. melanarius*, to elicit positive results in PCR-based molecular tests for slug DNA in the guts of predators. The results of the experiment indicate that PCR-based estimates of predation are unlikely to include incidences of aborted attacks. This is a positive result for the integrity of PCR-based gut content analysis. Particularly in light of the other observations made during experimentation, that attacks by *P. melanarius* had no effect on *D. reticulatum* survival. Therefore, aborted attacks are unlikely to contribute to pest control.

The observations reported in section 2.3 on the prey-interaction excluding feeding (PIEF) between *P. melanarius* and live *D. reticulatum* indicated that the mean PIEF duration was 20.8 s, although not all of the PIEF duration was attacking behaviour. That was longer than the duration of the interactions made during this experiment, but the attacks made during this experiment were more severe than those in the previous experiment. The increased severity was due to the beetles being held up against slugs, inducing them to make maximum mandible-gape bites. Thus, the attacks made by *P. melanarius* during this experiment might be considered as being at the high end of the spectrum on severity of aborted attacks, in terms of the severity of the wounds inflicted upon the slugs. Despite this, survival of attacked slugs was not different to that of non-attacked slugs. This can be seen as robustness in *D. reticulatum* that might make survival of *P. melanarius* attacks in a field environment highly likely. Therefore, it is important for the integrity of predation estimates made by PCR-based gut content analysis, that aborted attacks are unlikely to elicit positive results for the presence of slug DNA.

Carabids that are specialist slug predators target their attacks at vulnerable locations on the torsos of slugs (Pakarinen, 1994a). Generalists, including *P. melanarius*, do not employ this strategy (Pakarinen, 1994a; Mair and Port, 2002; Chapter 2); *P. melanarius* mainly attacks the posterior of slugs. *Pterostichus niger* was reported to do the same (Pakarinen, 1994b), to which

the main responses of *D. reticulatum* are to produce mucus, lift and wag its tail, and flee (Rollo and Wellington, 1979). Attacks directed at the posterior may also induce some slug species to autotomize that part of the body. *Pterostichus* niger was observed to induce autotomy in *D. reticulatum* (Pakarinen, 1994a, b). Slugs survived after autotomy, and *P. niger* concentrated on feeding on the autotomized section of tail rather than pursuing the fleeing slug. Consumption of an autotomized section of tail would be sufficient to elicit a positive result in molecular testing, although the slug is unlikely to die; therefore, positive molecular test results of this type would be false positives in terms of establishing predation rates and contributions to integrated pest management programmes. However, the incidences of autotomy induced by predators were not high; only one out of 13 attacked *D. reticulatum* in both studies (Pakarinen, 1994a, b), but mechanical stimulation using forceps caused autotomy in 52 out of 106 *D. reticulatum.* Additionally, in a wider context, *Arion fasciatus* and *Arion subfuscus* did not autotomize. Consumption of autotomized sections of slug tail would be beneficial to *P. melanarius* in an environment where food might be scarce (Sunderland, 1975; Van Dijk, 1982, 1996). The results of the study reported here suggest that there is negligible nutritional benefit to be gained by *P. melanarius* in only making a bite, because the foregut mass of beetles that had bitten *D. reticulatum* was not greater than that of control beetles.

The defence mechanisms of *D. reticulatum* do not prevent attacks occurring, although it has been reported that their tentacles can detect *P. melanarius* (Dodds, 1997), and they may avoid areas containing carabid trails (Armsworth 2005; Armsworth *et al.*, 2005), but rather they seek to rapidly terminate attacks, and deter subsequent attacks (Rollo and Wellington, 1979). The results presented here suggest that *D. reticulatum* survival after attacks was not significantly affected by body mass (within the range of masses tested). Even the smallest slugs (28 mg) survived a large *P. melanarius* bite. Thus, for any slug ≥ 28 mg, an unavoidable initial attack by *P. melanarius* should be survivable, allowing *D. reticulatum* to utilize its defence mechanisms to escape, and survive (for at least 72 h). In their investigation into the effects of slug size on predation by *P. melanarius*, McKemey *et al.* (2001) reported that, generally, *P. melanarius* were able to kill most *D. reticulatum* < 40 mg within 24 h, but only killed *D. reticulatum* > 40 mg after prolonged exposure inside an experimental

arena, in which the slugs could not hide. This allows for the suggestion to be made that perhaps repeated attacks on larger slugs were necessary before they could be killed by *P. melanarius*. In Petri dish arenas repeated attacks are possible, but in the field environment they may not. It is possible to suggest therefore, that the laboratory-based observations made by McKemey *et al.* (2001) might not extrapolate well to the field environment, because the complexity of that environment allows slugs to utilize their defence mechanisms, and make good their escape. The observations made in this experiment extend that assertion, by adding that escapees are likely to survive.

Chapter 4 -

Olfactory detection of *Deroceras reticulatum* by *Pterostichus melanarius*

4.1 Introduction

Pterostichus melanarius and slugs have both been reported to exhibit within-field spatial aggregations (Hunter 1966; Bohan *et al.*, 2000b), and the aggregations of the two taxa may be coincident (Symondson *et al.*, 1996; Bohan *et al.*, 2000a; Bell *et al.*, 2010). Favourable environmental factors may lead to aggregations of primary consumers (Bryan and Wratten, 1984; Harwood *et al.*, 2001b; Winder *et al.*, 2001, 2005; Bell *et al.*, 2010). Those factors may also be conducive for predators, but predators must locate to where prey are available, thus coincident spatial aggregations may be driven mainly by predators actively locating to sites of general prey aggregation (Den Boer, 1982; Sunderland, 2002). The processes involved in prey searching by carabids include locomotion and sensing (Mols, 1993). In turn, the mechanisms employed might be random walking across an area (Reynolds and Rhodes, 2009), and chemoreception to detect the presence of prey (Den Boer, 1982). Chemoreception has been reported in carabids (Wheater, 1989; Digweed, 1994; Ayre, 1995; Kielty *et al.*, 1996; Mundy *et al.*, 2000), and is facilitated by the antennae and palps (Wheater, 1989; Merivee *et al.*, 2002; Giglio *et al.*, 2003; Symondson and Williams, 1997; Giglio *et al.*, 2010). Carabid responses to slug mucus have also been reported (Wheater, 1989; Digweed, 1994; Ayre, 1995). *Pterostichus melanarius* was reported to be unable to detect the mucus of *Arion subfuscus* (Wheater, 1989), but could detect the mucus of *Deroceras reticulatum* (McKemey *et al.*, 2004).

Electroantennogram tests performed on the antennae of *P. melanarius*, in which responses to dead slugs were elicited, led McKemey *et al.* (2004) to assert that chemical cues are the mechanisms by which *P. melanarius* locate aggregations of slugs in arable field environments. However, electroantennograms provide an indication of physiological, but not behavioural responses. This important distinction was illustrated in the behavioural responses of *P. melanarius* to two synthesized preparations of aphid alarm pheromone with different constitutions; beetles were attracted by one of the preparations, but repelled by the other (Kielty *et al.*, 1996). This underlines that

prediction of in-field behaviour, through extrapolation of electroantennogram test results, is not straightforward.

The observations made in Chapter 2 indicated that there was no difference in initial attraction to the four prey types offered to *P. melanarius*, but there was a subsequent feeding preference. Of particular relevance was the observation that during dead-prey bioassays, *P. melanarius* did not differentiate among prey in terms of the prey type first contacted. Yet one of the prey types was a dead *D. reticulatum*, which reportedly *P. melanarius* can detect (McKemey *et al.*, 2004). Despite not displaying a preference during initial attraction, beetles did display a significant feeding preference for dead *D. reticulatum*. This would imply that, although *P. melanarius* antennae can detect dead *D. reticulatum*, and beetles fed on dead *D. reticulatum* in preference to the other prey types on offer, starved beetles were not immediately attracted to the slug cadavers. This seemed to be a curious observation, and one deserving of further investigation. Therefore, the aim of this experiment was to test the behavioural responses of adult *P. melanarius* in the presence of odours from live and dead *D. reticulatum*.

Chemoreception in *P. melanarius* has been suggested through olfactometer and electroantennogram tests. Slug specific responses have been reported in larvae through electroantennograms and behavioural bioassays, and in adults through electroantennograms only. In those tests, larvae responded to live and dead *D. reticulatum* (Thomas *et al.*, 2008), but adults only responded to dead slugs (McKemey *et al.*, 2004). Based on the previous chemoreception work on *P. melanarius*, it might be expected that adults should display behavioural response to the presence of dead *D. reticulatum*. Dead slugs in the early stages of decay are readily consumed by *P. melanarius* (Foltan *et al.*, 2005; Chapter 2); therefore, the response to relatively fresh cadavers should be one of attraction. The absence of electroantennogram response to live *D. reticulatum* (McKemey *et al.*, 2004) suggests there should be no behavioural response to live slugs.

In this study, behavioural responses to olfactory sensing by *P. melanarius* were tested using a four-arm, continuous air-flow olfactometer, under the null hypothesis that *P. melanarius* would not display preference for olfactometer arms containing *D. reticulatum*, over empty arms.

4.2 Methods

4.2.1 Collection, maintenance, and preparation of predators and prey

Pterostichus melanarius were collected by pitfall trapping in fields on Rothamsted Farm during the first two weeks of June 2009. Captured *P. melanarius* were separated according to sex, and maintained in individual containers under the same conditions as those described in section 2.2. Food was provided in the form of cat food, initially provided *ab libitum*. The final feeding took place five days before the bioassays. The food provided in the final feeding was removed after 24 h, in order that the beetles were starved during the four days prior to being used in bioassays.

Deroceras reticulatum were collected during June 2009 from fields on Rothamsted Farm. Slugs to be used as dead-prey in bioassays were killed immediately on return to the laboratory, by freezing at -43 °C, and then maintained at that temperature until required in a bioassay. Slugs to be used as live-prey in bioassays were maintained under the same conditions as those described for live-prey in section 2.2.1. Food was provided *ab libitum* in the form of *Brassica chinensis*.

Deroceras reticulatum used as dead-prey were removed from the freezer 12 h prior to being used in bioassays, placed upon the surface of soil held in an open plastic container, and maintained at room temperature (approximately 15 – 20 °C). The soil was taken from the field in which the slugs had been collected. The purpose of this part of the procedure was to allow decomposition of the slugs in a way simulating that of slug decomposition in a field situation. The 12 h duration for decomposition was based on the report of Foltan *et al.* (2005), that the mean residence time of slug carrion in experimental fields was 11.1 h.

4.2.2 Olfactometer configuration and testing

The experiment was conducted under red light, in a controlled environment room at 17 °C, using a pair of four-arm, continuous-airflow olfactometers (Pettersson, 1970; Vet *et al.*, 1983) (maximum internal length = 283 mm, internal height = 14 mm) (Figure 4.1).

A pump was used to draw-in air from the room, through the apparatus attached to the olfactometer, then through the main body of the olfactometer, and finally out through a hole in the top. Air entering the system first passed through charcoal filters to remove any odour present in the airstream drawn from the room. The air then passed through air-flow meters to check that a constant and equal air-flow was passing through each arm. The air-flow rate used was 4 x 0.3 $l.min^{-1}$. Finally, before entering the main body of the olfactometer, the air passed through the four prey chambers.

In order to facilitate scoring of beetle position within the olfactometer, a sheet of white paper, onto which had been drawn a square delineating five sectors, one sector pertaining to each of the fours arms of the olfactometer, and one central sector, was placed under the transparent olfactometer. Beetles were present in olfactometer sectors, and the prey (or blanks) were present in prey chambers on the olfactometer arms, however, for the sake of readability in the text that follows, when describing the location of beetles, sectors and arms are used interchangeably.

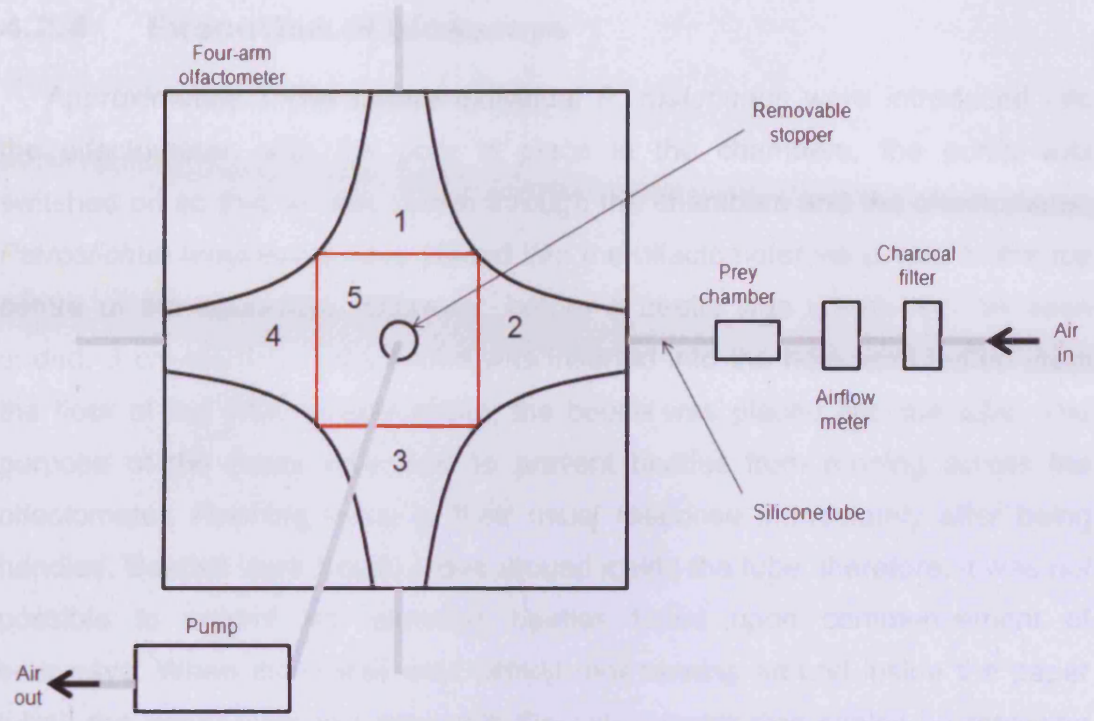


Figure 4.1 Four-arm, continuous-airflow olfactometer configuration. A similar set of apparatus as that shown attached to olfactometer Sector 2 was also attached to each of the other three sectors. A red square was drawn on to a piece of paper placed under the olfactometer to act as a sector delineation mark.

Before conducting the bioassays that would test whether *P. melanarius* could detect prey that were present in the olfactometer arms, the apparatus and environment were tested for bias in a set of preliminary blank bioassays ($n_m = n_f = 8$). These were performed using the olfactometer configuration described above, with all four prey chambers empty. The procedure used was the same as that described below for the bioassays testing detection of prey. The olfactometer and sex of beetle used were randomized, and sixteen such preliminary test bioassays were performed.

4.2.3 Experimental design

Three prey treatments levels were applied in prey detection bioassays: one live *D. reticulatum* with three empty chambers ($n_m = n_f = 15$), one dead *D. reticulatum* and three empty chambers ($n_m = n_f = 15$), and four empty chambers ($n_m = 8$, $n_f = 7$). The latter was a control to enable monitoring of bias within the apparatus throughout the experiment.

4.2.4 Execution of bioassays

Approximately 1 min before individual *P. melanarius* were introduced into the olfactometer, with the prey in place in the chambers, the pump was switched on so that air was drawn through the chambers and the olfactometer. *Pterostichus melanarius* were placed into the olfactometer via a hole in the top centre of the apparatus. However, before a beetle was introduced, an open ended, 3 cm-diameter paper tube was inserted into the hole, and rested upon the floor of the olfactometer arena; the beetle was placed into the tube. The purpose of the paper tube was to prevent beetles from running across the olfactometer. Running away is their usual response immediately after being handled. Beetles were free to move around inside the tube, therefore, it was not possible to control the direction beetles faced upon commencement of bioassays. When the beetle was settled (not running around inside the paper tube), the paper tube was removed, the olfactometer was sealed by replacing the stopper, airflow through the apparatus was resumed, and the bioassay commenced. Recording of beetle behaviour commenced immediately. The movement of beetles among sectors, and the duration spent in each sector was recorded. All bioassays ran for a duration of 10 min. After each bioassay the olfactometer and chambers were washed using 50 % ethyl alcohol solution, rinsed with deionized water, and dried.

4.2.5 Statistical analysis

4.2.5.1 Behavioural responses of *Pterostichus melanarius* when all olfactometer arms were blank

Friedman's non-parametric ANOVA was used to test whether there was a difference in the duration beetles spent in each of the four olfactometer arms. On an individual beetle basis, the cumulative duration spent in each of the olfactometer arms was input into Friedman's non-parametric ANOVA tests as the response variate data, and the olfactometer arms were considered the treatments. Values quoted for Friedman's test results are those after adjusting for ties (Siegel and Castellan, 1988).

4.2.5.2 Olfactometer arm first visited by *Pterostichus melanarius*, when in the presence of live or dead *Deroceras reticulatum*

First arm visitation was tested in order to assess whether the initial behaviour of *P. melanarius* was in response to the detection of prey. Chi-square goodness-of-fit tests calculated using the maximum likelihood method were applied to the frequency data of beetle first visitations to olfactometer arms in order to test whether there were differences between prey-containing, and blank olfactometer arms. The observed frequencies of bioassays in which prey-containing and blank olfactometer arms were the first to be visited, were compared against expected frequencies, for which first visit would be random, i.e., the prey-containing arm would be first visited in 25 % of bioassays, and blank olfactometer arms would be first visited in 75 % of bioassays.

4.2.5.3 Behavioural responses of *Pterostichus melanarius* over the duration of bioassays, when in the presence of live or dead *Deroceras reticulatum*

The behavioural responses of *P. melanarius* over the duration of bioassays were tested by Friedman's non-parametric ANOVA, using the same method as described in section 4.2.5.1. The olfactometer arm chosen to contain the prey was randomly selected for each bioassay, therefore, when testing for differences among the duration *P. melanarius* spent in each of the four olfactometer arms, it was necessary to arrange the data such that the olfactometer arm containing the prey item was consistently identified as such. This was done by allocating the duration *P. melanarius* spent in the olfactometer arm in which the prey was present to Treatment Level 1, the duration spent in the olfactometer arm that was adjacent in a clock-wise direction from that containing the prey was allocated to Treatment Level 2, and so on for the data of the other two olfactometer arms.

4.2.5.4 Location of *Pterostichus melanarius* at the termination of bioassays, when in the presence of live or dead *Deroceras reticulatum*

The locations of *P. melanarius* at the termination of bioassays was tested in order to be used as an indication of delayed detection of *D. reticulatum* by beetles, i.e., after having had the opportunity to explore the entire olfactometer arena. Chi-square goodness-of-fit tests calculated using the maximum likelihood method were applied to the frequency data of olfactometer arms in which beetles were located at the termination of bioassays. The observed frequencies of bioassays in which *P. melanarius* were located in prey-containing, and blank olfactometer arms at the termination of bioassays, were compared against expected frequencies in which location would be random, i.e., the prey-containing arm would be the site of *P. melanarius* location at the termination of bioassays in 25 % of bioassays, and blank olfactometer arms would be the site of *P. melanarius* location at the termination of bioassays in 75 % of bioassays.

4.3 Results

4.3.1 Behavioural responses of *Pterostichus melanarius* when all olfactometer arms were blank

There was no significant difference among male (Q_3 = 1.75, P = 0.627) or female (Q_3 = 3.18, P = 0.365) *P. melanarius* (combined: Q_3 = 0.94, P = 0.816) in the duration spent in each of the olfactometer arms during the bioassays run to test for bias in the olfactometer apparatus.

Similarly, there was no significant difference among male (Q_3 = 5.23, P = 0.156) or female (Q_3 = 2.06, P = 0.560) *P. melanarius* (combined: Q_3 = 4.52, P = 0.210) in the duration spent in each of the olfactometer arms during the blank bioassays performed in parallel with live- and dead-prey detection bioassays. Thus, there appeared to be no bias in the olfactometer apparatus throughout the period of experimentation.

4.3.2 Olfactometer arm first visited by *Pterostichus melanarius*, when in the presence of live or dead *Deroceras reticulatum*

The differences between the frequencies of bioassays in which beetles first visited the olfactometer arm containing the prey and those that first visited a blank arm, for both live- and dead-prey bioassays (Figure 4.2), were not significantly different to the expected random frequencies (Live-prey: χ^2_1 = 0.83, P = 0.361; Dead-prey: χ^2_1 = 0.23, P = 0.631). Therefore, *P. melanarius* did not exhibit a preference for the olfactometer arm containing *D. reticulatum*, either alive or dead, at the commencement of bioassays.

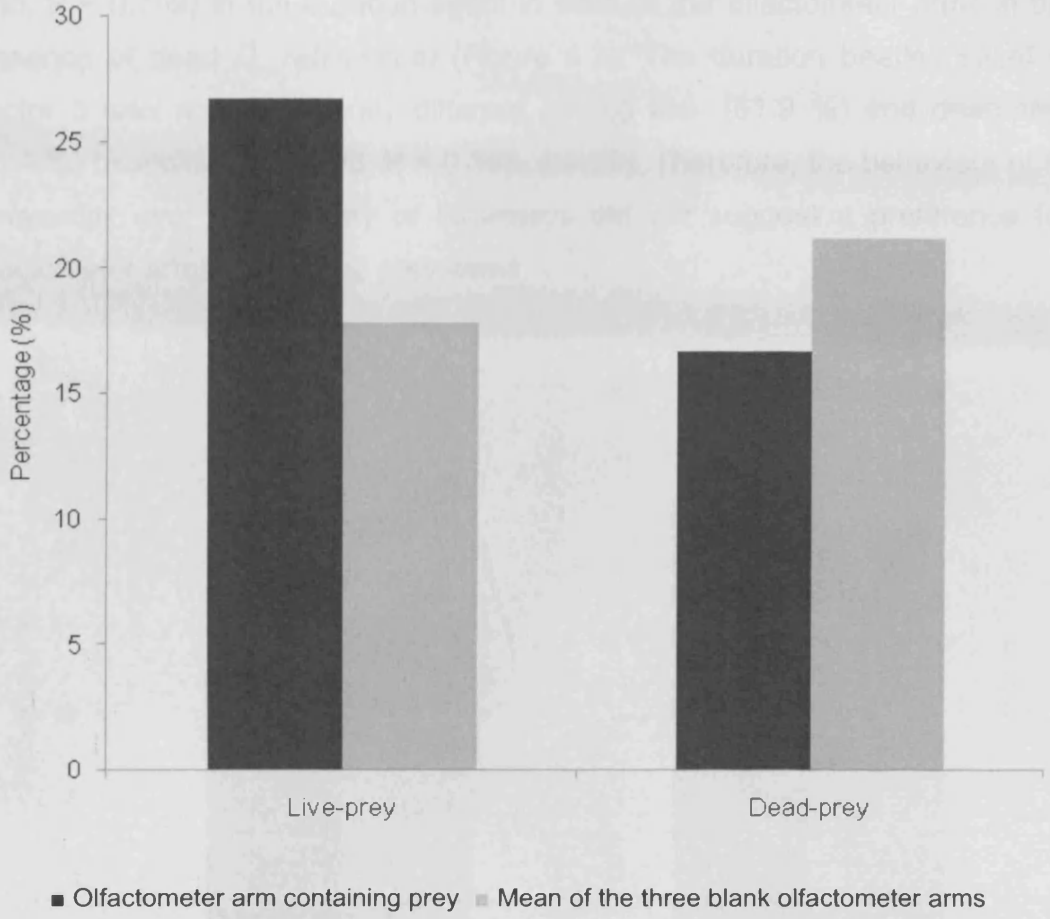


Figure 4.2 Percentage of bioassays in which *Pterostichus melanarius* first visited the olfactometer arms containing live or dead *Deroceras reticulatum*, or any one of the three blank arms. The actual percentage of bioassays in which a blank arm was first visited has been divided by three to provide a mean for clarity of presentation. The bioassays in which beetles did not vacate Sector 5 were included in the calculations (Live-prey bioassays = 20.0 %, and dead-prey bioassays = 20.0 %), but not shown on the graph. Pooled data for male and female beetles.

4.3.3 Behavioural responses of *Pterostichus melanarius* over the durations of bioassays, when in the presence of live or dead *Deroceras reticulatum*

There was no significant difference among male (Q_3 = 3.86, P = 0.277) or female (Q_3 = 5.92, P = 0.116) *P. melanarius* (combined: Q_3 = 0.25, P = 0.969) in the duration spent in each of the olfactometer arms in the presence of live *D. reticulatum*. Similarly, there was no significant difference among male (Q_3 = 1.12, P = 0.771) or female (4.46, P = 0.216) *P. melanarius* (combined: Q_3 =

1.38, P = 0.709) in the duration spent in each of the olfactometer arms in the presence of dead *D. reticulatum* (Figure 4.3). The duration beetles spent in Sector 5 was not significantly different among live- (51.9 %) and dead-prey (41.4 %) bioassays (U = 393, P = 0.398, n = 60). Therefore, the behaviour of *P. melanarius* over the entirety of bioassays did not suggest a preference for olfactometer arms containing prey items.

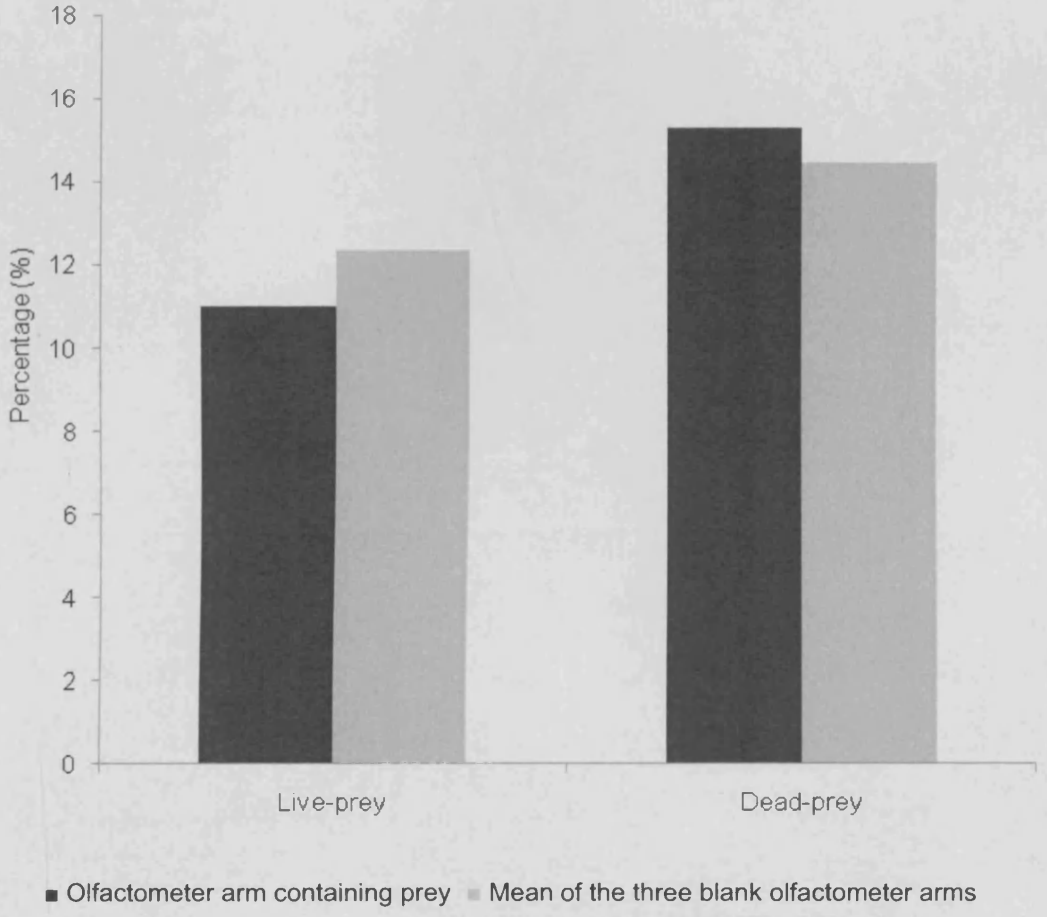


Figure 4.3 Percentage of total bioassay duration *Pterostichus melanarius* spent in the olfactometer arm containing the prey, and the mean duration spent in each of the remaining three blank arms. The percentage of bioassay time spent in Sector 5 was: live-prey bioassays = 51.9 %; dead-prey bioassays = 41.4 %. Pooled data for male and female beetles.

4.3.4 Location of *Pterostichus melanarius* at the termination of bioassays, when in the presence of live or dead *Deroceras reticulatum*

The frequencies of bioassays in which beetles were located in each of the olfactometer arms at the termination of bioassays, for both live- and dead-prey bioassays (Figure 4.4), were not significantly different from the expected random frequencies (Live prey: $\chi^2_1 = 0.53$, P = 0.467; Dead prey: $\chi^2_1 = 0.28$, P = 0.597). Therefore, the location of *P. melanarius* at the termination of bioassays did not indicate a preference for the olfactometer arms in which prey were present.

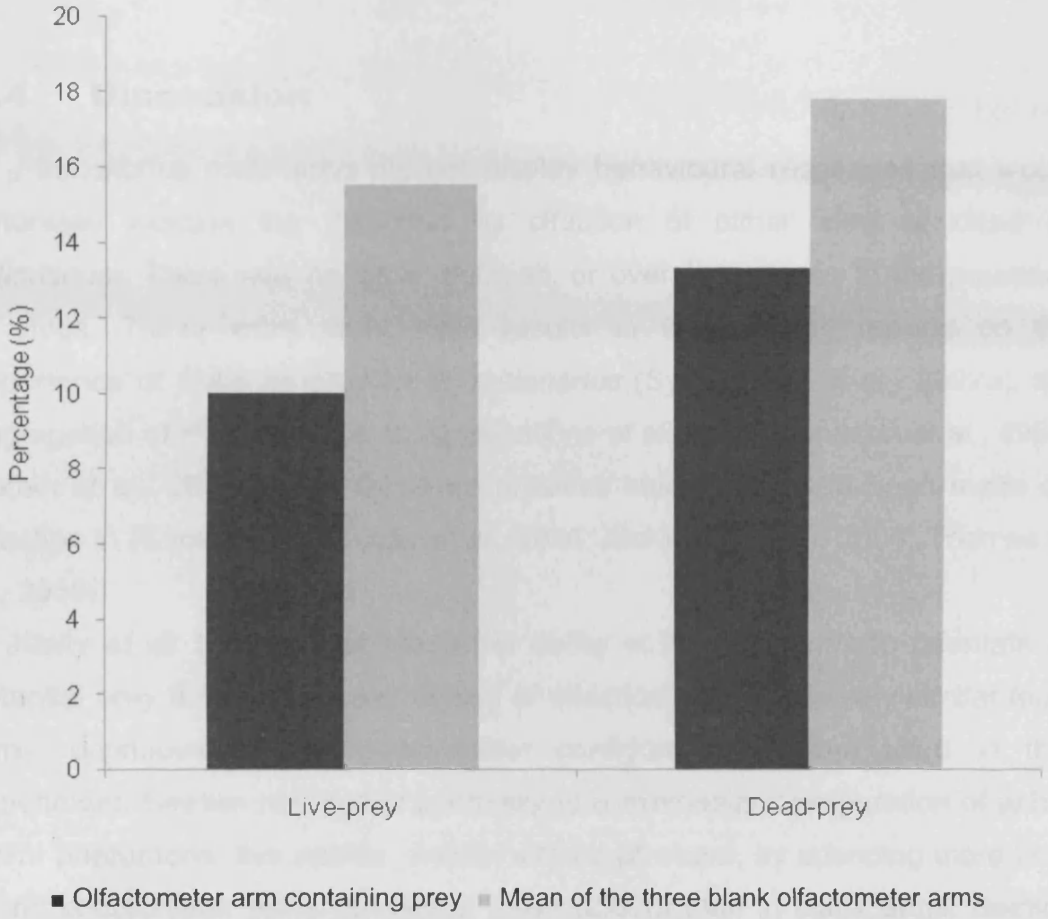


Figure 4.4 Percentage of bioassays in which, at the termination of bioassays, *Pterostichus melanarius* were located in the olfactometer arm containing the live or dead *Deroceras reticulatum*, or one of the three blank arms. The actual percentage of bioassays in which beetles were located in blank arms at the end of bioassays has been divided by three to provide a mean for clarity of presentation. The bioassays in which beetles were located in Sector 5 at their termination were included in the calculations (Live-prey bioassays = 43.3 %, and dead-prey bioassays = 33.3 %), but not shown on the graph. Pooled data for male and female beetles.

4.4 Discussion

Pterostichus melanarius did not display behavioural responses that would otherwise indicate the detection by olfaction of either alive or dead *D. reticulatum.* There was no initial, delayed, or overall response to the presence of slugs. These were unexpected results in view of the reports on the importance of slugs as prey for *P. melanarius* (Symondson *et al.,* 2002a), the aggregation of *P. melanarius* to aggregations of slugs (Symondson *et al.*, 1996; Bohan *et al.,* 2000a), and the three previous studies that had been made on olfaction in *P. melanarius* (Kielty *et al.,* 1996; McKemey *et al.,* 2004; Thomas *et al.,* 2008).

Kielty *et al.* (1996) established the ability of *P. melanarius* to orientate to potential prey through the mechanism of olfaction, by using a very similar four-arm, continuous air-flow olfactometer configuration to that used in this experiment. Beetles responded positively to a synthesized preparation of aphid alarm pheromone, live aphids, and an extract of wheat, by spending more time in the olfactometer arms containing those odours than in blank arms. Beetles were repelled by a second synthesized preparation of aphid alarm pheromone. When collembolans were present in one of the olfactometer arms, there was no difference between the durations *P. melanarius* spent in the olfactometer arms containing prey and those that were blank. Thus, *P. melanarius* appeared to be able to detect certain odours, and respond accordingly.

McKemey *et al.* (2004) then went on to establish, through the use of electroantennograms, that the antennae of adult *P. melanarius* responded to the odours emitted from *D. reticulatum* that had been decaying for 24 – 48 h at room temperature. However, the response of beetle antennae to *D. reticulatum* was limited, because beetles did not respond to slugs that were: live, injured, diseased (nematode infected: *Phasmarhabditis hermaphrodita*), advanced diseased, dead, or cut open dead. It should be noted, however, that McKemey *et al.* (2004) removed the tips of antennae, which contain sensilla (Symondson and Williams, 1997), in order to attach electrodes. This had the potential to limit the responsiveness of antennae. By responding to only decayed slugs, the

hypothesis can be formed that *P. melanarius* antennae were responding to the odour of putrefaction in general (Houston, 1986; DeVault *et al.*, 2003). Thus, the response might not be slug specific. The odour of putrefying carrion could be attractive or repulsive depending on the duration of putrefaction (Foltan *et al.*, 2005). Additionally, the 24 – 48 h putrefaction period allowed by McKemey *et al.* (2004) was rather longer than that reported as the mean residence time (11.1 h) of slug carrion in arable fields (Foltan *et al.*, 2005), and invertebrate carrion in general (Seastedt *et al.*, 1981; Fellers and Fellers, 1982), before removal by scavengers. Therefore, the odours emitted might be different, either in compounds present or in relative concentrations of compounds, to those of fresher carrion. Although, these studies monitored surface dwelling cadavers, it is possible cadavers in more cryptic locations, e.g. in the soil matrix, may be in residence for longer periods, but then the proliferation of emitted odours might be reduced.

Thomas *et al.* (2008) reported a greater response to *D. reticulatum* odour in the antennae of *P. melanarius* larvae than had been previously reported by McKemey *et al.* (2004) in *P. melanarius* adults. The antennae of larvae responded to odours from *D. reticulatum* that were: live, < 24 h-dead, and > 24 h-dead. Additionally, antennae responded to live, and < 4 h-dead *Calliphora* spp. (Diptera). Thomas *et al.* (2008) went further, and tested the behavioural responses of larvae. They performed bioassays in plastic boxes. In each was a thin soil substrate under which had been placed two Petri dishes containing either (1) a live slug and a control, or (2) a dead slug and a control, or (3) a live slug and a dead slug. *Pterostichus melanarius* larvae were released upon the soil surface, and their locations relative to the two dishes were then assessed after a predetermined duration. It was reported that, in experiment (1), there were significantly more larvae in the halves of the boxes that contained the live slugs. However, the result was only significant because the probability was calculated in a one-tail test; recalculating the P-value in a two-tail test makes the result non-significant. In experiment (2), again there were more larvae in the halves of the boxes containing the dead slugs. However, again the reported significant result is disputed. The value reported was $\chi^2_1 = 2.9512$, and the P-value quoted was 0.0212, which is incorrect. The correct P-value is 0.086, which is clearly not significant at the 5 % level. Halving the P-value, as was

done for the experiment (1) result, would give P = 0.043, which is statistically significant, but is a result that should be treated with caution. Thus, the application of a more prudent approach to the data from experiments (1) and (2) would provide non-significant results in both, and the conclusion that *P. melanarius* larvae did not display a behavioural response to the presence of alive or dead *D. reticulatum.* The result of experiment (3) was a non-significant difference in locations of larvae relative to the live and dead *D. reticulatum.*

Collectively, the work by Kielty *et al.* (1996), McKemey *et al.* (2004), and Thomas *et al.* (2008) indicates that the antennae of *P. melanarius* larvae respond to the odours emitted from live- and dead-prey, the antennae of adult *P. melanarius* respond to putrefied prey, and adults also exhibit a behavioural response to some potential-prey odours. The results from the experiment reported here add to this body of research by asserting the *P. melanarius* adults do not exhibit a behavioural response to the presence of live or dead *D. reticulatum*, and therefore, it is unlikely they are able to detect the odour from these prey. Had the dead slugs been allowed to putrefy for a longer period, *P. melanarius* may have been able to detect them, but the putrefaction period used was realistic of field available carrion.

Electroantennogram responses have been reported to vary in relation to sex, age, feeding history, level of starvation, previous experience, and life history related activities (Crnjar *et al.,* 1990; Vet *et al.,* 1990; Den Otter *et al.,* 1991; Digweed, 1994). Therefore, prey detection mediated by olfaction also may be contingent upon these factors. The data from this experiment confirmed that beetle sex was not an important variable, because the responses among male and female beetles were not different. The food eaten by beetles in the field prior to capture is of course unknown. Beetles were caught early in the season, soon after emergence, and then maintained under controlled environment conditions for numerous weeks before being subjected to bioassays. Therefore, their opportunity for prey consumption in the field was minimized, and during the maintaining period all beetles were fed the same artificial diet. Thus, the differences between beetles' feeding histories during the adult stage should have been minimal for beetles of the same cohorts, but there was potential for greater disparity among cohorts. An interesting follow-up to the experiment reported here, would be to perform the same olfactometer tests on

P. melanarius caught in-field towards the end of the summer, to assess whether their response is different to those caught early in the season. Alternatively, conduct similar olfactometer tests on groups of *P. melanarius* that have been differentially diet-conditioned.

The absence of an olfactory derived behavioural response to the odours of live and dead *D. reticulatum* does not rule out the possibility that *P. melanarius* can detect the presence of slugs using other means. Antennae of ground beetles typically contain sensilla that perform many different functions, including: chemoreception, mechanoreception, thermoreception, and hygroreception; where the chemoreception may be olfaction and gustation (Merivee *et al.*, 2002). In addition to antennae, beetles possess labial and maxillary palps which contain sensilla that may also function in chemoreception (olfaction and gustation), mechanoreception, thermoreception, and hygroreception (Giglio *et al.*, 2003; Giglio *et al.*, 2010). Thus, in terms of prey sensing, antennae and palps may respond to gustatory as well as olfactory stimuli. Indeed, McKemey *et al.* (2004) reported that *P. melanarius* were able to detect slug mucus trails, and Guy *et al.* (2008) reported the ability of *P. melanarius* to detect areas that had previously been walked over by conspecifics. Both of these suggest a role for gustatory sensing in *P. melanarius*.

It is significant that *P. melanarius* appears to be unable to detect the odour of *D. reticulatum*, since they are a potential source of food, and a role for olfaction in carabids has been previously reported (Bryan and Wratten, 1984; Wheater, 1989; Kielty *et al.*, 1996; Mundy *et al.*, 2000). Therefore, the emphasis in this discussion has concentrated on the lack of behavioural response in *P. melanarius* as being rather unexpected. However, it must be remembered that *P. melanarius* is a generalist, and opportunist, with a habit that has been described as "ultra-generalist, eating almost anything it can subdue" (Den Boer, 1982; Symondson *et al.*, 2002b). Thus, judged under these classifications, the negative results perhaps should not be considered so unexpected. Although slugs have been suggested to constitute a significant proportion of the diet for *P. melanarius* (Symondson *et al.*, 1996; Bohan *et al.*, 2000a; Symondson *et al.*, 2002a), this might be due to opportunistic exploitation of available resources at certain points in time, at other times, alternative prey might be more available

and exploited (Winder *et al.*, 2001; Winder *et al.*, 2005). Thus, general chemoreception responses might be expected, where sensilla respond to many odours rather than a specific few. In turn, it might be expected that such generally responding sensilla might be less sensitive, and thus, require higher concentrations of chemicals to elicit responses. Thus, scale may be an important factor in the chemoreceptive ability of *P. melanarius*.

Chemoreception is one component of the mechanisms employed by *P. melanarius* in prey finding. Olfaction and gustation would be combined with some form of random walk, in order to locate prey. The principle behind the random walk is to traverse a given area of habitat in the most efficient and effective way, in order to maximize the likelihood of encountering prey items. The Lévy flight paradigm of searching behaviour predicts a series of randomly orientated straight-line movements, in which most of the straight-line movements are short, but interspersed within these movements are less frequent, longer, straight-line movements. Still longer straight-line movements are made even less frequently, and so the pattern repeats (Reynolds and Rhodes, 2009). Observations made by Den Boer (1982) and Mols (1993), on the behaviours of carabids, transpose well onto the Lévy flight paradigm. Den Boer (1982) postulated that, as a response to the energetic costs involved in walking, when carabids search for food, they focus on finding aggregations of prey. Once an aggregation is found, carabids remain in the area and conduct restricted searching for more of the same prey (Mols, 1993). The same prey type is sought because a search image (Den Boer, 1982), or increased sensilla sensitivity (Vet *et al.*, 1990) is established. Whilst engaged in localized searching at the site of prey aggregation, the behaviour exhibited by carabids is to walk at reduced velocity (Mols, 1993), in a tortuous manner, with antennae bent downward, searching for more of the same prey (Den Boer, 1982). At this time, chemoreception would likely be used. The antennae are bent downwards to increase proximity to likely prey location, perhaps for olfactory sensing, and on to the substrate for gustatory sensing. In the absence of further prey encounters inside a given time period, carabids depart from the area. During their walk away from the area, until the next area randomly selected for tortuous walking and searching, velocity is greater (Mols, 1993), and antennae are held pointing forward rather than downwards. The lack of sensitivity at these times

meant potential prey may be run over without being attacked (Den Boer, 1982). The differential aspect of antennal orientation at the different stages of the searching process tends to suggest greater reliance upon the chemoreceptive organs during the localized searching activities, rather than during the longer movement activities. Thus, chemical reception in *P. melanarius* may be effective only at a small scale, when perhaps sensory organs are actually, or very nearly, in contact with the chemical source. It may be asserted that the olfactometer configuration used in this experiment was simulating a large scale, and tested detection through olfaction at that large scale. The behaviour protocols being executed by beetles inside the olfactometers were perhaps those associated with searching outside of prey aggregation areas; then it is possible to argue that, during this stage of the searching process, detection of *D. reticulatum* through olfaction is not possible in *P. melanarius*.

Chapter 5 -

The effects of feeding history on prey selection in *Pterostichus melanarius*

5.1 Introduction

It is suggested that prey differ in their nutritional quality for arthropod generalist predators (Toft, 1996; Toft and Wise, 1999a; Bilde *et al.*, 2000; Fagan *et al.*, 2002), and that feeding on differential quality foods, and mixed diets, may lead to differential growth, development, fecundity, and survival (Van Dijk, 1982, 1994; Wallin *et al.*, 1992; Toft, 1995; Toft and Wise, 1999a, b; Harwood *et al.*, 2009). In response, generalist arthropods may select among prey types in order to maximize the quality of their diets (Greenstone, 1979; Toft, 1996; Mayntz *et al.*, 2005; Raubenheimer *et al.*, 2007). This theory may be seen to be somewhat at variance with traditional ideas of the searching and feeding behaviour of generalist predator carabids, which suggest that once a prey item has been located and fed upon, beetles remain in the same area, and conduct "restricted area searching" (Mols, 1993) for more of the same prey (Den Boer, 1982).

The traditional model of prey searching and feeding behaviour in generalist predators is the ideal to be hoped for from *Pterostichus melanarius* in terms of its utility as an agent of slug pest control, because slugs are usually reported to be present in aggregations (Hunter, 1966; Bohan *et al.*, 2000a, b). Therefore, a given *P. melanarius* individual will make its maximum impact on reducing the slug population size by remaining in an identified area of slug aggregation, searching for more slug prey, and then feeding on them. Conversely, the assertion that generalists select among prey in order to maximize their quality of diet might result in sub-optimal pest control performance by generalists. For *P. melanarius* to fulfil its potential as an agent of slug pest control in arable fields, beetles must not select against future feeding on slugs after having previously fed on slug tissue. Similarly, feeding on prey types that might be nutritionally complementary to slug prey should not diminish the propensity of *P. melanarius* to then feed on slugs.

Scavenging on slug carrion might compound this potential problem. *Pterostichus melanarius* readily consumes slug cadavers (Foltan *et al.*, 2005; Chapter 2), and it has been suggested that beetles use chemical cues from slug carrion to locate aggregations of slugs (McKemey *et al.*, 2004). Although, in

Chapter 4 it was suggested that the hypothesis of McKemey *et al.* (2004) was perhaps too general, and scale might be an important factor in detection. Since live slugs are reported to be present in aggregations, have low rates of dispersal (South, 1965; Armsworth, 2005), and even exhibit homing behaviour (South, 1992), it might be expected that dead slugs should be aggregated coincidently with live slug aggregations. Additionally, lower handling costs are associated with feeding on carrion (Bilde *et al.*, 2000; Lang and Gsodl, 2001; Chapter 2), and there may be constraints on the sizes of slugs *P. melanarius* can prey upon (McKemey *et al.*, 2001; McKemey *et al.*, 2003; Symondson *et al.*, 2006). Collectively, these factors could be interpreted to form the hypothesis that *P. melanarius* which are aggregated coincidently with slugs, and have fed on slug tissue (Symondson *et al.*, 1996; Bohan *et al.*, 2000a), could have fed significantly on slug carrion. In this case, *P. melanarius* seeking to feed on a range of prey might select against feeding on live slug tissue after having fed upon slug carrion, and thus having made no contribution to pest management. Additionally, prey taxa in general may often be co-aggregated in arable fields (Bryan and Wratten, 1984; Harwood *et al.*, 2001b; Winder *et al.*, 2001; Bell *et al.*, 2010), therefore, alternative prey to slugs would likely be present in areas of slug aggregations, and provide *P. melanarius* with alternative options for feeding.

The aim of this experiment was to determine the effects of previous food intake on current prey selection by *P. melanarius*; specifically, whether beetles select against foods upon which they have most recently fed. This was tested in laboratory-based feeding trials on *P. melanarius* that had been conditioned to different diets. Given that generalists may seek to feed on a range of foods in order to maximize the overall quality of their diet, it might be expected that diet-conditioned *P. melanarius* would select against the food upon which they had fed most recently. The feeding behaviour of *P. melanarius* was tested against the null hypothesis that there would be no significant difference in prey selection among differently diet-conditioned groups of beetles.

5.2 Methods

5.2.1 Collection, maintenance and preparation of *Pterostichus melanarius* and prey

Pterostichus melanarius were collected from two fields on Rothamsted Farm during the period from 14 July 2009 to 16 July 2009. Beetles were housed individually in plastic containers (diameter = 6 cm, depth = 7.5 cm), with 3 cm moist peat-based substrate. The beetles (n = 200) were divided into five groups, each containing equal numbers of males and females, and kept in a controlled environment room at 17 ± 1 °C, 60 – 75 % humidity, and on a reversed light regime (darkness commenced at 9 am) of 16 h:8 h (light:dark). Over the following 32 to 38 days, before being the subject of a bioassay, individual *P. melanarius* were fed a diet consisting of only one prey type. Five such conditioning diets were applied: dead *Deroceras reticulatum*, dead *Aporrectodea caliginosa*, dead *Calliphora vomitoria* larvae, *Brassica napus* (Linnaeus) (Brassicales: Brassicaceae) seeds, and cat food (Whiskas Supermeat). The first of the four conditioning-diet types were chosen because they may be prey for *P. melanarius* under natural conditions (Symondson *et al.*, 1996; Menalled *et al.*, 1999; Honek *et al.*, 2003; Koprdova *et al.*, 2008; King *et al.*, 2010). Cat food was used because it is non-natural, therefore, it could be assured that beetles had no prior history of feeding on such food.

Deroceras reticulatum and *A. caliginosa* were collected from fields on the Rothamsted Farm. Upon return to the laboratory they were killed by freezing at -43 °C, and then maintained at that temperature. *Calliphora vomitoria* larvae were purchased from a local fishing tackle supplier, immediately killed by freezing at -43 °C, and then maintained at that temperature. *Brassica napus* seeds were obtained from stock held at Rothamsted Farm. Approximately 150 mg of food was given to each beetle at intervals of between four and six days. The final portion of food was provided five or six days prior to beetles being used in bioassays, thus ensuring that the beetles were in a state of hunger at

the time of bioassay. This was done with the expectation that they would then be more likely to feed during the 10 min bioassay.

5.2.2 Execution of bioassays

Bioassays commenced on 17 August 2009, and were conducted under red light in a controlled environment room set to 17 °C. Test arenas were 9 cm-diameter Petri dishes into which had been placed a filter paper, moistened with a few drops of water. *Pterostichus melanarius* were presented with four prey items: *D. reticulatum*, *A. caliginosa*, *C. vomitoria*, and *B. napus*. Approximately 100 mg of each prey type was provided, but perhaps more importantly, each item was about the same size in terms of the area of substrate surface covered, and therefore apparency to the beetles. This was to avoid potential selection bias by *P. melanarius* for the largest prey item, by virtue of it being more likely to be encountered. Prey items were arranged randomly and placed approximately 1 cm from the perimeter of the Petri dish at the four cardinal points.

An open ended 3 cm-diameter paper tube was placed at the centre of the arena and *P. melanarius* was placed into the tube. The purpose of the paper tube was to prevent beetles from running across the arena. Running away is their usual response immediately after being handled. Beetles were free to move around inside the tube, therefore, it was not possible to control the direction beetles faced upon commencement of bioassays. When the beetle was settled (not running around inside the paper tube), the tube was removed, the Petri dish lid was replaced, and the bioassay commenced (Plate 5.1). Bioassays were allowed to run for 10 min, and were recorded on an Archos 604 Portable Multimedia Player and DVR Station, via a video camera (Sanyo B/W CCD camera, model number: VCB-3412P) placed overhead.

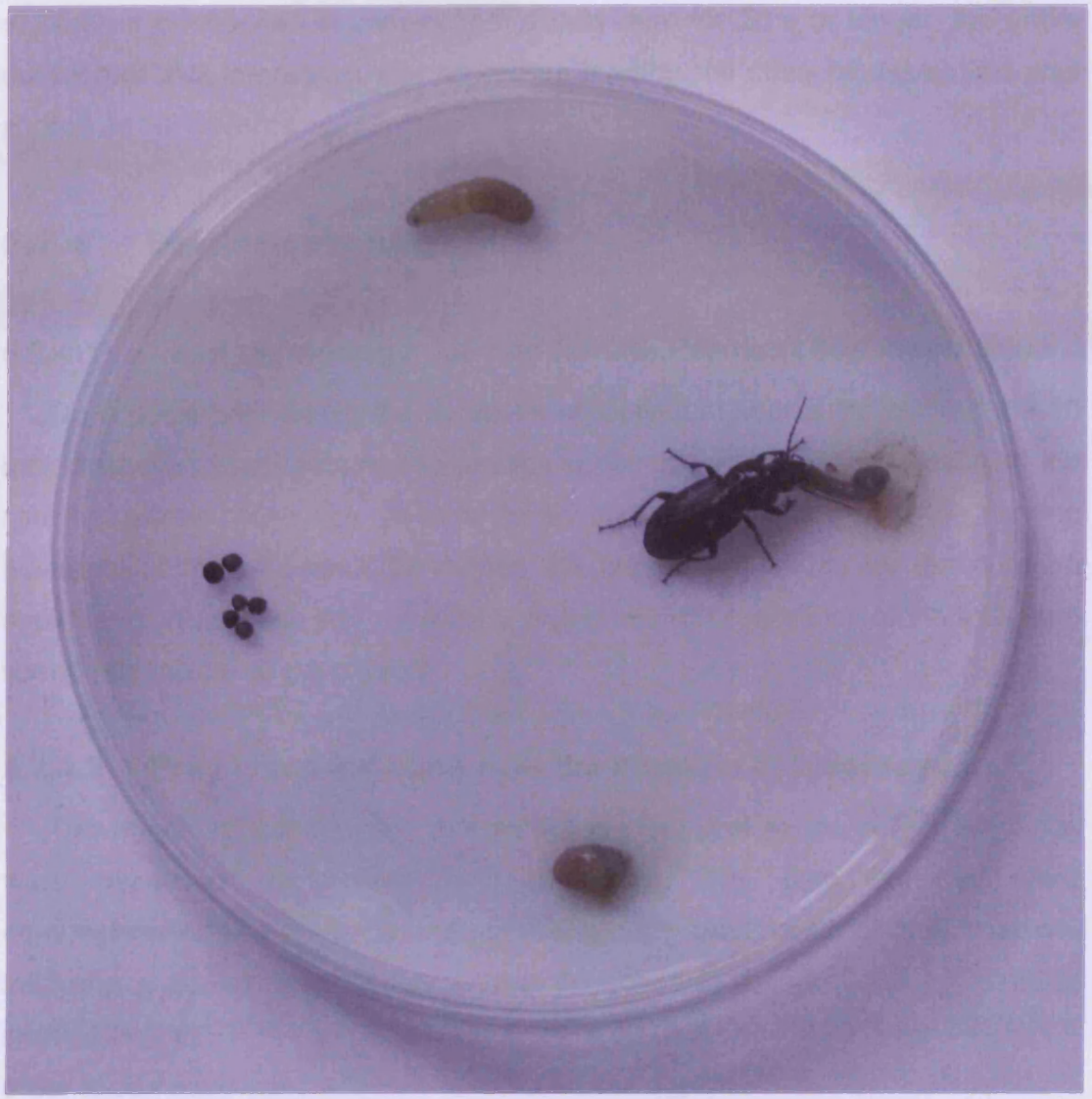


Plate 5.1 Experimental arena, consisting of a 9 cm-diameter Petri dish containing a moist filter paper substrate. Four prey items (clock-wise from upper centre: *Calliphora vomitoria*, *Aporrectodea caliginosa*, *Deroceras reticulatum*, and *Brassica napus*) were randomly arranged, and placed at the four cardinal points, approximately 1 cm from the perimeter of the Petri dish.

5.2.3 Data collection from digital videos

Using the digital video clips made of the bioassays, the behaviours of *P. melanarius* throughout the duration of the 10 min bioassays were scored as being one of nine behaviour categories: four categories (one for each prey type) of prey-interaction excluding feeding (PIEF), four categories of feeding (one for each prey type), or non-prey-interaction (NPI). Prey-interaction events excluding feeding occurred when a beetle was in contact with a prey item for less than 20

s. When a beetle was in contact with a prey item for 20 s or longer, the entire duration of that interaction was scored as feeding. All other bioassay time was scored as NPI events.

5.2.4 Statistical analysis

5.2.4.1 Feeding among male and female *Pterostichus melanarius*

On a per beetle basis, the durations of individual events throughout the 10 min bioassays were summed to provide a cumulative duration for each of the nine behaviour categories. In order to enable comparisons of just the feeding elements of *P. melanarius* behaviour, the cumulative values for the duration spent feeding on each prey type were expressed as proportions of the total time spend feeding on all prey types.

5.2.4.2 Prey types fed upon over the duration of bioassays

The effects of conditioning diet on the feeding preference of *P. melanarius* were assessed using MANOVA and CVA. The data obtained were compositional. Therefore, a log-ratio approach was used in the analysis, following a similar methodology to that described in section 2.2.5.1. On a per beetle basis (n = 170), the replacement method (Aitchison, 1986) was applied in order to eliminate zero values from data across the nine behaviour categories (δ = 0.5). Eight log-ratios were then calculated from the corrected data, using the category of NPI as the denominator. The four ratios calculated for the four feeding behaviour categories were then input into the MANOVA and CVA (Beaghen, 1997; Gardner *et al.*, 2006). The treatment structure in the MANOVA was: prey status x sex of *P. melanarius.* The grouping factor in the CVA was conditioning diet type.

Certain behaviours may occur before a beetle feeds on a prey item: one is the attraction of the beetle to a prey item, and another is the motivation to feed once in contact with a prey item. The effects of conditioning diet on the primary incidences of these behaviours were then analysed.

5.2.4.3 Primary prey investigations

The effect of diet-conditioning on which prey type was the first to be investigated was tested using chi-square goodness-of-fit (maximum likelihood). Within each diet-conditioned group of beetles, the observed frequencies for which each of the prey types had been the first investigated were compared against expected frequencies in which selection of prey to be primarily investigated was random (with four prey types, each would be the first to be investigated during 25 % of bioassays). The significance of difference between observed and expected values was tested against a chi-square distribution. The process was then repeated for each of the four remaining diet-conditioned groups of beetles.

5.2.4.4 Primary feeding events

Tests for effects of diet-conditioning on which prey type was the first to be fed upon were also tested using chi-square goodness-of-fit (maximum likelihood). The tests were performed using the same methodology as that described in section 5.2.4.3. That is, the observed frequencies for which each of the prey items were the first to be fed upon, within a diet-conditioned group of beetles, were compared against expected frequencies in which beetles' selection of prey type to be first fed upon was random (each of the four prey types would be first fed upon during 25 % of bioassays).

5.3 Results

5.3.1 Data used in analyses

After mortality during the conditioning period, and exclusion of beetles that were inactive during bioassays, data from a total of 170 bioassays were used in the statistical analyses (Table 5.1).

Table 5.1 The number of replicate beetles from each of the five conditioning diet types that were used in statistical analyses.

Beetle sex	Conditioning diet type				
	D. reticulatum	*A. caliginosa*	*C. vomitoria*	*B. napus*	Cat food
Male	20	17	14	16	18
Female	20	15	12	20	18

5.3.2 Feeding among male and female *Pterostichus melanarius*

Generally, the feeding behaviour among male and female *P. melanarius* was similar. In comparing the duration spent feeding on prey types as a proportion of total duration spent feeding, for each combination of conditioning diet type and prey type fed upon during bioassays (Table 5.2), the only statistically significant difference was that between male and female beetles conditioned to a diet of *D. reticulatum*, and then feeding on *A. caliginosa* during bioassays (male = 14.7 %, female = 38.6 %). With only one difference out of 20 combinations, i.e. there was a significant difference among only 5 % of combinations, the decision was made to pool the data from male and female beetle bioassays for the remainder of the analyses.

Table 5.2 Mann-Whitney U-test values (after adjusting for ties) with their associated probability values for tests done to assess whether there was a difference between male and female *Pterostichus melanarius* in the duration they spent feeding on each prey type as a proportion of total time spent feeding.

Conditioning diet type	Prey type fed upon in bioassays							
	D. reticulatum		*A. caliginosa*		*C. vomitoria*		*B. napus*	
	U	P	U	P	U	P	U	P
D. reticulatum	185	0.520	131	0.030	143	0.103	197	0.729
A. caliginosa	93.0	0.122	123	1.000	87.5	0.088	108	0.416
C. vomitoria	83.5	0.996	73.0	0.569	72.5	0.537	77.0	1.000
B. napus	130	0.221	147	0.664	145	0.606	160	1.000
Cat food	151	0.680	146	0.599	141	0.488	153	1.000

5.3.3 Prey types fed upon over the duration of bioassays

Within each diet-conditioned group of beetles there was a highly significant difference (Table 5.3) in the mean feeding duration among the four prey types presented in the bioassays (Figure 5.1). Therefore, all groups exhibited a feeding preference among the prey types presented. Across all conditioning groups, the ranking (highest to lowest) of the prey eaten during bioassays was fairly consistent, and was *C. vomitoria*, *A. caliginosa*, *D. reticulatum*, and *B. napus*. Deviations from these rankings occurred in the groups of beetles conditioned to *C. vomitoria* and *B. napus*; within these two groups, the rankings of *C. vomitoria* and *A. caliginosa* were reversed.

Within the group of *P. melanarius* diet-conditioned to *C. vomitoria*, the mean duration spent feeding on *C. vomitoria* as a proportion of total duration spent feeding was 37.6 %, which was to all intents and purposes the same as the proportional mean duration spent feeding on *A. caliginosa* (38.6 %) by the same group of beetles. Therefore, within the group of beetles conditioned to *C. vomitoria*, the joint favoured prey type was that to which they had been conditioned. For the beetles diet-conditioned to *B. napus*, the reverse was true; those beetles did not feed at all (0 %) on the prey type to which they had been conditioned. *Pterostichus melanarius* conditioned to *D. reticulatum* fed on *D. reticulatum* for 18.2 % of the time they spent feeding, and beetles conditioned to *A. caliginosa* fed on *A. caliginosa* for 21.9 % of the time they spent feeding.

Therefore, the beetles within these two groups were neither favouring nor discriminating against the prey type to which they had been conditioned.

The feeding behaviour of the cat food 'control' group followed the general trend set by the beetles of the other diet-conditioned groups, in that the prey types of *C. vomitoria* and *A. caliginosa* were favoured, with *D. reticulatum* third choice, and *B. napus* the least favoured.

Table 5.3 Kruskal-Wallis test statistics (after adjusting for ties) for comparisons made within differently diet-conditioned groups of *Pterostichus melanarius*, on whether there was a difference in the mean duration spent feeding, as a proportion of total duration spent feeding, on each of the prey types presented in bioassays. Data for male and female beetles combined.

	Conditioning diet				
	D. reticulatum	*A. caliginosa*	*C. vomitoria*	*B. napus*	Cat food
H_3	17.0	17.9	16.6	28.7	36.5
P	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
n	40	32	26	36	36

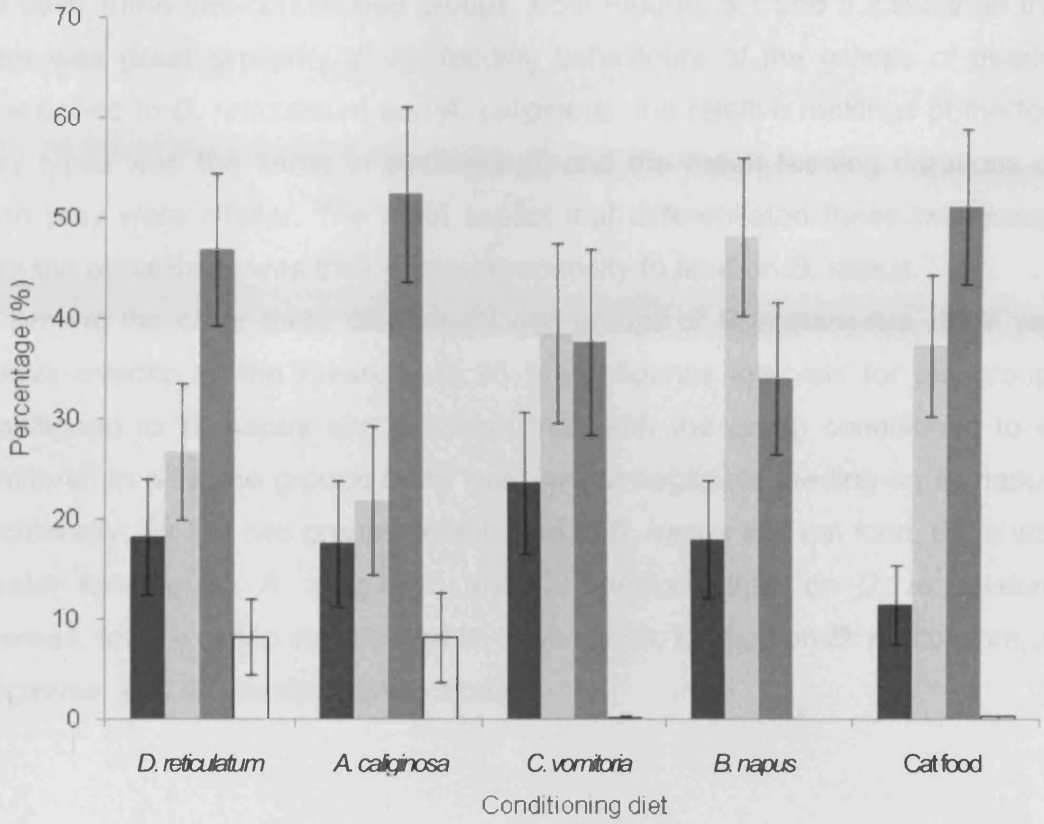
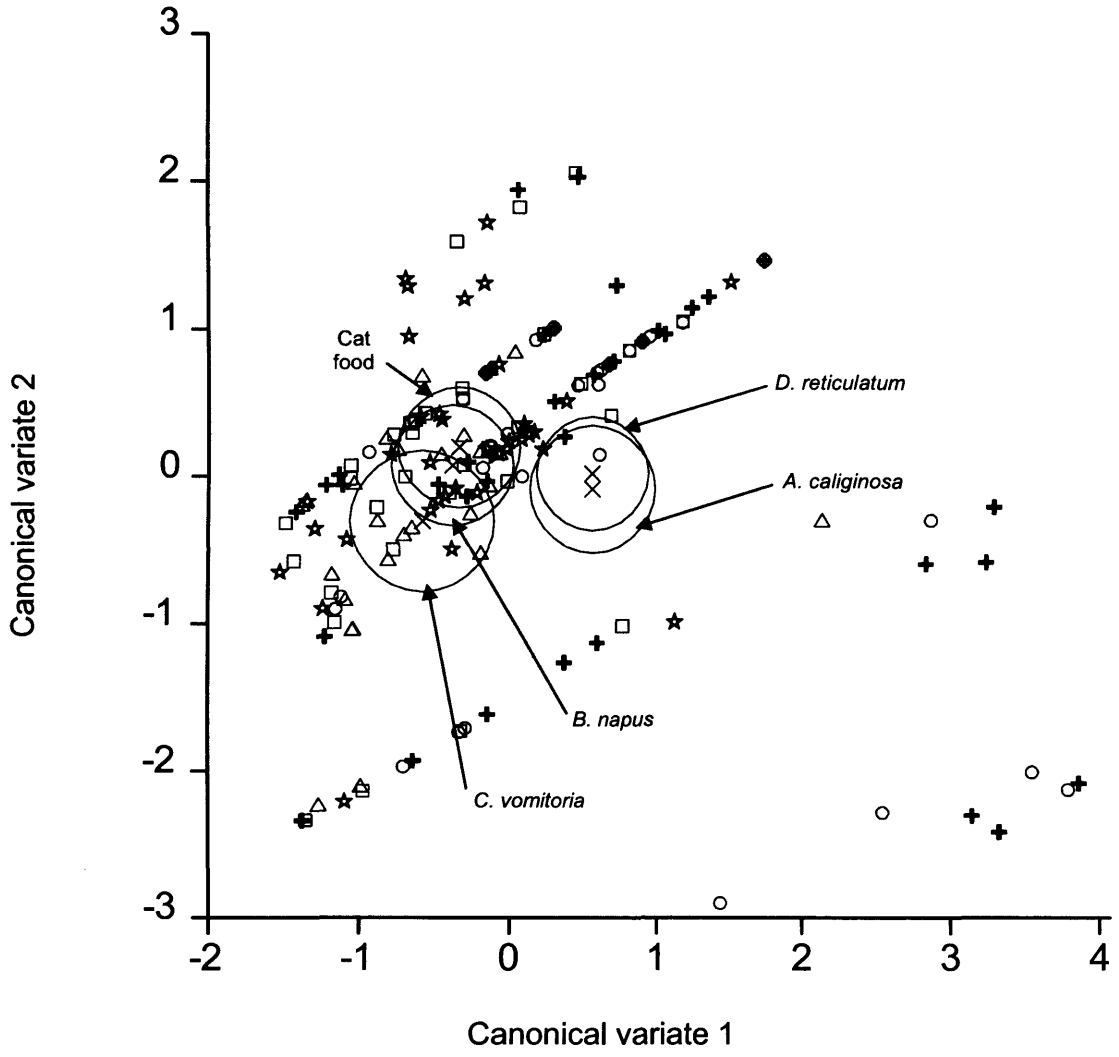


Figure 5.1 Mean (± SEM) feeding duration on individual prey items as a proportion of total duration spent feeding by *Pterostichus melanarius*. The mean duration was calculated by expressing the cumulative duration each beetle spent feeding on each of the prey types, as a proportion of total duration that beetle spent feeding, then averaging across all beetles (data for males and females combined).

A MANOVA identified that the there was a significant difference ($F_{16,480}$ = 2.76, P < 0.001) among the diet-conditioned groups of beetles in their feeding preference among the four prey types presented in bioassays, and confirmed there was not a significant difference among male and female beetles ($F_{4,157}$ = 1.32, P = 0.264), neither was there an interaction ($F_{16,480}$ = 0.79, P = 0.700). Thus, conditioning diet did have some effect on the subsequent food selection of *P. melanarius*. A CVA of the data on beetles' feeding behaviour, in which canonical variate 1 accounted for 87.2 % and canonical variate 2 accounted for 8.98 % of the variation in the data (Figure 5.2), illustrated that the main difference was that which distinguished the feeding behaviour of the two groups of beetles conditioned to *D. reticulatum* and *A. caliginosa* from that of beetles in

the other three diet-conditioned groups. Both Figures 5.1 and 5.2 illustrate that there was great similarity in the feeding behaviours of the groups of beetles conditioned to *D. reticulatum* and *A. caliginosa*; the relative rankings of the four prey types was the same in each group, and the mean feeding durations on each prey were similar. The main aspect that differentiated these two groups from the other three was their greater propensity to feed on *B. napus*.

Among the other three diet-conditioned groups of *P. melanarius*, there was greater overlap of the means and 95 % confidence intervals for the groups conditioned to *B. napus* and cat food, than with the group conditioned to *C. vomitoria*. In all three groups there was zero or negligible feeding on *B. napus*. Additionally, for the two groups conditioned to *B. napus* and cat food, there was greater feeding on *A. caliginosa* and *C. vomitoria* than on *D. reticulatum*; whereas, for the group conditioned to *C. vomitoria*, feeding on *D. reticulatum*, *A. caliginosa*, and *C. vomitoria* were similar.



Data points: + *D. reticulatum* ○ *A. caliginosa* Δ *C. vomitoria* □ *B. napus* ✲ Cat food

x mean canonical variate for each group

○ 95 % confidence interval for means

Figure 5.2 Canonical variates analysis two-dimension plot of data from all bioassays. The data input into the CVA were, on a per beetle basis, log-ratios of cumulative feeding duration on each of the prey items as proportions of non-prey-interaction duration. The grouping factor applied to the data was conditioning diet type.

5.3.4 Primary prey investigations

For *P. melanarius* diet-conditioned to *D. reticulatum*, *A. caliginosa*, and *C. vomitoria*, the differences between observed and expected frequencies of prey types subjected to primary investigation were not significantly different from

random (Table 5.4). Therefore, these groups of beetles did not significantly discriminate among prey types during primary investigation (Figure 5.3). Generally, beetles within these three groups were less likely to make *B. napus* the subject of their primary investigation. Additionally, the group conditioned to *A. caliginosa* made *C. vomitoria* the subject of primary investigation more frequently than the other three prey items. *Pterostichus melanarius* diet-conditioned to *B. napus* appeared to discriminate among prey types. The beetles in this group made their primary investigation on *B. napus* significantly less frequently than on the other three prey types. Therefore, they discriminated against the prey type to which they had been conditioned. None of the other diet-conditioned groups discriminated against the prey to which they had been conditioned. The trend among the cat food conditioned beetles followed the general trend set by the groups of beetles conditioned to *D. reticulatum*, *A. caliginosa*, and *C. vomitoria*; beetles made primary investigations of *D. reticulatum*, *A. caliginosa*, and *C. vomitoria* most frequently, and *B. napus* least frequently, but the observed frequencies were not significantly different to the expected random frequencies.

Table 5.4 Chi-square goodness-of-fit test statistics for comparisons made within differently diet-conditioned groups of *Pterostichus melanarius*, on the frequencies of bioassays in which prey types were the subject of primary investigation. Data for male and female beetles combined.

	Conditioning diet				
	D. reticulatum	*A. caliginosa*	*C. vomitoria*	*B. napus*	Cat food
χ^2_3	2.03	6.67	6.04	14.2	5.44
P	0.567	0.083	0.110	0.003	0.142

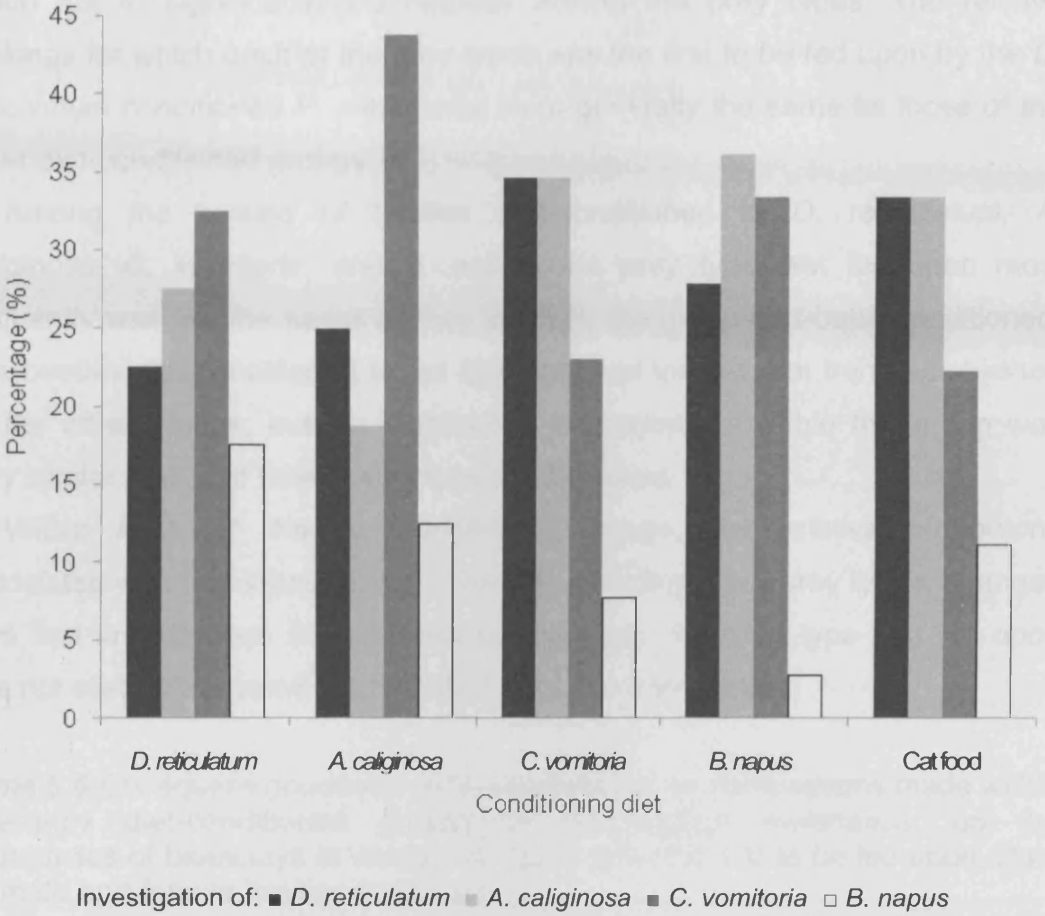


Figure 5.3 Comparison within differently diet-conditioned groups of *Pterostichus melanarius*, on the percentage of bioassays in which prey types were the subject of primary investigation. Data for male and female beetles combined.

5.3.5 Primary feeding events

In all groups of diet-conditioned beetles, with the exception of the group conditioned to *D. reticulatum*, there were significant differences between observed and expected frequencies of occasions on which each prey type was the first to be fed upon (Table 5.5). Therefore, these groups of diet-conditioned beetles discriminated among prey types presented in bioassays in terms of the first to be fed upon (Figure 5.4). They were consistent in selecting to first feed upon *B. napus* least frequently. In terms of relative rankings, the occurrences of primary feeding upon the other three prey types was fairly consistent across the groups, with the exception of the group diet-conditioned to *A. caliginosa*, which exhibited a greater than expected propensity for primary feeding on *C. vomitoria*. *Pterostichus melanarius* conditioned to *D. reticulatum* were the only

group not to significantly discriminate among the prey types. The relative rankings for which each of the prey types was the first to be fed upon by the *D. reticulatum* conditioned *P. melanarius* were generally the same as those of the other diet-conditioned groups.

Among the groups of beetles diet-conditioned to *D. reticulatum*, *A. caliginosa*, *C. vomitoria*, and *B. napus*, the prey type first fed upon most frequently was not the same as that to which the group had been conditioned. The beetles diet-conditioned to cat food followed the general trend established by the other groups, but the distribution of proportions within this group was very similar to that of those conditioned to *B. napus*.

Within each of the diet-conditioned groups, the relative proportions associated with first investigation of, and first feeding upon prey types, changed from first investigation to first feeding. Therefore, the prey type first fed upon was not always the same as that which was first investigated.

Table 5.5 Chi-square goodness-of-fit test statistics for comparisons made within differently diet-conditioned groups of *Pterostichus melanarius*, on the frequencies of bioassays in which prey types were the first to be fed upon. Data for male and female beetles combined.

	Conditioning diet				
	D. reticulatum	*A. caliginosa*	*C. vomitoria*	*B. napus*	Cat food
χ^2_3	6.21	10.1	9.23	22.2	21.2
P	0.102	0.018	0.026	< 0.001	<0.001

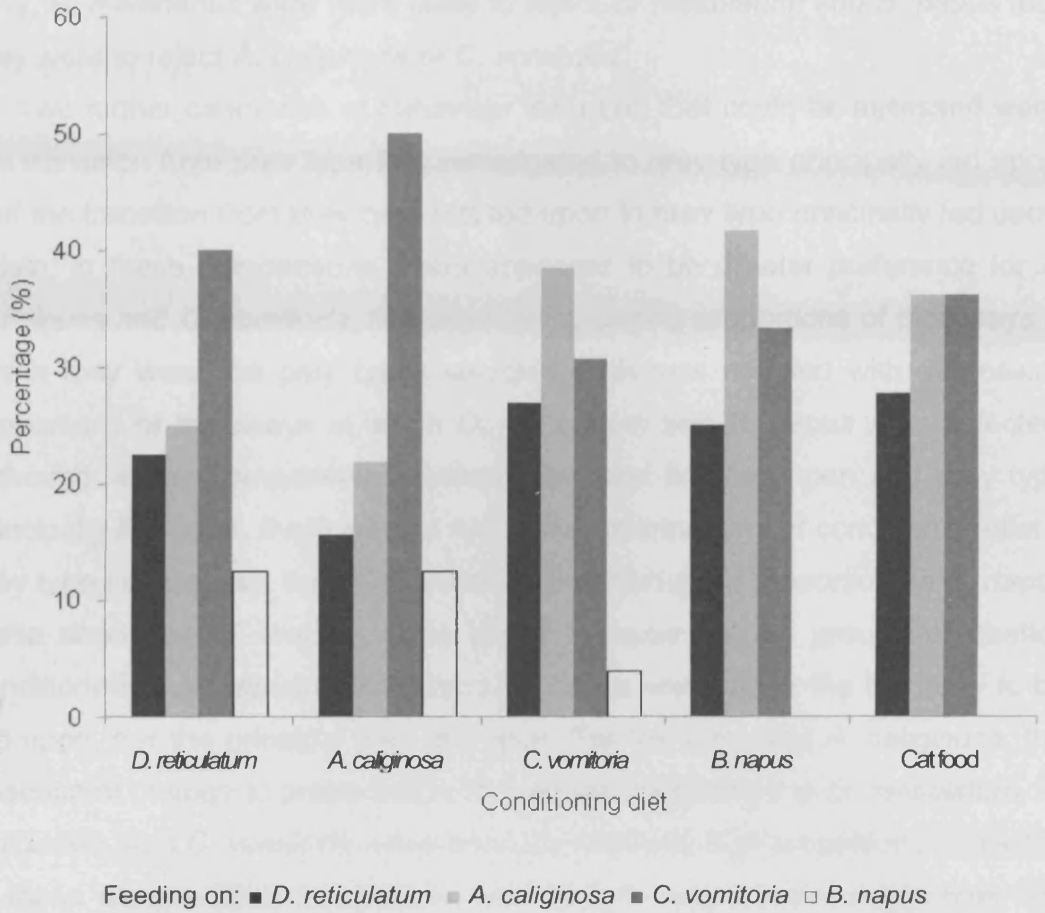


Figure 5.4 Comparison within differently diet-conditioned groups of *Pterostichus melanarius*, on the percentage of bioassays in which prey types were the first to be fed upon. Data for male and female beetles combined.

5.3.6 Synthesis

Within each of the diet-conditioned groups of beetles, a fairly consistent difference was observable for the prey types of *D. reticulatum* and *B. napus*, when comparing the data for prey type first investigated (Figure 5.3), with that for the subsequent behaviour of prey first fed upon (Figure 5.4). The relative proportions of bioassays in which these two prey types were the subject of primary investigation were generally higher than the relative proportions of bioassays in which they were the first to be fed upon. The reverse relationship applied to the prey types of *A. caliginosa* and *C. vomitoria*; there was an increase in the relative proportion from primary investigation to being the first prey fed upon for these prey types (Table 5.6). Therefore, upon investigation of

prey, *P. melanarius* were more likely to reject *D. reticulatum* and *B. napus* than they were to reject *A. caliginosa* or *C. vomitoria*.

Two further categories of behaviour transition that could be assessed were: the transition from prey type first investigated to prey type principally fed upon, and the transition from prey type first fed upon to prey type principally fed upon. Again, in these comparisons, there appeared to be greater preference for *A. caliginosa* and *C. vomitoria*, indicated by increasing proportions of bioassays in which they were the prey types selected. This was coupled with decreasing proportions of bioassays in which *D. reticulatum* and *B. napus* were selected. Although, in the comparison between prey type first fed upon and prey type principally fed upon, there were a few more combinations of conditioning-diet – prey type in bioassay, for which there was no change in proportion. In *B. napus* these absences of change came about because for the groups of beetles conditioned to *B. napus* and cat food, *B. napus* were never the first prey to be fed upon, nor the principal prey fed upon. For the prey type *A. caliginosa*, the absence of change in proportion in the groups conditioned to *D. reticulatum*, *A. caliginosa*, and *C. vomitoria* arose because relatively high proportions of beetles in those groups (25.0 %, 21.9 %, and 38.5 % respectively) made their first feeding events upon *A. caliginosa* and continued to feed upon it throughout the majority of the respective bioassays.

Table 5.6 Summary of the changes in prey types selected by groups of differently diet-conditioned *Pterostichus melanarius*, according to the three key behavioural processes of: prey type first investigated, prey type first fed upon, and prey type principally fed upon (for a given beetle, this was the prey type upon which the longest cumulative feeding duration occurred). Within each group of diet-conditioned beetles, values were calculated for the relative proportion of bioassays in which each of the prey types presented in bioassays was the: first investigated (θ), first fed upon (ξ), and principally fed upon (φ). Changes in proportions between the three key behavioural processes were then calculated as follows: (a) ξ - θ, (b) φ - θ, and (c) φ - ξ.

▲ Proportion increase ▼ Proportion decrease — No change in proportion

Prey item in bioassay	Conditioning diet: *D. reticulatum*	*A. caliginosa*	*C. vomitoria*	*B. napus*	Cat food
(a) Change in proportion between prey type first investigated and first fed upon					
D. reticulatum	—	▼	▼	▼	▼
A. caliginosa	▼	▲	▲	▲	▲
C. vomitoria	▲	▲	▲	—	▲
B. napus	▼	—	▼	▼	▼
(b) Change in proportion between prey type first investigated and principally fed upon					
D. reticulatum	▼	▼	▼	▼	▼
A. caliginosa	▼	▲	▲	▲	▲
C. vomitoria	▲	▲	▲	▲	▲
B. napus	▼	▼	▼	▼	▼
(c) Change in proportion between prey type first fed upon and principally fed upon					
D. reticulatum	▼	▲	▼	▼	▼
A. caliginosa	—	—	—	▲	▲
C. vomitoria	▲	▲	▲	▲	▲
B. napus	▼	▼	▼	—	—

5.4 Discussion

Pterostichus melanarius exhibited a feeding preference hierarchy among the prey types presented in bioassays that was generally consistent across all diet-conditioned groups. This suggests that, broadly, *P. melanarius* favours certain prey types over others, irrespective of the feeding histories of beetles. Nevertheless, MANOVA and CVA identified that there were significant differences in feeding behaviour among the diet-conditioned groups, suggesting that *P. melanarius* discriminates among prey according to its previous feeding history. Therefore, feeding histories did affect prey selection, but within the framework of the afore-mentioned overarching prey preference hierarchy.

During the introduction to this Chapter it was suggested that there is a potential dichotomy between reports that generalists select among prey for quality and variety, and that generalist predators remain in an area searching for more of the same prey. The two sets of behaviours may be mutually exclusive (Mayntz *et al.*, 2005). Selection among prey may be limited to certain situations: when there are significant differences in quality among the prey types available (Toft and Wise, 1999b), or at critical life history stages (Raubenheimer *et al.*, 2007). At other times, and for prey that are generally nutritionally complete, selection among prey might not be important. Then, generalist arthropod predators may optimize for rate of prey capture (e.g. MacArthur and Pianka, 1966), and feed on a restricted number of prey species. In this sense, *D. reticulatum*, as well as *A. caliginosa*, and *C. vomitoria*, might all be considered nutritionally complete for *P. melanarius*, because they were not selected against by conditioned beetles.

Pterostichus melanarius that had fed solely on *D. reticulatum* for five weeks were just as likely to feed on *D. reticulatum* in bioassays as were those beetles that had been conditioned to the other prey types. Therefore, after feeding on *D. reticulatum*, *P. melanarius* do not always then seek to feed on something different. This is an encouraging result in terms of the utility of *P. melanarius* as an agent of slug pest control, particularly since slugs tend to aggregate (Hunter, 1966; Bohan *et al.*, 2000a, b), and generalist predators tend to remain in areas

of aggregated prey (Den Boer, 1982; Mols, 1993). Additionally, opportunistic feeding on slug carrion by *P. melanarius* should not then make individuals discriminate against subsequent feeding on live slug prey.

It has been reported that generalist predators may seek to feed on a range of prey types in order to maximize the overall quality of their diet (Toft, 1996), or they may select among prey in order to redress imbalances in amino acids (Greenstone, 1979), and protein:lipid ratios (Mayntz *et al.*, 2005; Raubenheimer *et al.*, 2007). The causes of imbalances may be attributed to feeding history, or life history events such as diapause. Thus, it may be suggested that, for individuals at similar stages of life history, when a choice of prey is available, those that have a history of feeding on good quality food may be more likely to accept a wider variety of prey types that includes lower quality prey (Toft and Wise, 1999a). Conversely, those individuals that have previously fed on lower quality food may be more selective; selecting for higher quality prey. On the basis of this principle, it may be suggested that *D. reticulatum* and *A. caliginosa* are higher quality prey types than *C. vomitoria*, *B. napus*, and cat food, because the beetles that had been diet-conditioned to the former two prey types were more likely to feed on a wider range of prey types; they fed on *B. napus* when the other groups exhibited zero or negligible feeding on *B. napus*. Additionally, the beetles conditioned to *D. reticulatum* were the only group not to make a significant distinction among prey types in prey type first fed upon. Harwood *et al.* (2009) reported there to be no difference in mass among *P. melanarius* fed solely on slugs, earthworms, or Diptera for a period of eight weeks, but all were more massive than beetles fed on aphids. Additionally, females fed earthworms produced significantly more eggs than females fed any of the other diets, thus, confirming slug, and particularly earthworm, to be higher quality diets. Toft and Bilde (2002) also suggested that slugs and snails should be high quality food for carabids. As might be expected of putative high quality prey, both slug (Ayre, 1995; Ayre and Port, 1996; Symondson *et al.,* 1996; Bohan *et al.*, 2000a; Dodd, 2004), and earthworm (Symondson *et al.*, 2000; Harper *et al.*, 2005; King *et al.* 2010) tissue are consumed by *P. melanarius* in the field.

Although it can be suggested that *D. reticulatum* and *A. caliginosa* might be relatively high quality foods for *P. melanarius*, it must be noted that there appear to be anomalies surrounding the behaviours of beetles in regard to *D.*

reticulatum. Firstly, all diet-conditioned groups ranked *D. reticulatum* only third in order of preference among the four prey types on offer. Secondly, *D. reticulatum* was often rejected after being first investigated or first fed upon.

As *D. reticulatum* was often rejected after first investigation and first feeding, so the same was true for *B. napus* seeds. It has been reported that *P. melanarius* and other carabids will feed on seeds in the field as well as in the laboratory (e.g. Honek *et al.*, 2003; Koprdova *et al.*, 2008), but based on the assumption that the overriding selection criteria of beetles was food quality, it may be inferred from the behaviours of beetles, that *B. napus* seeds are a relatively low quality food resource. Therefore, it is difficult to reconcile the similarities in behavioural responses by *P. melanarius* towards the two foods, with the purported differential quality between them.

Laboratory based trials have reported mixed diets to be beneficial for generalists (Wallin *et al.*, 1992; Harwood *et al.*, 2009), but in the reality of the arable field environment, when predators may be in a general state of hunger (Sunderland, 1975; Chapter 6) due to low availability of prey (Van Dijk, 1982, 1996), selection for diversity seems an unlikely event. Indeed, during this experiment, many beetles fed on the same prey to which they had been conditioned rather than seeking diversity.

The propensity of *P. melanarius* to consume *B. napus* seeds in the presence of animal prey types exemplifies why *P. melanarius* really is deserving of the epithet 'ultra-generalist', and illustrates why *P. melanarius* might not be relied upon to predate heavily upon slugs. However, the low level of feeding on *B. napus* seeds during bioassays suggests low preference for this type of food. Nevertheless, the ability to feed on a wide range of food types, even those of low quality, may be beneficial for generalist predators. Toft (1995) reported that, for a generalist predator whose diet otherwise consisted of one prey type only, the addition of a small quantity of low quality aphid prey increased egg hatching rate. This ability of *P. melanarius* to utilize such a wide range of prey types can also be beneficial for the purposes of pest control. Preying upon various pest taxa may assist in the control of these other pests: e.g. preying upon *B. napus* seeds may reduce the emergence of volunteers in the subsequent crop in a rotation (Koprdova *et al.*, 2008). Additionally, alternative prey can help to sustain a generalist predator population during periods when preferred prey

may be at low density (Symondson *et al.*, 2000). In this way, the predator population size may remain relatively large, and may then be more effective in controlling an emerging pest population (Symondson *et al.*, 2002b).

Beetles conditioned to the *D. reticulatum*, *A. caliginosa*, *C. vomitoria*, and cat food diets did not exhibit significant preferences among prey types primarily investigated. There were, however, significant differences among the prey primarily investigated by the beetles conditioned to *B. napus*. Those beetles were significantly less likely to primarily investigate *B. napus*, the food to which they had been conditioned. In order for those beetles to be able to positively discriminate against a food type suggests that *P. melanarius* were, to some extent, able to detect the prey items without physically contacting them. Olfactory response to *B. napus* has been reported in non-Carabidae Coleoptera (e.g. Blight *et al.*, 1995). Alternatively, beetles were able to detect by chemoreception the *D. reticulatum*, *A. caliginosa*, and *C. vomitoria* prey types, and were attracted to these, but could not detect the *B. napus*, and so did not exhibit attraction to the seeds. The second scenario seems less likely because beetles from the other diet conditioned groups investigated the seeds. Any chemoreception of the other prey types was not discriminated for or against by beetles that had been conditioned to those other prey types.

In this experiment, current prey selection was being tested in adult beetles that had been diet-conditioned for five weeks. However, the importance of feeding history may go back further than that which occurred in the adult stage. Feeding during the larval stage has been reported to carry over effects into adulthood. Adult body mass was reported to be mainly determined by feeding during the larval stage (Nelemans, 1987; Van Dijk, 1982, 1994; Fernandez-Montraveta and Moya-Larano, 2007). Additionally, differential nutritional requirements catalysed by life history events may arise during discrete periods within the adult stage (Raubenheimer *et al.*, 2007). Emergence of the entire adult population can occur over a protracted period (Ericson, 1977; Matalin, 2006), but beetles used in this experiment were caught during the middle of July. Therefore, some individuals had potentially been active as adults since the end of May, but others might have emerged much later, into June or perhaps even July. Additionally, some individuals were likely to have been members of the cohort that overwintered as adults. Thus, the potential exists for longer term

feeding history effects, which occurred before capture, to have differentially influenced the behaviours of beetles during bioassays. Experimentation avoiding such confounding effects would necessitate beetles being reared under controlled conditions from the egg stage. However, since the feeding history of adult females might have effects on egg production, hatching success rate, and juvenile size (Wallin *et al.*, 1992; Toft, 1995), bioassays conducted on third generation captive beetles would be preferable.

Beetles conditioned to cat food behaved broadly in accordance with those that had fed on 'natural' prey types. Hence, rearing *P. melanarius* under controlled conditions on a diet of cat food should not induce feeding behaviours that are extraordinary. Therefore, acceptability in the use of cat food as an easily accessible food source in the rearing of *P. melanarius* is highlighted.

Chapter 6 -

Slug control by *Pterostichus melanarius* in an arable field environment

6.1 Introduction

Since it was hypothesized that *Pterostichus melanarius* could potentially have negative effects on slug populations (Symondson *et al.*, 1996), research interest in this generalist predator has grown. The body of research has since provided a great deal of useful data in many areas of the ecology surrounding this interspecific relationship. However, synthesis of these data does not lead to a clear indication of the field scale efficacy of *P. melanarius* as a slug predator, nor the extent to which predation on slugs may occur. Ultimately, the utility of this generalist predator in contributing to integrated pest management programmes targeted at slug populations remains unclear.

A review by Symondson *et al.* (2002b) emphasized that generalist predators can be highly effective pest control agents, and cited examples where control had occurred. Indeed, taken in isolation, various works on *P. melanarius* suggest apparently strong evidence for slug predation and slug population control, although counter arguments can be made on the interpretation of data or methodologies utilized. Other studies have suggested a weak trophic link between *P. melanarius* and slugs, and lack of controlling effects on slug population size. The following is a brief summary of some of the main works, and highlights the ambiguity in current understanding of the trophic interaction.

Crucially, *P. melanarius* does consume slug tissue in the field environment. Slug remains have been detected in the guts of field-caught beetles (Ayre, 1995; Ayre and Port, 1996; Symondson *et al.,* 1996; Bohan *et al.*, 2000a; Dodd, 2004; Bell *et al.*, 2010), but importantly, predation cannot be confirmed absolutely, because detection methods cannot distinguish among predated, secondarily predated and scavenged material (Harwood *et al.,* 2001a; Foltan *et al.*, 2005; Sheppard *et al.*, 2005). Symondson *et al.* (2002a) argued for the existence of very strong linkages between the two taxa; they reported that a dynamic relationship existed between the populations of *P. melanarius* and its slug prey over a five year period. Slugs were suggested to be of such importance as prey for *P. melanarius*, that the numbers of beetles were dependent upon the numbers of slugs. Additionally, the between-year

population growth in slugs was suggested to be dependent upon predation by *P. melanarius*. However, tests for the presence of slug tissue in the guts of beetles was performed only on beetles caught during the first year (Symondson *et al.*, 1996), therefore, the actual extent of predation on slugs by *P. melanarius* during the remaining four years could not be confirmed. Bohan *et al.* (2000a) reported coincident spatial aggregations of slugs and *P. melanarius*, and beetles with the greatest foregut masses were spatially associated with slugs (Symondson, 2002a; Armsworth, 2005). However, Mair *et al.* (2001) repudiated the findings of Bohan *et al.* (2000a) (response to the repudiation in Bohan *et al.*, 2001), for reasons of methodology and interpretation of results. One area of contention was the use of environmental data, and the rejection of effect by environmental factors in driving the distributions of slugs and beetles. Favourable environmental or microclimate conditions that may have been responsible for the aggregations of slugs may have also produced aggregations of other invertebrates. Indeed, it has been reported that primary consumers are highly aggregated (Bell *et al.*, 2010). Aggregated prey communities may attract aggregations of predators, including *P. melanarius* (Bryan and Wratten, 1984; Harwood *et al.*, 2001b; Winder *et al.*, 2001, 2005; Sunderland, 2002). Additionally, slug carrion may be aggregated coincidently with live slugs, and be an attractant for *P. melanarius*. The antennae of *P. melanarius* were reported to respond to decaying dead slugs (McKemey *et al.*, 2004), but beetles were unable to detect live or 12 h-decayed dead *D. reticulatum* by olfaction (Chapter 4). *Pterostichus melanarius* was reported to be unable to detect the mucus of *Arion subfuscus* (Wheater, 1989), but could detect the mucus of *Deroceras reticulatum* (McKemey *et al.*, 2004). *Deroceras reticulatum* tentacles responded to volatile chemicals emitted from *P. melanarius* (Dodds, 1997), and avoided areas in test arenas over which *P. melanarius* had walked (Armsworth, 2005; Armsworth *et al.*, 2005). These observations can be interpreted to suggest that predation by *P. melanarius* on slugs might be significant enough to have selected for this predator avoidance behaviour. *Pterostichus melanarius* reduced slug population size in mini-plots when alternative prey were absent (McKemey *et al.*, 2003; Symondson *et al.*, 2006), but in the presence of alternative prey, *P. melanarius* did not have a significant effect on slug population size (Symondson *et al.*, 2006). These observations suggest that

slugs are an acceptable food item, but live slugs may not feature highly in a hierarchy of potential food types for *P. melanarius* (Chapter 2). Slug defence mechanisms may be a significant factor influencing the feeding behaviour of *P. melanarius* (Foltan, 2004; Chapter 2). Additionally, *P. melanarius* may be limited to preying mainly on small slugs (McKemey *et al.*, 2001), but may experience greater difficulty in locating small slugs in field environments (Symondson *et al.*, 2006). Slug carrion may be a significant source of food for *P. melanarius*. Beetles preferentially fed on dead prey rather than live prey (Foltan *et al.*, 2005), the antennae of adults responded to decaying dead, but not live slugs (McKemey *et al.*, 2004), and higher densities of *P. melanarius* were observed in areas baited with dead slugs (Foltan *et al.*, 2005). The propensity of a predator to scavenge has the potential to reduce its utility as a pest controller, if scavenged food is eaten in place of prey that would otherwise be killed by the predator. However, the potential also exists for carrion to act as a mechanism to attract predators to aggregations of live prey organisms. The extent to which scavenging on slug carrion affects *P. melanarius* slug-consumption rate estimates in-field (Sunderland, 1996) remains unknown (Foltan *et al.*, 2005).

When these works are viewed as a whole, they appear to show inconsistency in terms of understanding whether *P. melanarius* might exert significant predation pressure on field populations of slugs. Therefore, the aim of this experiment was to apply a direct approach to the problem, and test the ability of *P. melanarius* to exert controlling effects on slug populations through predation pressure, whilst in the presence of naturally occurring alternative prey, at the semi-field scale. This was achieved through the employment of a large scale manipulation experiment in a field of winter wheat, over a period of four months. The expectation, and null hypothesis tested, was that *P. melanarius* would have no significant controlling effect on slug numbers.

6.2 Methods

6.2.1 Experimental design, and set-up of the field experiment

Experimentation was conducted in West Barnfield on Rothamsted Farm. The field is well-drained with a clay soil. Following in rotation from oilseed rape, on 30 September 2007 the field was sown with winter wheat. During the period from the oilseed rape harvest (14 July 2007), until completion of the experiment, typical commercial applications of herbicides, fungicides, and fertilizers were made. Molluscicide was applied on 4 October 2007, in the form of 5 % w/w metaldehyde (Pathfinder Excel: Barclay Chemicals Manufacturing Ltd.) at a rate of 5.0 $kg.ha^{-1}$. No insecticides were applied.

On 21 April 2008, 120 experimental enclosures were erected (Plates 6.1, 6.2, and 6.3). The enclosures measured 3 m x 3 m and were bounded by partially buried polythene barriers (Polybags Ltd.) 30 cm above and below ground. Wooden posts positioned at each corner, along with a thin rope that was threaded between the double layered polythene and stapled to the posts, served to maintain an upright aspect to the barriers. At enclosure corners, the polythene was stapled to the wooden posts in order to maintain the integrity of enclosures, and thus prevent movement into, or out of enclosures by *P. melanarius*. The barriers were not expected to prevent slugs from climbing into or out of the enclosures. However, it was considered that transgressions would be of relatively low frequency given the limited dispersion in slugs (South, 1965, 1992; Fleming, 1989; Armsworth, 2005), and the in- and out-flows of slugs would be approximately equal.

On 2 June 2008, four dry pitfall traps (diameter = 6 cm, depth = 7.5 cm) were set-out around the internal perimeter of each enclosure; one trap was placed approximately mid-way along each of the enclosure sides. An up-turned 14 cm diameter flower pot holder, raised approximately 5 cm above the ground on metal wire supports, was placed over each pitfall trap to act as a rain cover and prevent pitfall traps filling with rain water. Initially, the purpose of the traps

was to capture all *P. melanarius* for removal from the enclosures. Pitfall traps were set-out in other fields on the farm in order to provide additional *P. melanarius* to be used in the experiment. Pitfall traps were emptied daily; the *P. melanarius* caught were maintained under the same controlled environment conditions as those described in section 2.2, and fed cat food *ab libitum*. All other invertebrates caught in pitfall traps were returned to their respective enclosures or fields. Pitfall trapping ceased after 19 days. At that time, *P. melanarius* were still being caught in the pitfall traps inside enclosures, although in smaller numbers than at the beginning of the trapping period. Therefore, it was acknowledged that the enclosures were not cleared of *P. melanarius*, but cessation of trapping was necessary in order to allow sufficient duration over which the experiment could be run.

After the cessation of pitfall trapping, on 1 July 2008, the experimental enclosures were augmented with *P. melanarius* in four treatment levels: zero *P. melanarius*, one *P. melanarius* m^{-2}, two *P. melanarius* m^{-2}, and four *P. melanarius* m^{-2}. It was expected that attempted removal of all *P. melanarius* from the enclosures was not 100 % successful, and that some would still be present in the enclosures at the time of *P. melanarius* augmentation. Therefore, augmented beetles were marked in order that upon recapture they could be distinguished, and the total number of *P. melanarius* present in each enclosure at the start of the experiment could be estimated. Two marks were made on each beetle: one white dot (white nail varnish, Boots p.l.c.) and one yellow dot (#1166 luminous marking paint, BioQuip Products Inc.).

A randomized block design was employed, and enclosures were arranged in five replicate blocks, with each block containing 24 enclosures (four beetle density treatment levels x six sampling time periods). This number of enclosures was required within each block because soil sampling for slugs is destructive, therefore, a separate enclosure was required for each treatment at every sampling time point.

Plate 6.1 One hundred and twenty polyethylene-barriered enclosures on 9 May 2008, 18 days after installation in a field of winter wheat (West Barnfield, Rothamsted Research). Enclosures measured 3 m x 3 m, and were separated by walkways (minimum width approximately 1.5 m) on all sides. Barrier height was 60 cm, of which 30 cm was above ground, and 30 cm was below ground.

Plate 6.2 A single 3m x 3m polyethylene-barriered enclosure on 9 May 2008, 18 days after installation in a field of winter wheat (West Barnfield, Rothamsted Research). Barrier height was 60 cm, of which 30 cm was above ground, and 30 cm was below ground.

Plate 6.3 A single 3 m x 3 m polyethylene-barriered enclosure on 14 June 2008, 54 days after installation in a field of winter wheat (West Barnfield, Rothamsted Research). The crop shows good recovery from disturbance caused during insertion of the barriers.

6.2.2 Collection of field samples

Sampling of invertebrates from enclosures was conducted six times during the experiment; firstly, at the time of *P. melanarius* augmentation, and then at 13 day intervals thereafter (Table 6.1).

Table 6.1 Dates at which sampling of enclosures occurred. The date given was the first day of the sampling effort, and the time elapsed is relative to the day on which the augmentation of *Pterostichus melanarius* occurred.

Sample number	Date of sample	Time elapsed (days)
1	02 July 2008	1
2	15 July 2008	14
3	28 July 2008	27
4	10 August 2008	40
5	23 August 2008	53
6	05 September 2008	66

Sampling for slugs occurred on the first three days of each sampling time period. Slugs were sampled using the mustard extraction method (Paulson-

Lawrence and Bowers, 2002). Samples were taken from an area of soil bound by a 50 cm x 50 cm quadrat. After checking for the presence of slugs, and collecting them as a part of the sample when present, the above-ground crop biomass was removed. Then, 6 l of mustard solution (50 g of mustard powder (R.M. Curtis & Co. Ltd.) dissolved in 6 l of tap water) was applied to the area of soil enclosed by the quadrat. All slugs, earthworms and soil-dwelling larvae that emerged within 10 min were collected. Each enclosure was sub-sampled using three randomly located quadrats. The sub-samples were pooled to provide one sample per enclosure. Upon return to the laboratory, organisms were stored in 70 % ethyl alcohol solution. Later, organisms were counted and identified as, *Deroceras* spp., *Arion* spp., earthworm, or larvae. Slugs were also weighed individually.

Pterostichus melanarius were sampled by dry pitfall trapping over a period of six days and nights. Pitfall traps that had previously been installed inside enclosures for the initial removal of *P. melanarius* from enclosures were re-used. Traps were opened on the day following completion of soil sampling, and emptied every morning (mean time approximately 10:30 am) during the sampling period. *Pterostichus melanarius* were weighed and sexed, then frozen at -43 °C immediately after capture to halt metabolism, and facilitate molecular analysis of beetle gut contents. Other organisms that were caught in pitfall traps were counted.

6.2.3 Molecular analysis on the foregut contents of field-caught *Pterostichus melanarius*

The processes of dissection-out of beetles' foreguts, DNA extraction from foreguts, PCR amplification of template DNA, and visualization of PCR products were conducted in the same way as was described in section 3.2.3. Slugs from the generas *Deroceras* and *Arion* are known to be present on Rothamsted Farm, therefore, aliquots from each sample were subjected to two singleplex PCR runs. In the first run, the DR11F and DR50R primer combination was used to test for the presence of *Deroceras* spp. DNA. The primer combination amplifies DNA from both *D. reticulatum* and *D. panormitanum* (Dodd, 2004). The second run used a general *Arion* primer combination of: Ai1F (5'-

CACATAAATGATAGTCACC-3') and AR2R (5'-ATACTTACAAGTCCATCTTT-3') that amplifies a 204 – 221 bp region of the mitochondrial 12S rRNA gene sequence (Dodd, 2004). The general *Arion* primers amplify the DNA of: *A. hortensis, A. distinctus, A. owenii, A. intermedius, A. silvaticus, A. circumscriptus, A. fasciatus, A. subfuscus, A. flagellus, A. lusitanicus* and *A. ater*. Between them, the two primer combinations would amplify DNA from all species known to be present on the farm. Cycling parameters for the *Arion* spp. primer combination were: 95 °C for 15 min, then 42 cycles of 94 °C for 30 s, 57 °C for 90 s, 72 °C for 90 s, and then a final extension of 72 °C for 10 min.

Samples that were slug DNA-negative after tests for *Deroceras* spp. and *Arion* spp. were tested in a third round of PCRs, using the general invertebrate DNA primer combination of LCO1490 and HCO2198, to check that the DNA extraction process had been successful.

6.2.4 Statistical analysis

6.2.4.1 *Pterostichus melanarius* and its interspecific effects

Initial analysis of beetle numbers showed that the beetle augmentation failed to yield the intended discrete treatment levels, due to continued beetle emergence after the cessation of the trapping-out effort. The mean densities of *P. melanarius* per enclosure, observed at sampling time two (the first sample taken post beetle augmentation), were not the expected mean densities (Table 6.2); there was no significant difference (H_3 = 1.17, P = 0.761, n = 20) in the mean numbers of beetles per enclosure among the four treatment levels. This observation revealed that the experimental design had been compromised, because the numbers of *P. melanarius* per enclosure were not present in four discrete treatment levels. Additionally, none of the beetles captured bore painted markings.

Table 6.2 The expected (augmented number), observed mean ± SEM, and range for the number of *Pterostichus melanarius* per enclosure (9 m^2) at sampling time period two. This was the first sampling event to take place after augmentation of *P. melanarius* had occurred.

Expected	Mean ± SEM	Range	
		Lowest	Highest
0	38 ± 8	22	67
9	40 ± 10	11	65
18	34 ± 9	17	63
36	29 ± 6	17	51

As a consequence of the absence of treatment levels, the blocking and treatment structure were ignored in all analyses. The analyses were, therefore, performed on 20 replicate enclosures at each sampling time period.

Pitfall trap and soil sampling provided estimates of *P. melanarius* activity-density, slug density, and abundance of alternative prey (all organisms other than *P. melanarius* and slugs that were obtained in the sampling effort) per enclosure. Changes in the magnitudes of these measurements through time were tested using one-way ANOVA. Data for *P. melanarius* and slugs were Log_{10} transformed to stabilize variance before being analysed.

Growth in density (G) was calculated by dividing the mean count of individuals per enclosure at a given time period (n_t) by the count of individuals per enclosure at the previous time period (n_{t-1}), and then taking the log_{10} of the resulting value, i.e.:

$$G = \log_{10}(n_t/n_{t-1})$$

Generalized linear modelling (McCullagh and Nelder, 1989) was used to test for relationships among the activity-density of *P. melanarius*, density of slugs, and abundance of alternative prey. Generalized linear modelling was used because normality of data is not implicit, and data do not have to be continuous. Response variable data were counts of individuals, therefore, each GLM was fitted utilizing the Poisson distribution and logarithm link function. Models were fitted using terms for the explanatory variable and time, and their interaction. Where applicable, non-significant terms were removed sequentially, and models refitted, until all terms in models were significant.

6.2.4.2 Molecular evidence for predation or scavenging on slugs by *Pterostichus melanarius*

Molecular analysis of foregut contents was performed on a sub-sample of *P. melanarius*. The distribution of the numbers of *P. melanarius* sampled from enclosures across sampling time periods was highly skewed, with instances of very low numbers of *P. melanarius* in some enclosures in later sampling time periods. The methodology utilized in selecting the sub-sample was designed to ensure that: sufficient samples were analysed from all enclosures, accurate estimates of mean and variance could be made, and discrete analyses could be made for male and female *P. melanarius*. Therefore, six beetles (three of each sex) from each enclosure (where available) were randomly selected for analysis of foregut contents. Subsequently, fourteen out of the 418 samples were rejected for failure to display bands after PCR amplification with all three primer combinations. Therefore, statistical analyses were performed on the remaining 404 *P. melanarius*. The proportions of slug-positive beetles within sub-samples were multiplied by the number of *P. melanarius* observed inside the respective enclosures to give an overall estimate for the number of slug-positive *P. melanarius* per enclosure.

Generalized linear modelling, utilizing the same methodology as that described in section 6.2.4.1, was used to test for relationships between slug density and slug-positive *P. melanarius*, and abundance of alternative prey and slug-positive *P. melanarius*.

6.2.4.3 Foregut mass of *Pterostichus melanarius*

In order to assess the recent feeding history (in terms of the quantity of food consumed) of field-caught *P. melanarius*, the foregut masses of beetles sampled from enclosures were compared to those of the laboratory maintained 72 h-starved beetles ($n_m = n_f = 44$) used in the experiment described in Chapter 3. Foregut mass data were $\log_{10}$ transformed to stabilize variance before being analysed with ANOVA. Relationships were tested using GLM (described in section 6.2.4.1).

6.2.4.4 Slug mass dynamics

Slug masses recorded were those after the slugs had been preserved in 70 % ethyl alcohol solution. Therefore, estimates of live masses were calculated by applying data to the function:

$$y = 1.41x$$

where y represents the live mass and x the preserved mass. This function was obtained by applying simple linear regression ($r^2 = 0.999$, n = 40, $P < 0.001$) to a random sample of *Deroceras* spp. and *Arion* spp. slugs that had been weighed prior to, and after preservation in 70 % ethyl alcohol solution. The live mass values were used in analyses.

Frequencies of slugs were calculated for the mass-class categories: 0 – 9, 10 – 19, 20 – 29, 30 – 39, 40 – 49, 50 – 99, 100 – 149, 150 – 199, and ≥ 200 mg. Chi-square tests of association (maximum likelihood) were then used to test for association between mass-class frequency and time. Where appropriate, mass-class categories were combined in order to eliminate low expected values.

6.3 Results

6.3.1 Data used in analyses

Weather conditions prevalent on the days before and during sampling time periods one and three were extremely hot and dry (Figure 6.1). The total number of slugs obtained from all enclosures on these sampling occasions was 43 (29 *Deroceras* spp. and 14 *Arion* spp.). The number of slugs obtained from all enclosures during the remaining four sampling time periods was 1,548 (668 *Deroceras* spp. and 880 *Arion* spp.). The data from sampling times one and three were excluded from analyses on the basis that they were unlikely to represent the true densities of slugs at those time periods. Consequently, data analyses were conducted using the 1,249 *P. melanarius* caught from sampling times two, four, five and six.

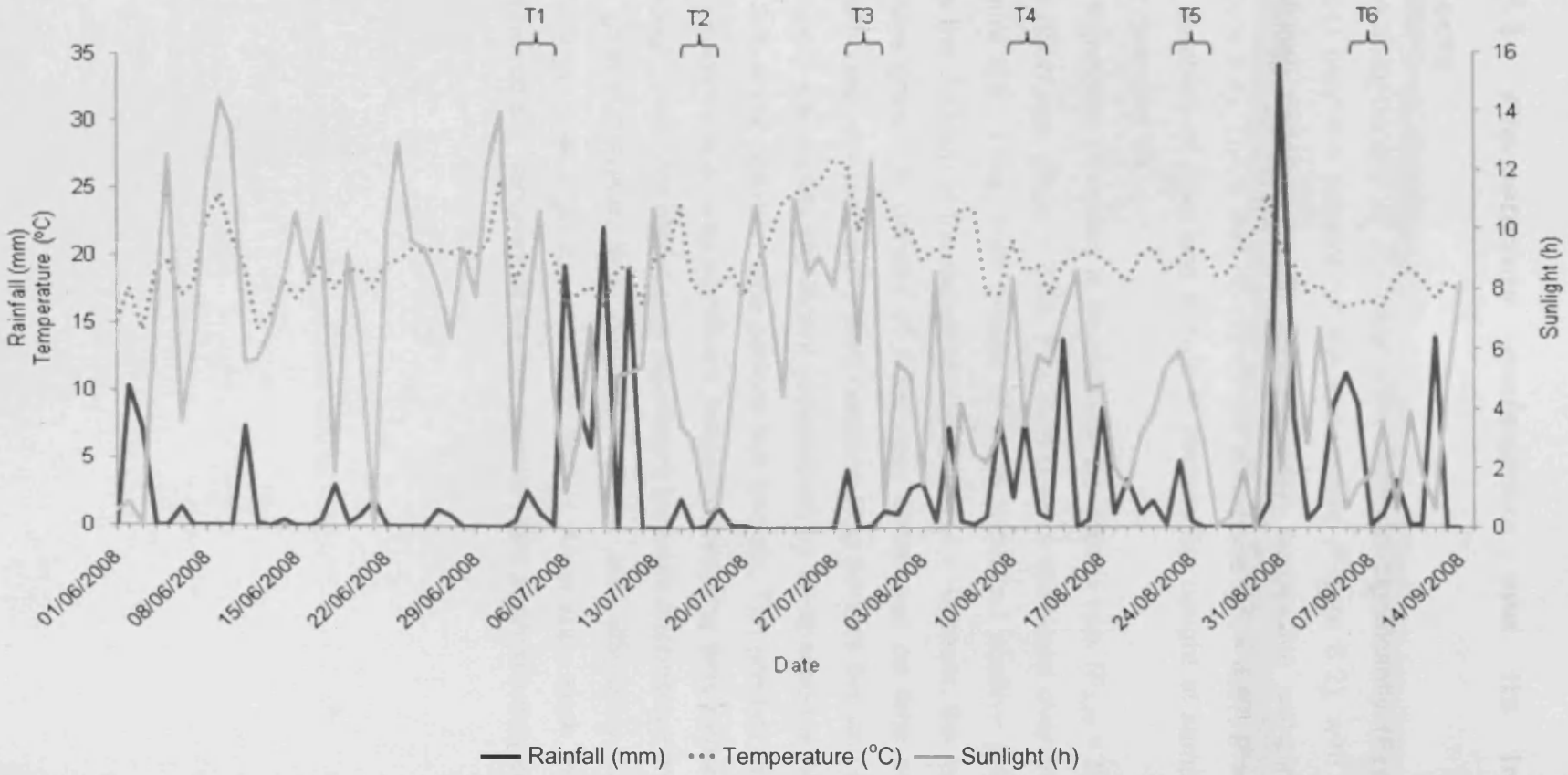


Figure 6.1 Daily rainfall, temperature (maximum), and sunlight on Rothamsted Farm during the period from 01/06/2008 to 14/09/2008. T1 – T6 indicate the days on which soil sampling occurred for each sampling time period. Pitfall trapping occurred during the following six days at each of the sampling time periods. Meteorological data were collected by Rothamsted Research.

6.3.2 *Pterostichus melanarius* and its interspecific effects

Activity-density of *P. melanarius* declined significantly ($F_{3,74}$ = 36.2, P < 0.001) over the duration of the experiment (Figure 6.2), with activity-density exhibiting negative growth between each successive sampling time period (Figure 6.4). There was no significant difference (χ^2_3 = 4.87, P = 0.181) among the numbers of male and female *P. melanarius* caught at sampling times two, four, five, and six.

Significant increases in densities of *Deroceras* spp. ($F_{3,76}$ = 8.88, P < 0.001) and *Arion* spp. ($F_{3,75}$ = 2.89, P = 0.041) were observed over the same period (Figure 6.3). Thus, both groups of slugs exhibited positive growth in density over the duration of the experiment (Figure 6.4). However, the magnitude of the positive growth in density of *Arion* spp. decreased as time proceeded, until growth was only just above zero between time periods five and six, and growth was only statistically significant (determined by comparison against the LSD at the 5 % level) between time periods two and six. The positive growth in density of *Deroceras* spp. was significant between sampling time periods two and six, although it was not statistically significant between successive time periods until the growth between time periods five and six. Ultimately, *Deroceras* spp. density grew at a greater rate than that of *Arion* spp., such that by sampling time period six, densities of the two genera were approximately equal.

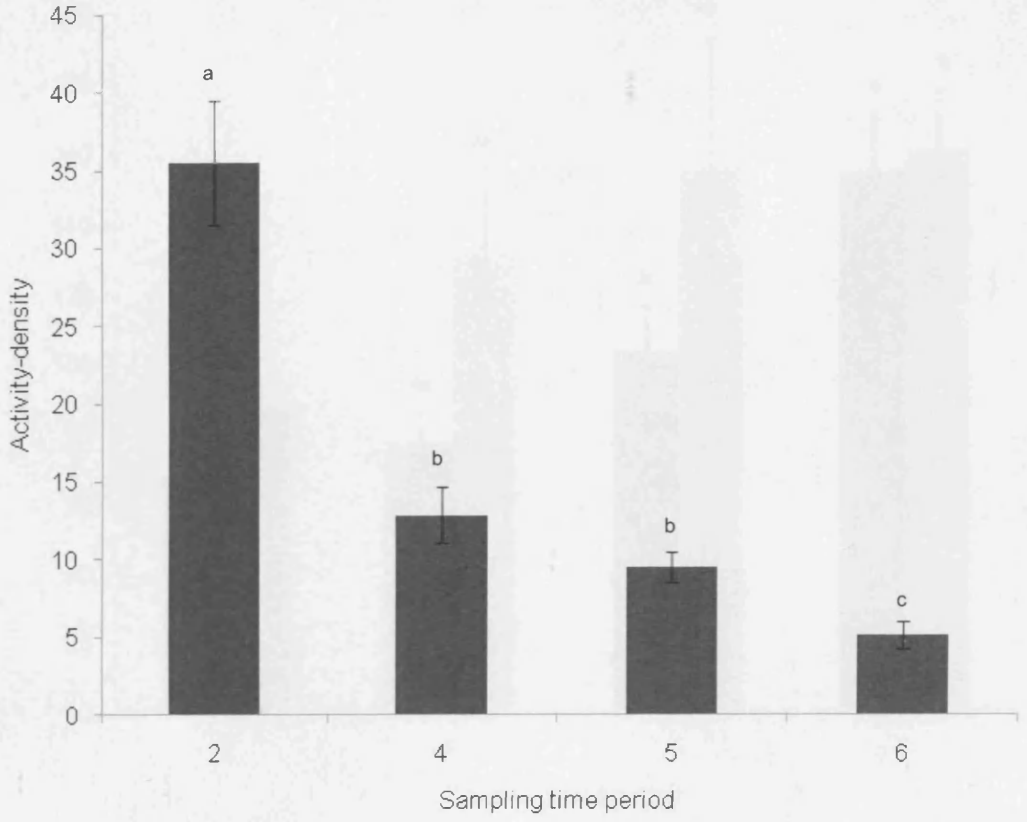


Figure 6.2 Mean activity-density (± SEM) of *Pterostichus melanarius* per enclosure (9 m^2) at sampling time periods two, four, five, and six. *Pterostichus melanarius* were sampled by pitfall trapping (four 6 cm-diameter traps per enclosure) over a period of six days and nights. Characters above bars indicate the result of comparisons among means, with respect to the LSD values (at 5 % level) obtained in one-way ANOVA of data (log_{10} transformed). The presence of the same character above bars indicates no significant difference among means.

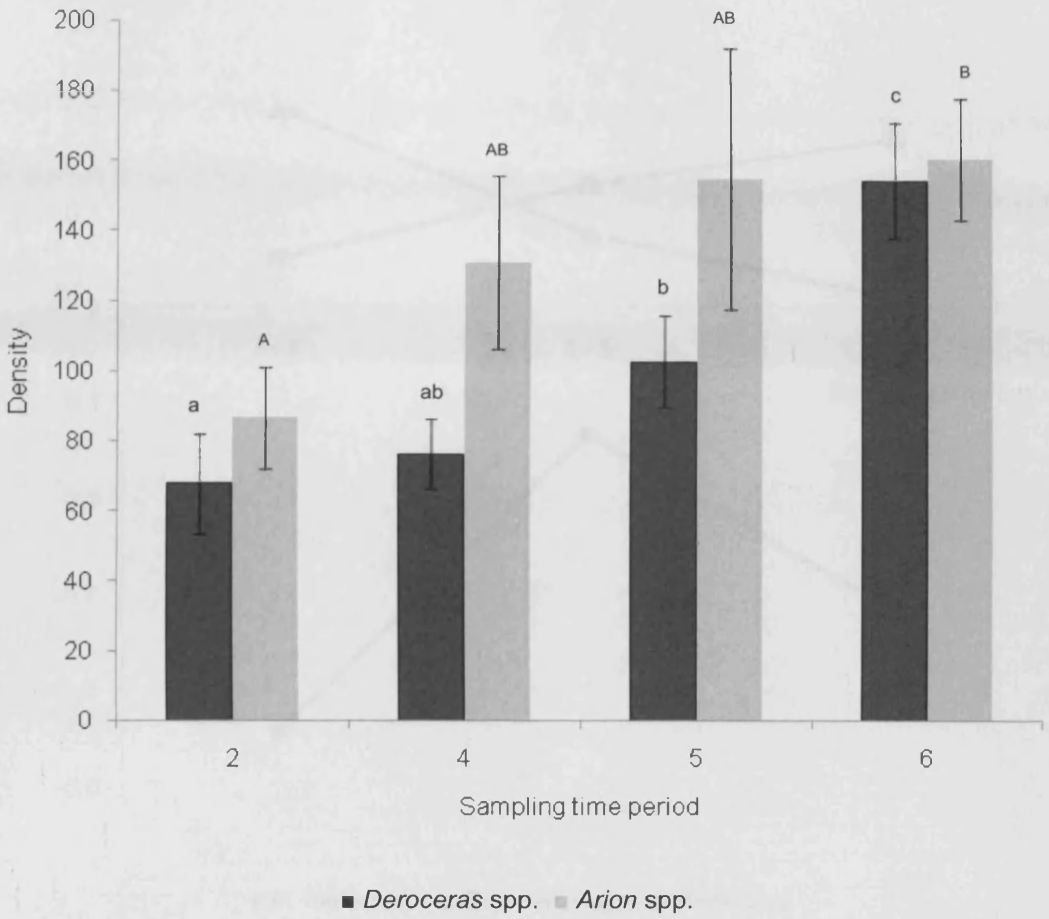


Figure 6.3 Mean densities (± SEM) of *Deroceras* spp., and *Arion* spp. at sampling time periods two, four, five, and six. Slugs were sampled from the soil using the mustard technique. Three 0.25 m^2-quadrats per enclosure (9 m^2) were taken. The number of slugs sampled from each enclosure was then multiplied by 12 to provide an estimate of total slug density per enclosure. Characters above bars indicate the result of comparisons among means, with respect to the LSD values (at 5 % level) obtained in one-way ANOVA for each set of data (log_{10} transformed). The presence of the same character above bars indicates no significant difference among means.

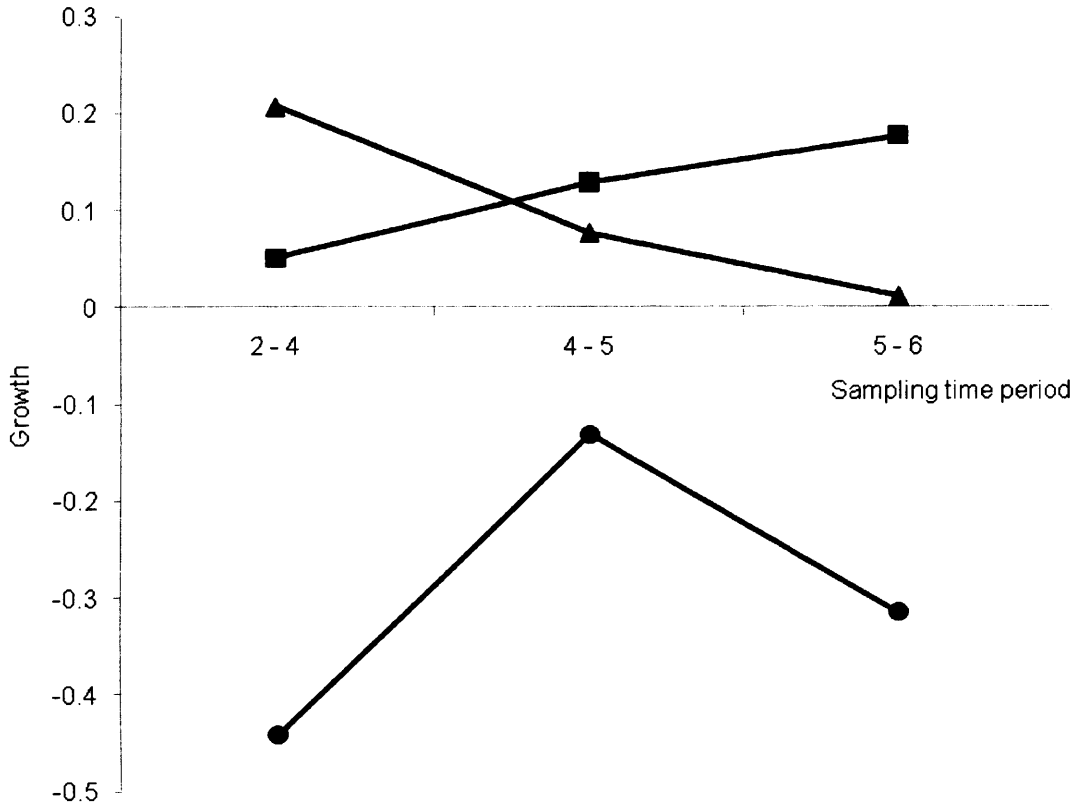


Figure 6.4 Growth in density of individuals per enclosure, for *Deroceras* spp. and *Arion* spp., and growth in activity-density of *Pterostichus melanarius*. The first set of data points indicate the growth in densities from sampling time periods two to four, due to the absence of data at the sampling time three. The second and third sets of data points indicate the growth between subsequent sampling time periods.

The density of slugs was negatively related, $y = e^{(-0.0163x + 3.19)}$ ($F_{1,78} = 9.92$, $P = 0.002$) to the activity-density of *P. melanarius* (Figure 6.5). Activity-density of *P. melanarius* was negatively related to the abundance of alternative prey, $y = e^{(-0.00930x + 4.10)}$, ($F_{1,78} = 60.5$, $P < 0.001$) (Figure 6.6). The abundance of alternative prey significantly ($F_{3,76} = 56.8$, $P < 0.001$) increased over time, although the difference between times five and six was not significant (Figure 6.7). The activity-density of *P. melanarius* was negatively related to the abundance of total prey (slugs plus alternatives), $y = e^{(-0.00872x + 4.16)}$, ($F_{1,78} = 63.4$, $P < 0.001$) (Figure 6.8).

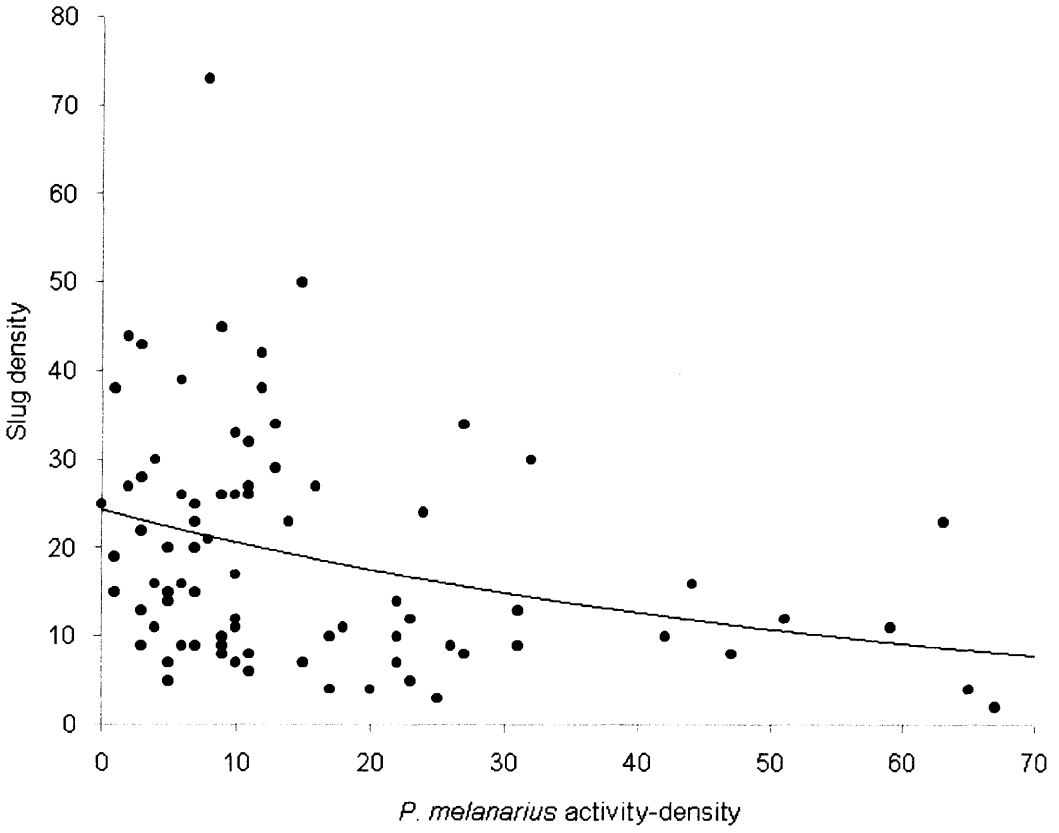


Figure 6.5 Relationship between activity-density of *Pterostichus melanarius* and slug density; expressed by the function: $y = e^{(-0.0163x + 3.19)}$. Twenty 9 m^2-enclosures were sampled at time periods two, four, five and six.

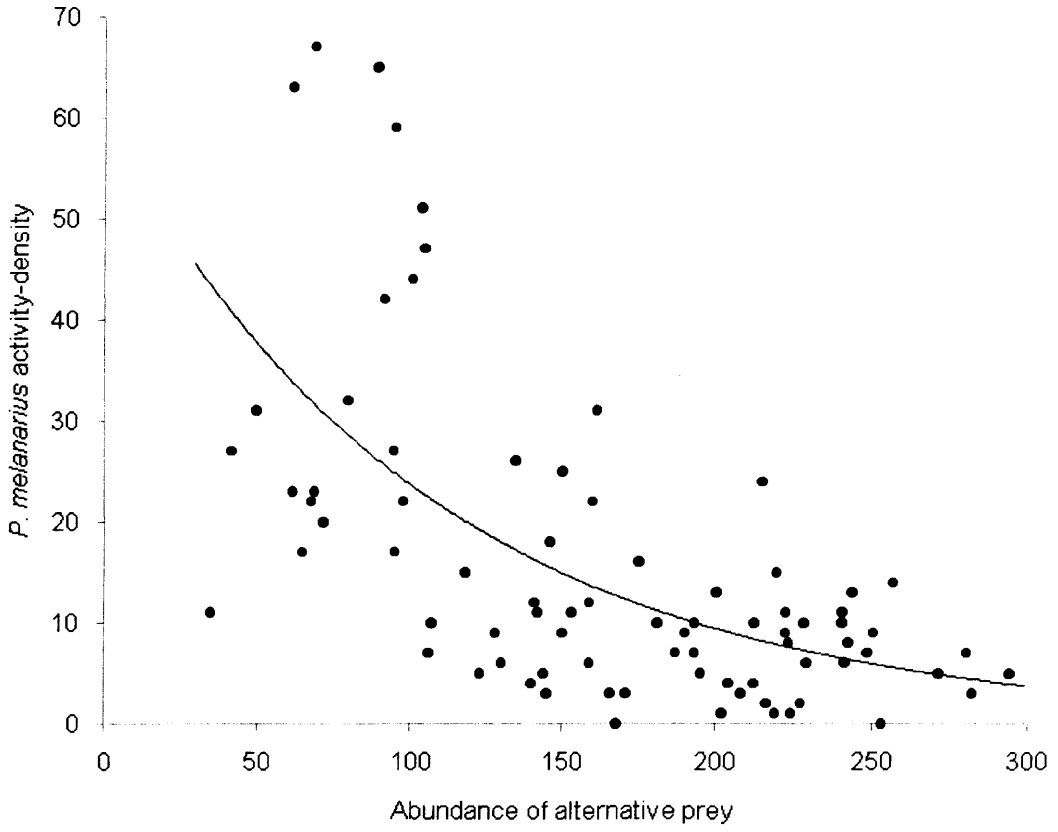


Figure 6.6 Relationship between the abundance of alternative prey, and the activity-density of *Pterostichus melanarius*, expressed by the function: $y = e^{(-0.00930x + 4.10)}$. Twenty 9 m^2-enclosures were sampled at time periods two, four, five and six. *Pterostichus melanarius* were sampled over a period of six days and nights using four 6 cm-diameter pitfall traps. The abundance of alternative prey was obtained in soil and pitfall trap samples. Soil was sampled with three 0.25 m^2-quadrats per enclosure, using the mustard technique.

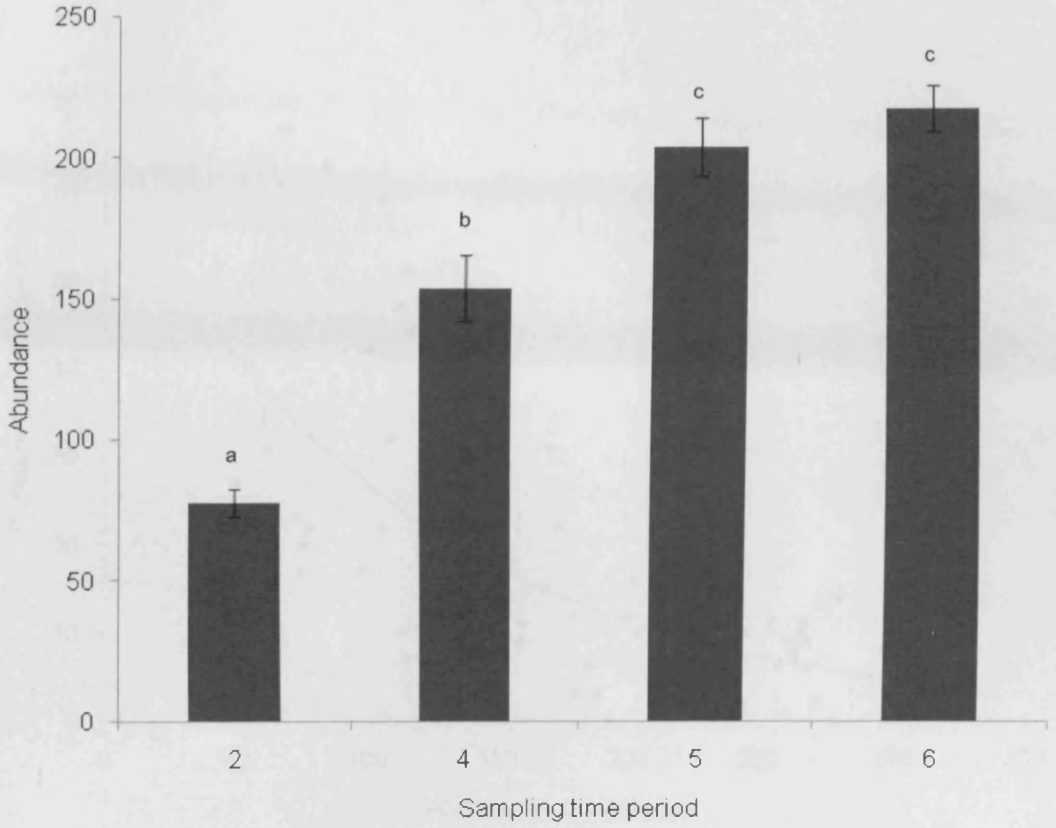


Figure 6.7 Abundance (± SEM) of alternative prey per enclosure (9 m^2) at sampling time periods two, four, five, and six. Characters above bars indicate the result of comparisons among means, with respect to the LSD values (at 5 % level) obtained in one-way ANOVA of data. The presence of the same character above bars indicates no significant difference among means.

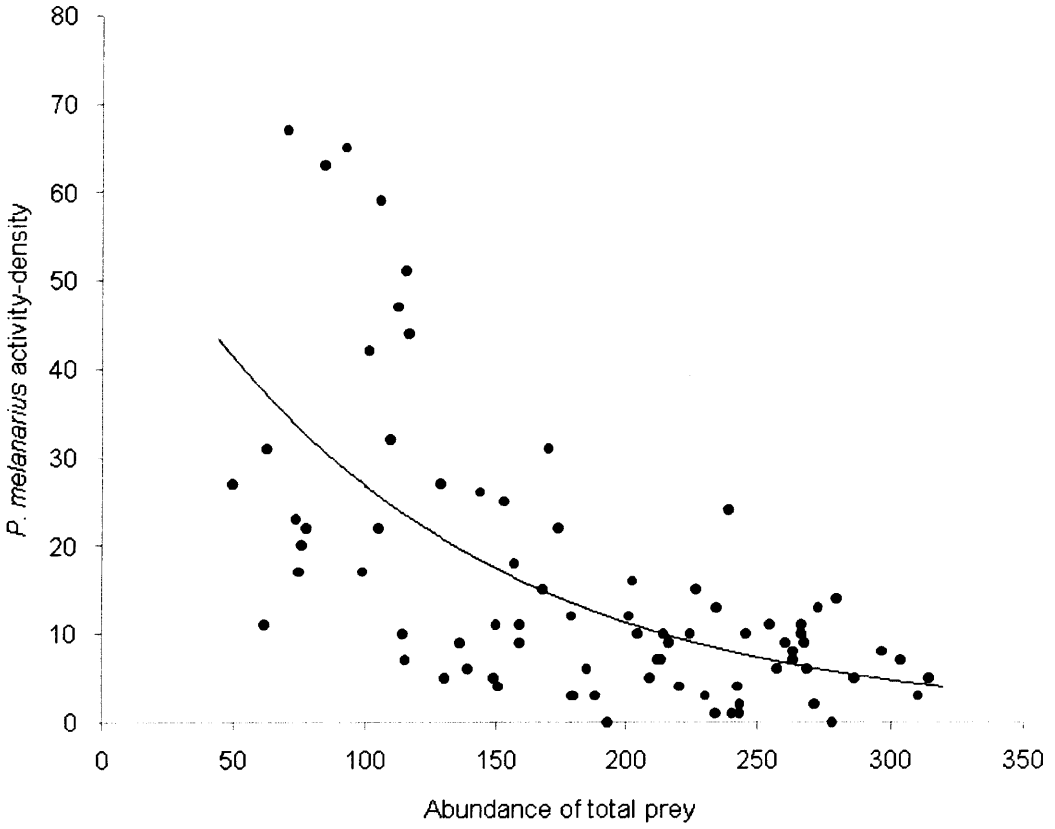


Figure 6.8 Relationship between the abundance of total prey, and the activity-density of *Pterostichus melanarius*, expressed by the function: $y = e^{(-0.00872x + 4.16)}$. Twenty 9 m^2-enclosures were sampled at time periods two, four, five and six.

Approximately 1 % of the organisms obtained in samples (excluding *P. melanarius* because their numbers were manipulated) were medium – large Coleoptera (Carabidae and Staphylinidae) and Arachnida. Within this group, 30.8 % were *Pterostichus madidus*. Approximately 6 % of all organisms were small Coleoptera, Arachnida and Chilopoda. The remaining 93 % of organisms were primary consumers. Within this grouping, the majority were earthworms (84.6 %), slugs (11.3 %), and bristletails and springtails (3.70 %).

A highly significant positive relationship, $y = e^{(0.00385x + 2.30)}$ ($F_{1,78} = 13.4$, $P < 0.001$) existed between the abundance of alternative prey and the density of slugs (Figure 6.9).

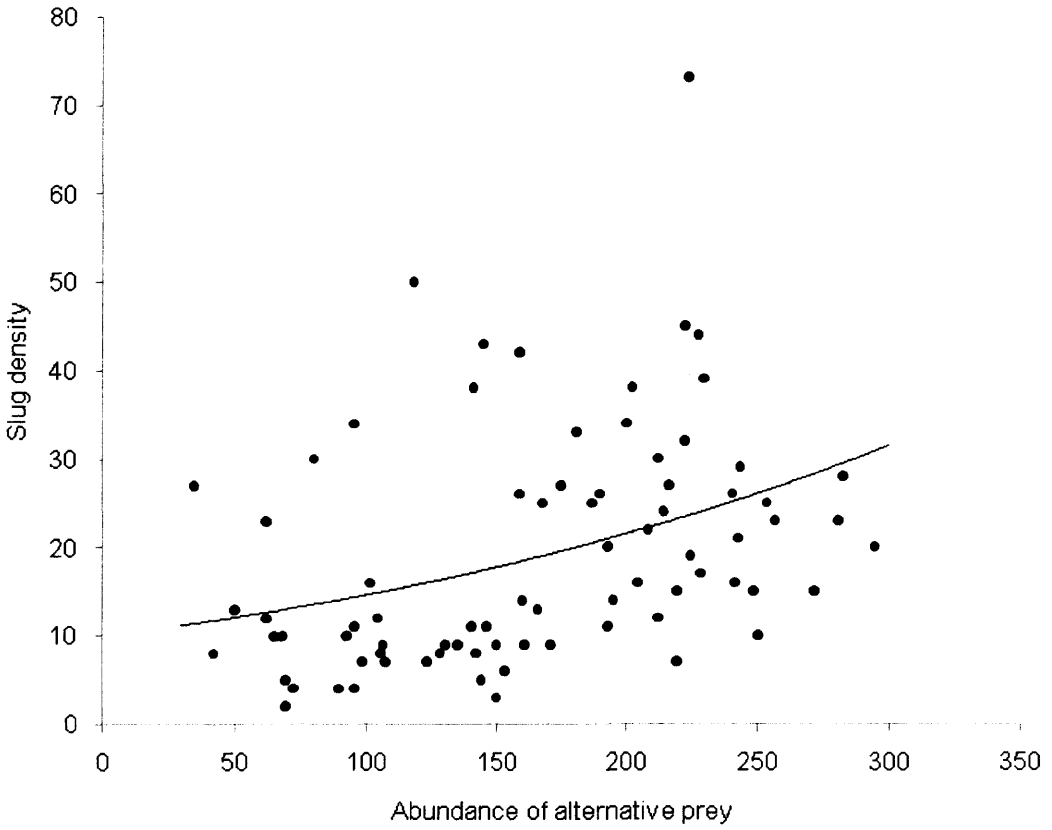


Figure 6.9 Relationship between the abundance of alternative prey and slug density, expressed by the function: $y = e^{(0.00385x + 2.30)}$. Twenty 9 m^2-enclosures were sampled at time periods two, four, five and six.

6.3.3 Molecular evidence for predation or scavenging on slugs by *Pterostichus melanarius*

Pterostichus melanarius inside enclosures did feed on slug tissue from *Deroceras* spp. (19 / 404 beetles) and *Arion* spp. (48 / 404 beetles). The numbers of *Arion* spp. DNA-positive beetles per enclosure were greater than those of *Deroceras* spp.-positive beetles ($H_1 = 8.61$, $P = 0.003$, $n = 80$). Over time there was a significant ($\chi^2_3 = 34.8$, $P < 0.001$) decline in *Arion* spp.-positive beetles, but the change in *Deroceras* spp.-positive beetles was not statistically significant ($\chi^2_3 = 2.75$, $P = 0.432$) (Figure 6.10). Additionally, at each sampling time period there were more *Arion* spp.-positive beetles than *Deroceras* spp.-positive beetles.

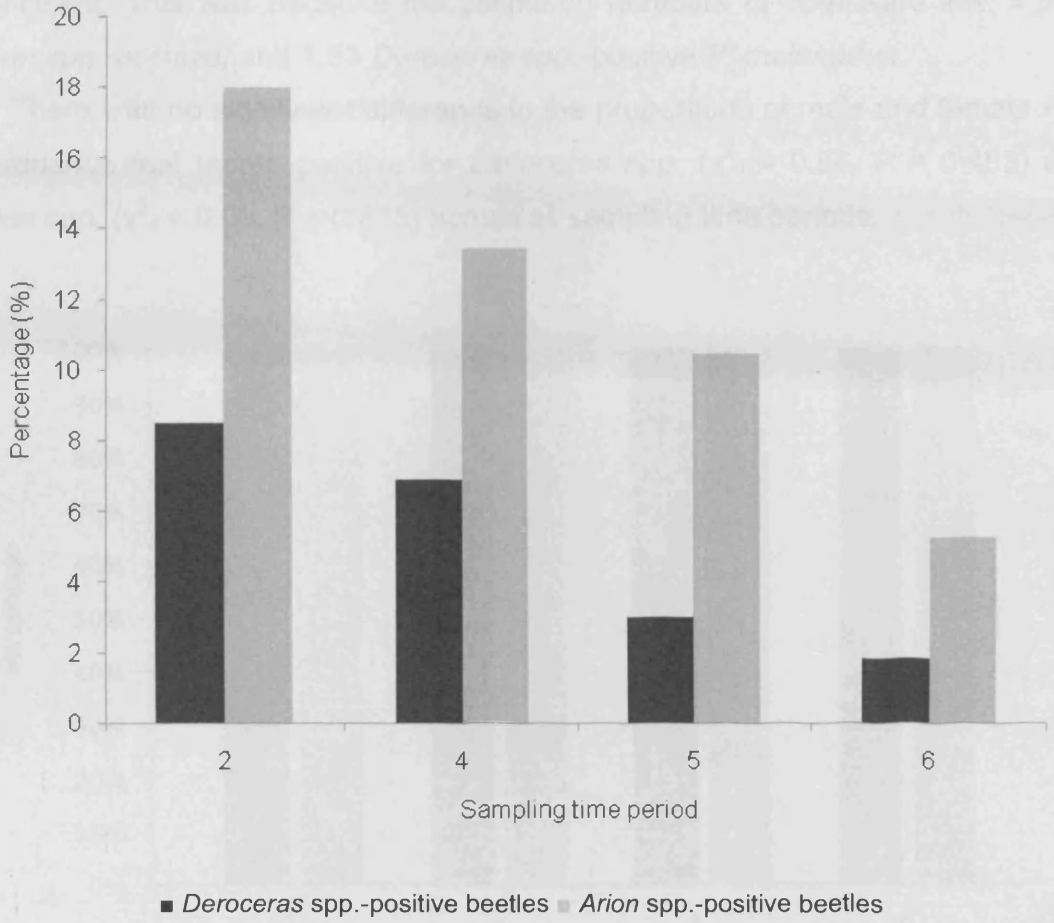
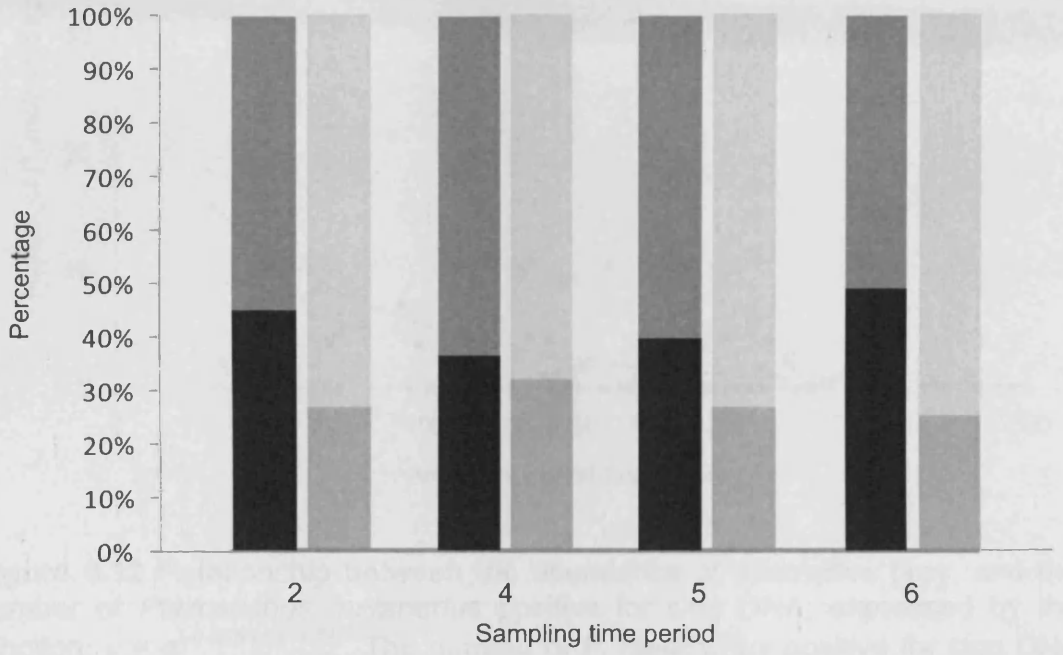


Figure 6.10 The percentage of *Pterostichus melanarius* that had consumed slug tissue. A sub-sample of 404 beetles, from the 1249 sampled in field-enclosures, were subjected to molecular analysis of foregut contents.

At sampling time two, the number of *Arion* spp.-positive *P. melanarius*, relative to the number of *Deroceras* spp.-positive beetles, was significantly (χ^2_1 = 14.0, P < 0.001) greater than expected according to the relative proportions of *Arion* spp. and *Deroceras* spp. observed in the enclosures (Figure 6.11). At sampling times four, five, and six, the relative abundance of *Arion* spp.- and *Deroceras* spp.-positive *P. melanarius* were not significantly (time four: χ^2_1 = 0.48, P = 0.487; time five: χ^2_1 = 1.80, P = 0.180; and time six: χ^2_1 = 1.47, P = 0.225) different to the expected values. In all cases the relative number of *Arion* spp.-positive *P. melanarius* was greater than the relative number of *Arion* spp. present inside enclosures. At time six, the relative proportion of *Arion* spp.-positive beetles appeared to be considerably greater than that of *Deroceras* spp.-positive beetles (Figure 6.11), but the difference was not statistically

significant. This was because the estimated numbers of both were low: 4.60 *Arion* spp.-positive, and 1.53 *Deroceras* spp.-positive *P. melanarius*.

There was no significant difference in the proportions of male and female *P. melanarius* that tested positive for *Deroceras* spp. ($\chi^2_1 = 0.56$, P = 0.453) or *Arion* spp. ($\chi^2_1 = 0.05$, P = 0.815) across all sampling time periods.



Relative proportion of slugs in field: ■ *Deroceras* spp. ■ *Arion* spp.

Relative proportion of slug-positive *P. melanarius*: ■ *Deroceras* spp. ■ *Arion* spp.

Figure 6.11 The relative percentage of *Deroceras* spp. and *Arion* spp. present inside field-enclosures, and the relative percentage of DNA-positive *Pterostichus melanarius* for the same two genera of slugs. The DNA-positive beetles were those in a sub-sample of 404 from the 1249 beetles sampled in enclosures across sampling time periods two, four, five, and six.

The abundance of alternative prey, and the number of *P. melanarius* positive for slug DNA (Figure 6.12) were negatively related, $y = e^{(-0.0156x + 3.23)}$ ($F_{1,78} = 40.28$, $P < 0.001$). A significant relationship did not exist between slug density and the number of *P. melanarius* positive for slug DNA ($F_{1,78} = 2.24$, P = 0.124).

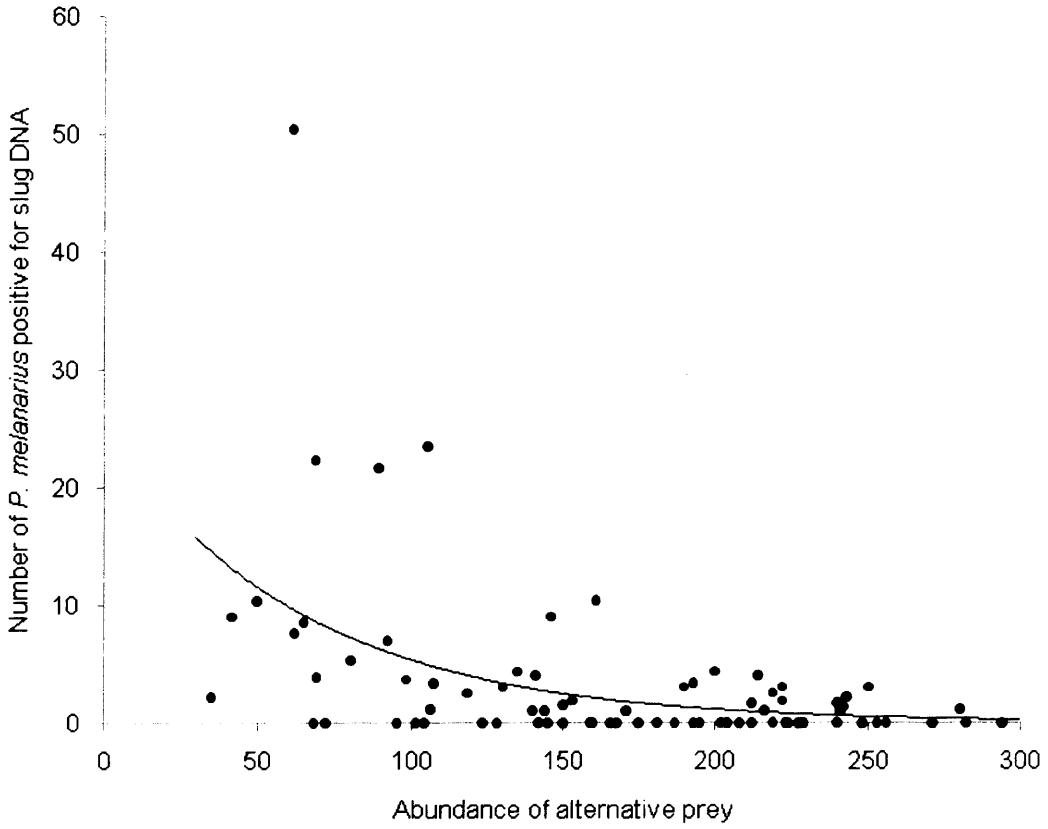


Figure 6.12 Relationship between the abundance of alternative prey, and the number of *Pterostichus melanarius* positive for slug DNA, expressed by the function: $y = e^{(-0.0156x + 3.23)}$. The number of *P. melanarius* positive for slug DNA was calculated as the proportion of sub-sampled beetles that were slug DNA-positive, multiplied by the number of *P. melanarius* that were observed in pitfall trap samples, all on a per enclosure basis. The abundance of alternative prey was the sum of those observed in soil and pitfall trap samples taken from each enclosure, across sampling time periods two, four, five, and six.

6.3.4 Foregut mass of *Pterostichus melanarius*

Analyses of variance indicated that there were significant differences (male: $F_{4,247} = 6.93$, $P < 0.001$; female: $F_{4,249} = 8.81$, $P < 0.001$) in mean foregut mass among beetles caught during different sampling time periods, and among field-caught beetles and 72 h-starved laboratory-maintained beetles (Figure 6.13). All male *P. melanarius* sampled from enclosures, and females from sampling time periods two, four and five had a mean foregut mass lower than that of the 72 h-starved beetles. Only the female beetles from sampling time point six had a mean foregut mass that was not significantly different to that of the 72 h-starved beetles.

There was a significant relationship between foregut mass and sampling time period in females ($F_{3,66}$ = 4.94, P = 0.004), but not males ($F_{3,67}$ = 1.83, P = 0.150). In both male and female beetles, mean foregut mass increased significantly (greater than the LSD value at the 5 % level) between sampling time periods two and four. For male beetles, there was then no difference in mean foregut mass between sampling time periods four, five, and six. In females, there was no difference between sampling time periods four and five, but there was a significant increase between times five and six, although the increased foregut mass at time six was not significantly greater than the foregut mass at time four.

No relationship existed between the proportion of *P. melanarius* that tested positive for slug DNA, and the mean foregut mass of *P. melanarius* (males: $F_{1,67}$ = 0.03, P = 0.873; females: $F_{1,66}$ = 0.01, P = 0.912).

A positive relationship, $y = e^{(0.00177x + 2.15)}$ ($F_{1,73}$ = 7.03, P = 0.010), existed between the abundance of alternative prey and the mean foregut mass of female *P. melanarius* (Figure 6.14). No significant relationship was observed in male beetles ($F_{7,73}$ = 1.20, P = 0.312). A significant relationship did not exist between slug density and beetle foregut mass for either male ($F_{7,68}$ = 1.54, P = 0.167) or female ($F_{7,67}$ = 1.68, P = 0.129) *P. melanarius*.

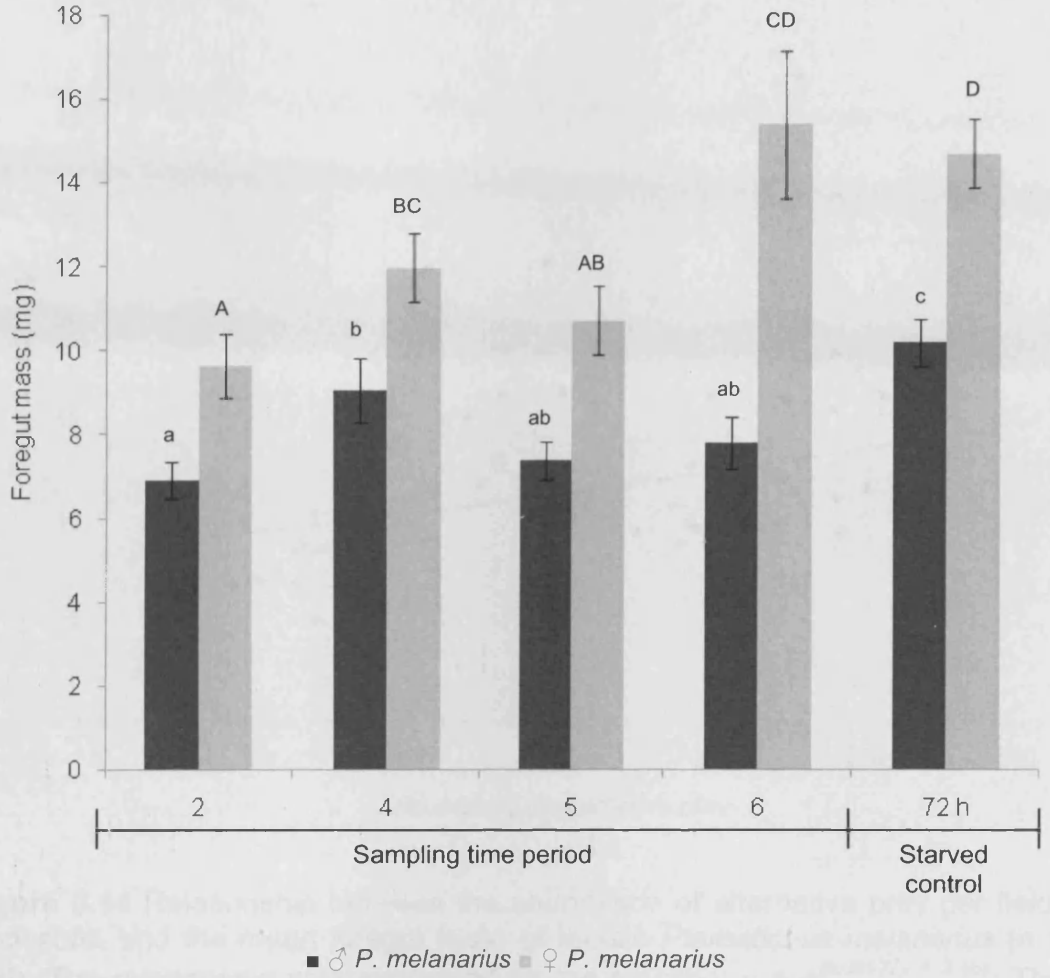


Figure 6.13 Mean foregut mass (± SEM) of *Pterostichus melanarius* sampled from field-enclosures (n_m = 208, n_f = 210), and 72 h-starved, laboratory-maintained beetles ($n_m = n_f$ = 44). Characters above bars indicate the result of comparisons among means, with respect to the LSD values (at 5 % level) obtained in a one-way ANOVA of each data set (log_{10} transformed). The presence of the same character above bars indicates no significant difference among means. Comparisons were made only among individuals of the same sex.

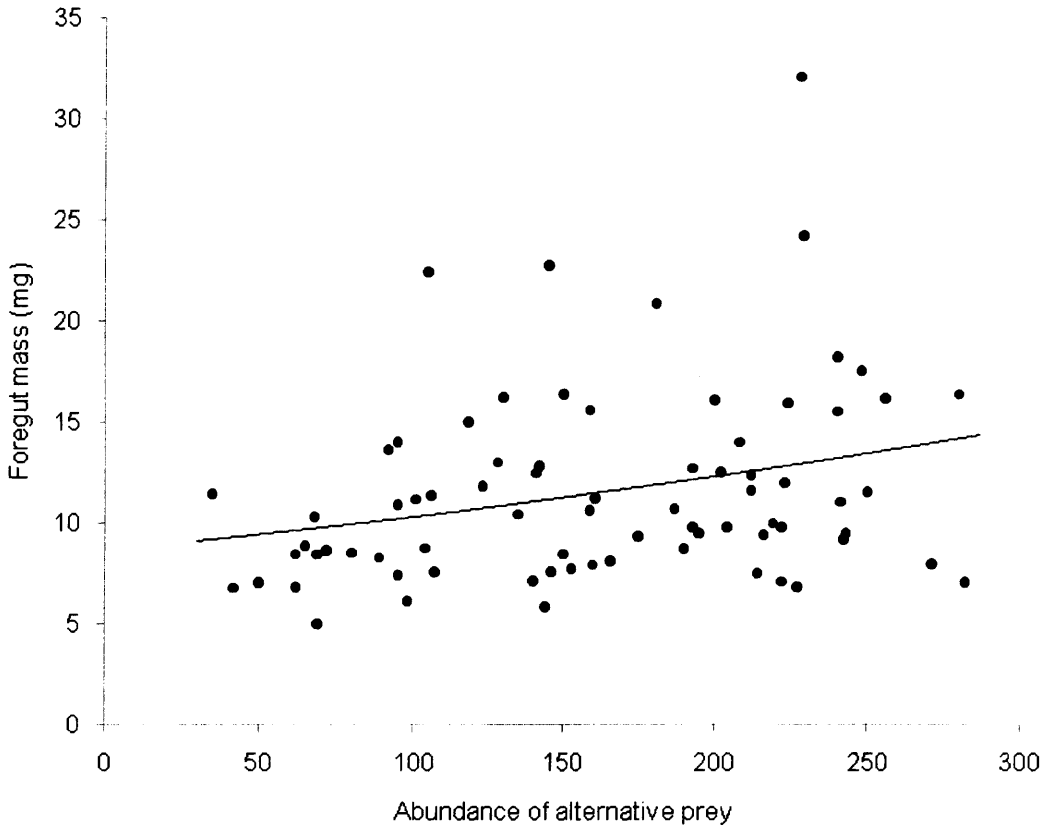


Figure 6.14 Relationship between the abundance of alternative prey per field-enclosure, and the mean foregut mass of female *Pterostichus melanarius* (n = 210). The relationship was expressed by the function: $y = e^{(0.00177x + 2.15)}$. The abundance of alternative prey was the sum of those observed in soil and pitfall trap samples taken from each enclosure, across sampling time periods two, four, five, and six.

The mean foregut mass of *Deroceras* spp.-positive male *P. melanarius* was significantly ($F_{2,198}$ = 14.1, P < 0.001) greater than that of *Arion* spp.-positive beetles, and those that were slug-negative (Figure 6.15). There was no significant difference in mean foregut mass between the latter two categories of beetles. Additionally, there was no significant difference ($F_{2,202}$ = 2.39, P = 0.095) in mean foregut mass of female *P. melanarius*, among those that were *Deroceras* spp.-positive, *Arion* spp.-positive, or slug-negative.

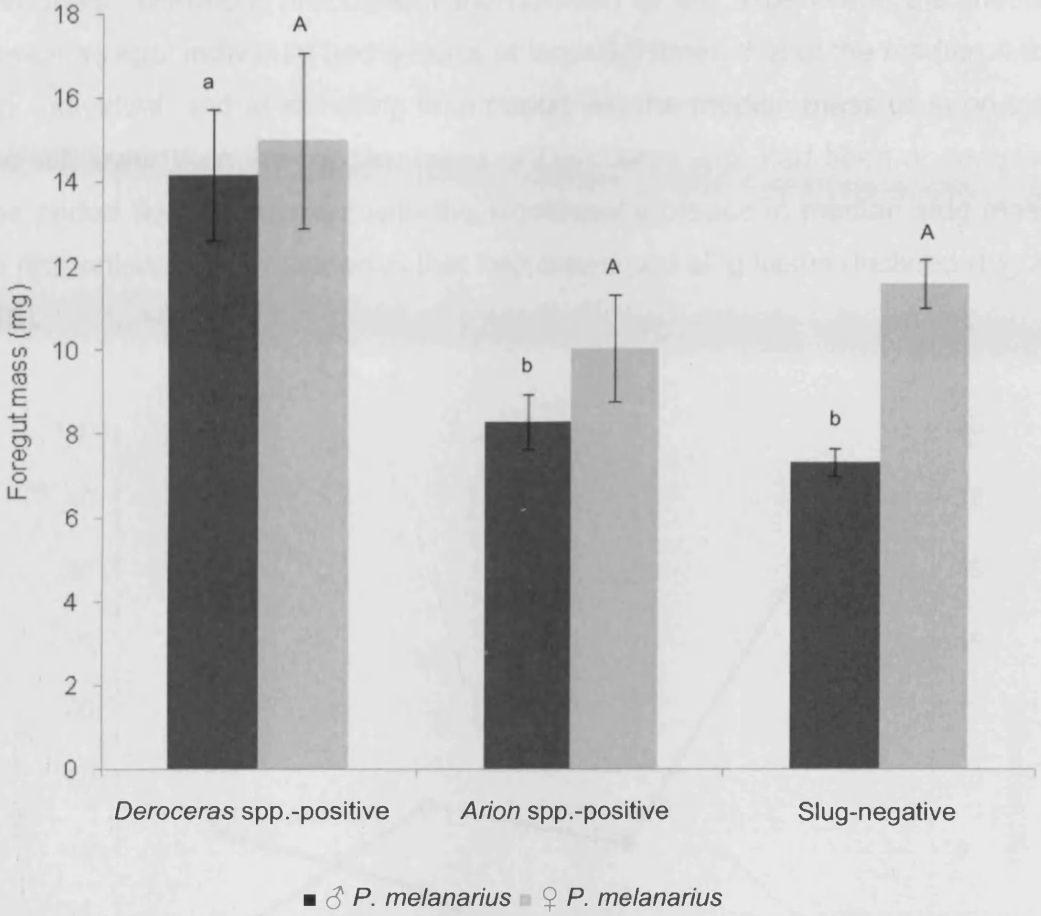


Figure 6.15 Mean foregut mass (± SEM) of slug DNA-positive (*Arion* spp.: n_m = 23, n_f = 25; *Deroceras* spp.: n_m = 11, n_f = 8) and DNA-negative (n_m = 167, n_f = 172) *Pterostichus melanarius* sampled from field-enclosures across sampling time periods two, four, five and six. Characters above bars indicate the result of comparisons among means, with respect to the LSD values (at 5 % level) obtained in one-way ANOVA of the data (log_{10} transformed). The presence of the same character above bars indicates no significant difference among means. Comparisons were made only among individuals of the same sex.

6.3.5 Slug mass dynamics

The median mass of slugs increased significantly over time, for both *Deroceras* spp. (H_3 = 87.7, P < 0.001, n = 668), and *Arion* spp. (H_3 = 168, P < 0.001, n = 880). The proportional increase in median mass of individuals between the first and final sampling events was similar for the two groups of slugs: the median mass of *Deroceras* spp. increased by 326 %, whilst the increase in median mass of *Arion* spp. individuals was 267 %. However, at the beginning of the experimental period, the median mass of *Deroceras* spp. individuals was approximately 3.5 times greater than that of *Arion* spp.

individuals. Therefore, throughout the duration of the experiment, the median *Deroceras* spp. individual had a mass at least 3.5 times that of the median *Arion* spp. individual, and at sampling time period six, the median mass of *Arion* spp. was still lower than the median mass of *Deroceras* spp. had been at sampling time period two. Concurrent with the significant increase in median slug mass, the proportions of *P. melanarius* that had consumed slug tissue declined (Figure 6.16).

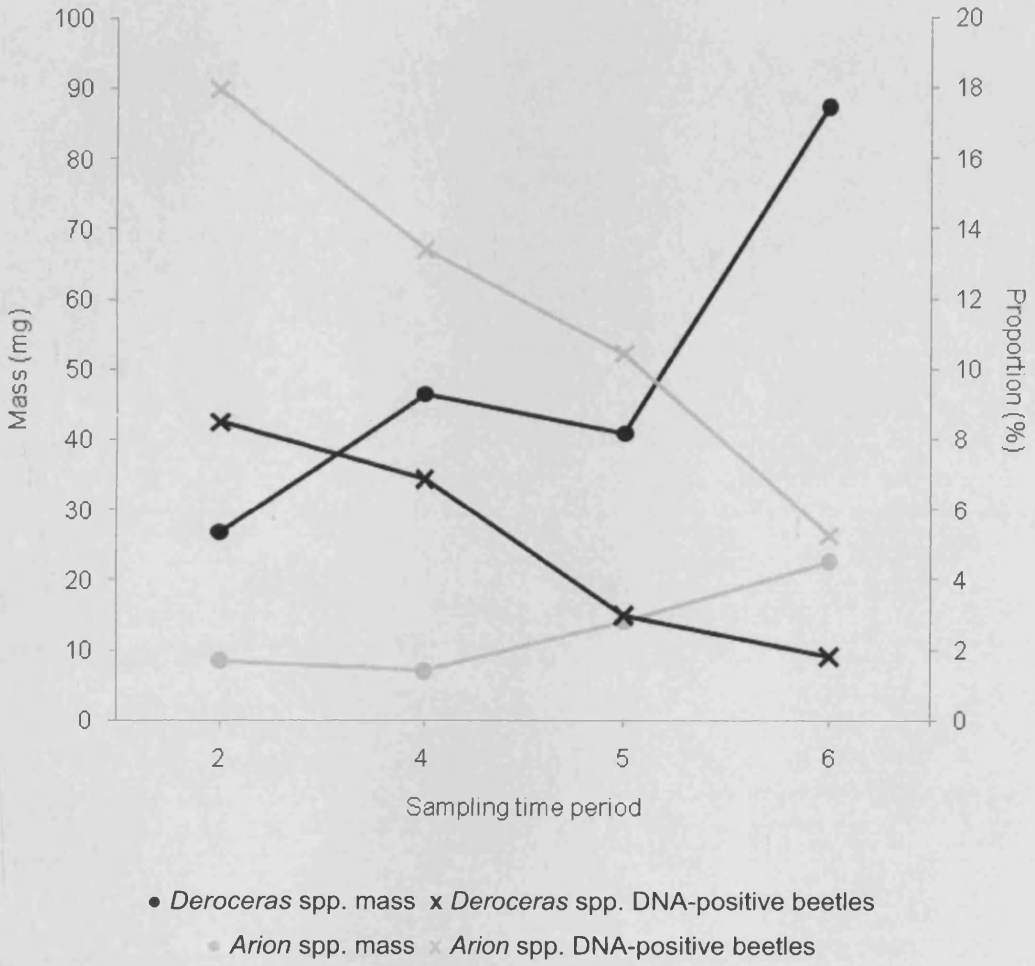


Figure 6.16 Relationship between median slug mass, and the proportion of *Pterostichus melanarius* that tested positive for the presence of slug DNA. Slugs were sampled from field-enclosures in three 0.25 m^2-quadrats per enclosure (9 m^2), using the mustard technique. A sub-sample of 404 beetles, from the 1249 sampled in field-enclosures, were subjected to molecular analysis of foregut contents.

There was a strong association between slug mass-class frequency and time for both *Deroceras* spp. (χ^2_{24} = 137, P < 0.001) and *Arion* spp. (χ^2_{21} = 329,

$P < 0.001$). The main contributing factors to the significant association in *Deroceras* spp. (Figure 6.17) occurred during sampling time periods two and six. There were higher than expected frequencies of slugs in mass-classes 0 – 9 and 10 – 19 mg at sampling time period two, and lower than expected frequencies of slugs in mass-classes 0 – 9, 10 – 19, and 20 – 29 mg at time six. Also, a lower than expected frequency of slugs ≥ 200 mg at time two, and a higher than expected frequency of slugs ≥ 200 mg at time six. In *Arion* spp. (Figure 6.18), the main contributing factors to the significant association occurred across all time periods. At sampling time periods two and four, there were greater than expected frequencies of slugs in the 0 – 9 mg mass-class, whereas at times five and six there were fewer than expected slugs in that mass-class. At times two and four there were fewer than expected slugs in the mass-classes 10 – 19, 20 – 29, and 30 – 39 mg, but at times five and six there were more slugs than expected in those same mass-classes. Finally, at times two and six there were greater than expected frequencies of slugs in some of the larger mass-classes.

At sampling time period two, the frequency distributions among mass-classes for the two groups of slugs were similar. The majority of individuals from both genera were in the smallest mass-classes (0 – 49 mg). However, after sampling time period two, there was a divergence between the two groups of slugs, in terms of the frequency distributions within mass-classes. The *Arion* spp. population continued to be dominated by slugs in the smallest mass-classes, and indeed increasingly so, for the frequency of individuals in the smallest mass-classes generally increased over time. In the *Deroceras* spp. population, the frequencies of individuals in the smallest mass-classes fluctuated, but remained relatively stable. However, the frequencies of individuals in the larger mass-classes increased.

Frequencies of the very smallest slugs, 0 – 9 mg, differed considerably among the two genera, with there being considerably higher frequencies of *Arion* spp. At sampling time periods two, four, five and six, the frequencies of *Deroceras* spp. in the 0 – 9 mg mass-class were 16, 7, 3, and 2 respectively. The corresponding frequencies of *Arion* spp. were 79, 121, 66, and 11.

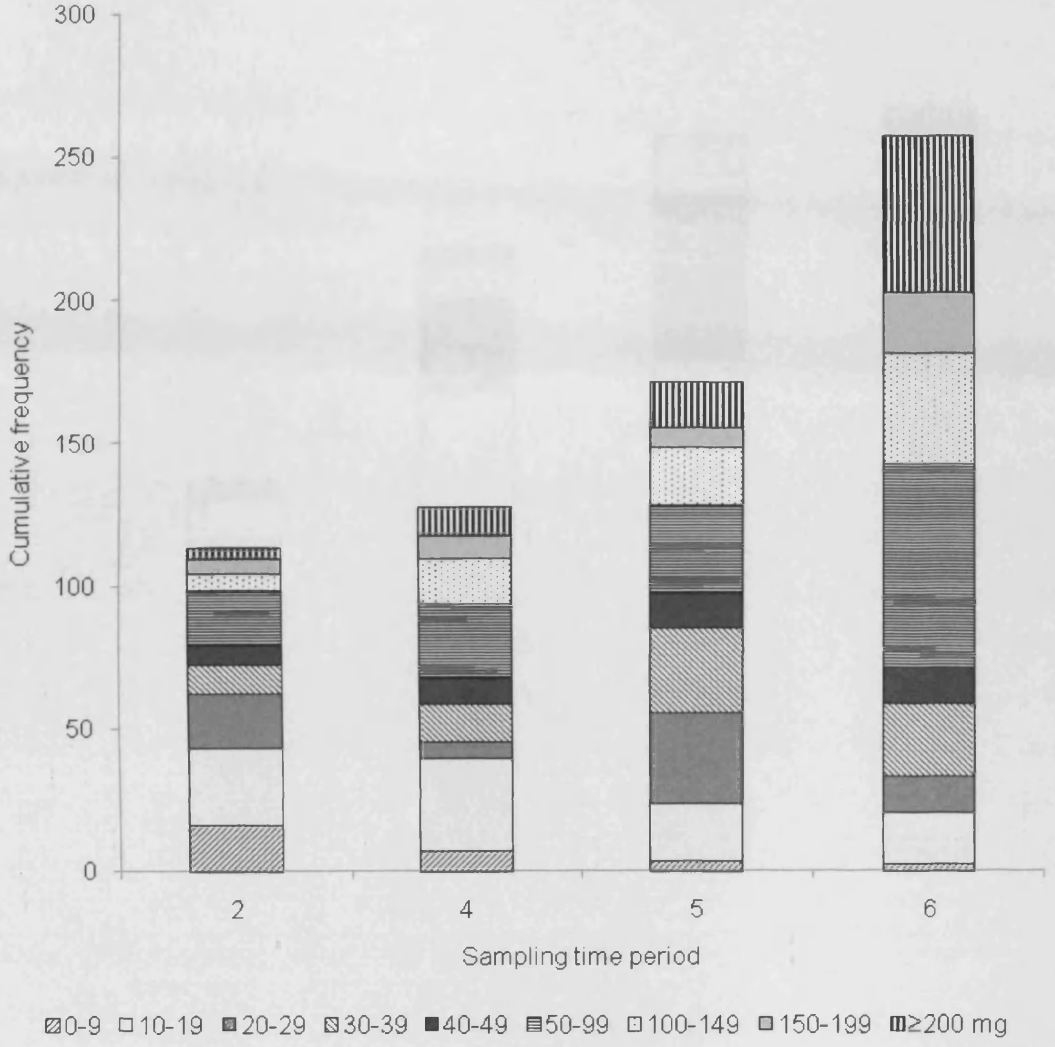


Figure 6.17 Mass-class frequency distribution of *Deroceras* spp. observed in field-enclosures across sampling time periods two, four, five, and six. Slugs were sampled in three 0.25 m^2-quadrats per enclosure (9 m^2), using the mustard technique.

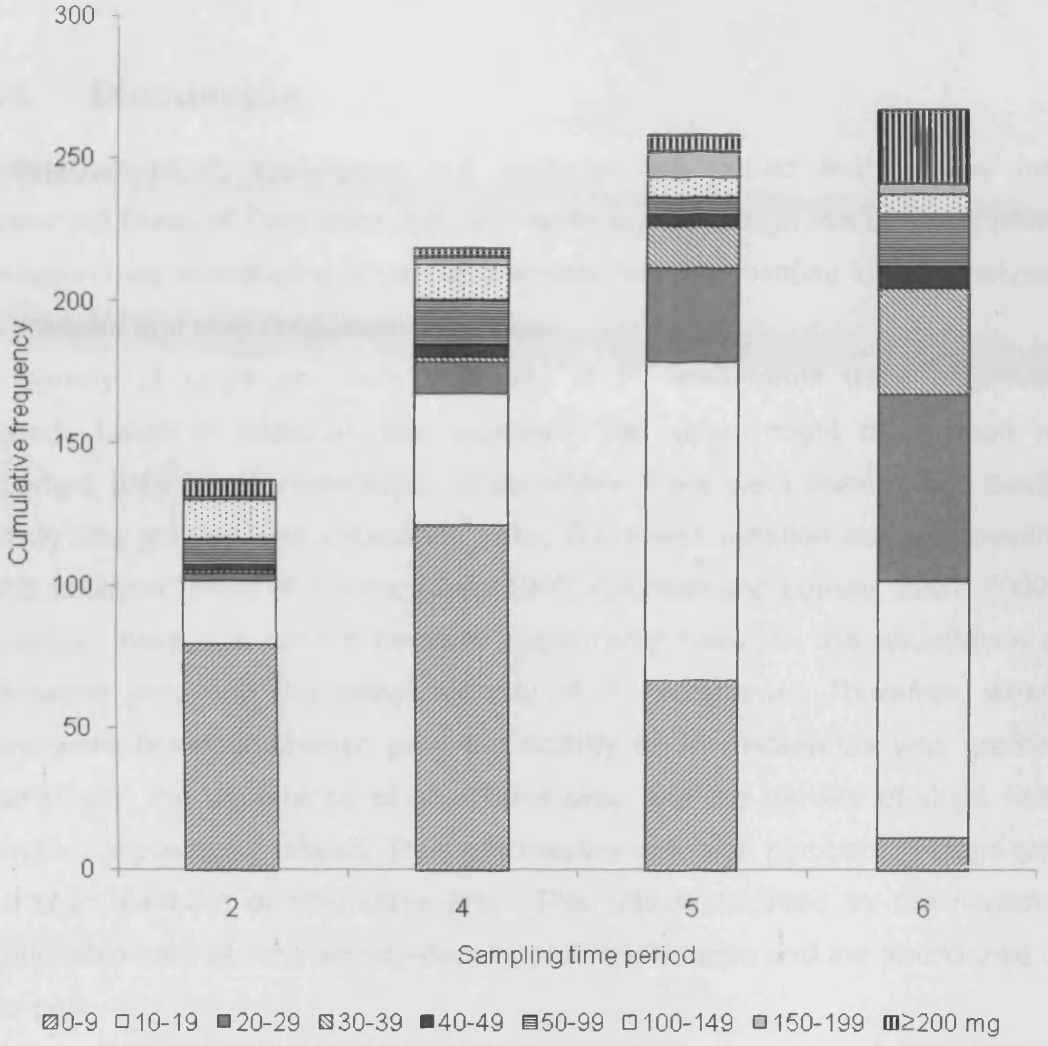


Figure 6.18 Mass-class frequency distribution of *Arion* spp. observed in field-enclosures across sampling time periods two, four, five, and six. Slugs were sampled in three 0.25 m^2-quadrats per enclosure (9 m^2), using the mustard technique.

6.4 Discussion

Analysis of *P. melanarius* gut contents established that beetles had consumed tissue of *Deroceras* spp. and *Arion* spp. Although this does not prove predation over scavenging, it proves a trophic link that justifies further analyses on predator and prey population dynamics.

Density of slugs and activity-density of *P. melanarius* were negatively related. Taken in isolation, this suggests that slugs might have been an important prey for *P. melanarius*, since where there were fewer slugs beetle activity was greater. The implication being that lower satiation status in beetles leads to higher levels of activity (Mols, 1993; Fournier and Loreau, 2001, 2002). However, there was also a negative relationship between the abundance of alternative prey and the activity-density of *P. melanarius*. Therefore, where there were fewer alternative prey the activity of *P. melanarius* was greater. Additionally, the abundance of alternative prey and the density of slugs were found to be positively related. Thus, enclosures with high numbers of slugs also had high numbers of alternative prey. This was highlighted by the negative relationship between the activity-density of *P. melanarius* and the abundance of total prey.

Pitfall trap catches are skewed towards higher frequencies of hungry beetles in search of food (Chiverton, 1984; Mols, 1993; Fournier and Loreau, 2001, 2002). Therefore, data from the field enclosures suggest that the general level of satiation in *P. melanarius* increased over time, since their activity-density decreased. This should not necessarily be manifest in increased gut mass of those beetles caught in pitfall traps, because captured beetles should be the part of the population with lowest satiation status. However, in female beetles there was some evidence of increased foregut mass over time. This suggests an increasing availability of prey. Abundance of both slugs and alternative prey increased with time, and both groups are potential prey. The foregut mass of female beetles was positively related to the abundance of alternative prey, but neither male nor female gut mass was related to slug density. This suggests that it was the increased availability of organisms other than slugs that led to

increased gut mass. Indeed, Symondson *et al.* (2006) reported that in mini-plots the presence of alternative prey diverted *P. melanarius* away from feeding on slugs. Armsworth (2005) reported that *P. melanarius* with the largest gut masses were spatially associated with slugs, but did not provide data on the alternative prey present. The data from this experiment showed that densities of slugs and alternative prey were positively related. Thus, were a similar relationship between slugs and alternative prey to have existed in the field from which the Armsworth (2005) data was obtained, an alternative interpretation on beetle gut mass may have been possible for that data. In future experiments of this type, it might be useful to place beetle refuges inside enclosures. Gut content analysis could then be performed on beetles captured from underneath the refuges. This would provide an analysis of gut contents that was not related to activity, and should facilitate a better understanding of prey availability and consumption inside enclosures.

Predation by *P. melanarius* on slugs was not sufficient to cause negative growth in slug population size. Both groups of slugs showed significant positive growth in population size over the duration of the experiment. In the *Deroceras* population, the magnitude of growth increased, but in the *Arion* population, growth declined. It remains possible that predation pressure by *P. melanarius* on slugs did slow slug population growth, but it is an unfortunate consequence of the absence of control enclosures (zero *P. melanarius* m^{-2}), that it is not possible to determine the existence or extent of this low level controlling effect. Enclosures that excluded *P. melanarius* would have provided slug population size growth rates against which growth rates for enclosures containing beetles could have been compared. The absence of a detectable controlling effect by *P. melanarius* was despite *P. melanarius* being present in field-realistic densities (Frank, 1967; Ericson, 1977; Thiele, 1977; Scheller, 1984; Ayre, 1995; Thomas *et al.*, 1998). Symondson *et al.* (2002a) reported detecting an effect of predation by *P. melanarius* on slug population growth between years, but did not detect an effect between months within years. Weather conditions became more favourable for slugs (South, 1989b; Young and Port, 1989) during the latter time periods; there was a decrease in the number of hours of sunlight each day, and an increase in rainfall. Increasingly favourable conditions could have changed slug behaviour (Hunter, 1966; Glen, 1989), and increased the chances of them

being collected in samples. This could have given the impression that slug density was increasing, whether it was or not. However, Paulson-Lawrence and Bowers (2002) tested the mustard technique for earthworm sampling, and reported that soil characteristics, including texture, organic matter and moisture content, did not have significant effects on the efficacy of the technique.

One of the effects of lower environmental moisture availability may be to reduce the ability of slugs to produce defence mucus. Production of this type of mucus is critical in deterring attacks by carabids (Rollo and Wellington, 1979; Pakarinen, 1994a; Mair and Port, 2001, 2002; Chapter 2). Thus, during periods of low rainfall and high sunlight, when environmental moisture decreases, slugs may be at increased risk of mortality by predation. During this experiment, such periods of increased attack susceptibility occurred mainly during June and July. At that time, slug density was lower than during later periods. Thus, during June and July, the effect of predation on the slug population might have been greatest. The response of slugs to harsh environmental conditions is to migrate down through the soil (Hunter, 1966; Glen, 1989). Although the mustard technique is not highly sensitive to soil moisture conditions (Paulson-Lawrence and Bowers, 2002), during the periods of extreme dryness, when slugs had migrated far underground, the mustard solution was unlikely to have been able to penetrate deeply enough to reach them. Thus, measurements of slug density were not possible during the extremely dry periods. However, carabids may utilize the cracks that form in dry soil to hunt subterranean prey, and slugs may emerge to feed during the night. Therefore, *P. melanarius* may still be able to prey on slugs during dry conditions. Indeed, other research has found slug-positive *P. melanarius* during periods when sampling for slugs failed to yield specimens (J.R. Bell, personal communication).

In those enclosures with highest prey availability, the activity-density of *P. melanarius* was reduced. The loosely defined group of 'alternative prey' contained other predators, some of which may be competitors and some potential prey for *P. melanarius*. Abundances of other predators were low in relation to primary consumers. No attempt was made to sub-categorize 'alternative prey', because of the complexities involved in the food-web interactions. Some species may be both competitor and potential prey to *P. melanarius*. Species that are not prey for *P. melanarius* may be prey for another

predator which in-turn may be prey for *P. melanarius* (Sunderland, 2002). Additionally, any organism may be a food resource in carrion form. Bell *et al.* (2010) reported that primary consumers were highly aggregated; it appears that the enclosures were successful at capturing the clumped distribution of primary consumers. In-turn, the differential availability of prey had an effect on *P. melanarius* activity-density.

Densities of slugs are relatively easy to measure and interpret, but densities of beetles can be difficult to interpret from pitfall trapping measurements, because pitfall trapping measures activity-density, which is a combined measure of activity and density (Mitchell, 1963). This experiment was designed to limit the complexities of interpreting beetle activity-density by having known densities of beetles inside enclosures at the start of the experiment. The extant beetles inside enclosures at the time of beetle augmentation were not a significant problem, because the presence of marks on the augmented beetles was expected to facilitate estimation of total beetle density, based on the ratios of marked and unmarked beetles captured during the pitfall trapping effort (e.g. Ericson, 1977). Thus, the failure of the markings made on beetles was a hindrance to understanding the initial beetle densities inside enclosures. Marking using alternative methods (Griffiths *et al.*, 2001; Winder, 2004) is recommended for future studies.

One of the main factors influencing activity of carabids is prey searching, which itself is motivated by hunger (Chiverton, 1984; Mols, 1993; Fournier and Loreau, 2001, 2002). The assertion is made, that beetles of lower satiation status, searching for food, are more likely to be caught in pitfall traps than beetles of higher levels of satiation. Therefore, it follows that, where prey are more numerous, relatively fewer predators should be captured, and *vice versa.* The interpretations of data reported in this experiment are based upon these principles. Therefore, it is implicit that density of *P. melanarius* across sampling time periods remained relatively stable. Luff (1982) suggested that the activity-density of *Harpalus rufipes* was strongly related to population density, but that was in an open-field environment, where beetles are very mobile (Symondson *et al.*, 1996, 2002a; Thomas *et al.,* 1998; Bohan *et al.*, 2000a; Winder *et al.*, 2001, 2005), which strongly influences density. Indeed, Purvis and Fadl (1996) reported that the emergence density of *P. melanarius* in winter wheat was 13.8

m^{-2}. This value for density at emergence is considerably higher than the majority of values reported for general density of adult *P. melanarius* (Frank, 1967; Ericson, 1977; Thiele, 1977; Scheller, 1984; Ayre, 1995; Thomas *et al.*, 1998). It suggests overwintering of beetles may occur in aggregations, which later disperse, thus, emphasizing the effects of mobility on density. The polythene barriers employed in this experiment reduced mobility of beetles, and prevented immigration and emigration. Therefore, the events affecting beetle population size were limited to emergence and mortality (see section 1.3.4). Mortality in carabid eggs and larvae are reported to be extremely high: 70 – 80 % for eggs and 90 % for larvae (Van Dijk and Den Boer, 1992), but within-year survival rates for adult carabids are reported to be high. Ericson (1977) estimated mean daily survival rates in *P. melanarius* as 0.905 (males) and 0.895 (females), and 0.913 in *Pterostichus cupreus*. Luff (1980) reported mortality of 10 % per month in *Harpalus rufipes*. Mortality rate in adults may, however, increase in the autumn (Luff, 1980). Therefore, adult population density should decline over time, but at a low and steady rate. Additionally, protracted emergence of beetles would enhance stability of population density by having an opposing effect to that of mortality. Emergence occurs mainly from the end of May through June (Thomas *et al.*, 2002a), but may be protracted (Ericson, 1977; Matalin, 2006), even extending into the autumn (Wallin, 1989).

The rate of decline in *P. melanarius* activity-density was greatest between sampling time periods two and four. Activity-density of carabids may be positively related to temperature (Luff, 1982; Ayre, 1995). The days over which the pitfall trapping was conducted at sampling time two, were hotter than those at sampling times four, five and six, although there was a short temperature spike during sampling at time five. Additionally, behaviour of teneral individuals may increase their chances of being caught (Vlijm and Dijk, 1967 cited in Ericson, 1977); an effect that would decrease with time. Also, greater activity-density might be expected after emergence, as the cohort that overwintered as adults seeks to find prey on which to feed for the first time since the previous autumn. Indeed, there was a significant increase in the foregut mass of both male and female beetles from sampling time point two to four. This was the only increase that occurred in the male population.

The data reported in this experiment illustrate a temporal effect in the proportions of *P. melanarius* that were slug-positive, with proportions of positives ranging from 1.81 % to 8.50 % for *Deroceras* spp., 5.25 % to 18.0 % for *Arion* spp., and 7.07 % to 26.5 % for slugs in general. Comparative values for *P. melanarius* from other studies in arable fields are: 9 % slug-positive using ELISA (Ayre and Port, 1996); 83.5 % mollusc-positive using ELISA (Symondson *et al.*, 1996); 11 % slug-positive using ELISA (Bohan *et al.*, 2000a); 3.8 % *D. reticulatum*-positive, and 1.0 % *Arion* spp.-positive using PCR, and 8.0 % *D. reticulatum*-positive, and 43 % mollusc-positive using ELISA (Dodd, 2004). Differences in sensitivity between PCR-based and ELISA-based techniques mean that comparison of consumption rate estimates is not straight forward. ELISA tends to have a longer detection period (Symondson and Liddell, 1995). However, the rates of slug-positive *P. melanarius* in this study were higher than those observed in the other study using PCR (Dodd, 2004), and higher than those observed by Ayre and Port (1996) and Bohan *et al.* (2000a), both of which used ELISA. Despite the relatively high proportions of slug-positive beetles, negative growth in the slug population did not occur.

The number of slug-positive *P. melanarius* per enclosure was negatively related to the abundance of alternative prey, i.e., where there was greater abundance of alternative prey there were fewer slug-positive beetles. However, there was no relationship between slug-positive *P. melanarius* and slug density. This tends to suggest that alternative prey were fed upon in preference to slugs.

Using DNA detection decay rates it is possible to estimate daily slug-consumption rates for *P. melanarius*. The decay half-life for detection (the time at which positive samples yielding positive results is reduced to 50 % of the total number of samples) of *Arion* spp. DNA in the guts of *P. melanarius* using the Ai1F and AR2R primer combination is 17.2 h from the time of feeding, with zero detection after two days. For detection of *Deroceras* spp. DNA using the DR11F and DR50R primer combination, the decay half-life is 19.7 h post-feeding, and there is zero detection after two days (Dodd, 2004). Therefore, all *Arion* spp. and *Deroceras* spp. were consumed within the two days prior to beetles being caught, but most were probably consumed during the 24 h prior to being captured and frozen. However, since *P. melanarius* are mainly active at night (on average night time was between the hours of 8:00 pm and 5:30 am during

the experimental period), and given that the mean time (t) at which pitfall traps were emptied was approximately 10.30 am, then during the two days prior to capture, their periods of main activity would have been approximately: t - 5 h to t - 14.5 h, and t - 29 h to t - 38.5 h. The probability of detecting DNA from slugs consumed during the period from t - 29 h to t - 38.5 h is considerably lower than that from t - 5 h to t - 14.5 h, therefore, in the majority of cases, slug tissue had been consumed during the previous night. Thus, the data for the proportion of slug-positive beetles provide an indication of the daily rate of slug consumption by a population of *P. melanarius*. However, the estimation may be lower than the actual consumption rate, because pitfall traps caught beetles that were generally of the lowest levels of satiation status. Therefore, slug consumption may have been higher in the part of the *P. melanarius* population not caught in the pitfall traps.

The term 'consumption rate' is used in preference to 'predation rate', because of the inability to distinguish true predation, secondary predation, and scavenging (Sunderland, 1996). It can be argued that the decreasing proportions of slug-positive *P. melanarius* with time are an indication that most slug-positive results were due to predation, because median slug mass increased over time, and there is evidence to suggest that *P. melanarius* preferentially predate on small slugs (McKemey *et al.*, 2001). Therefore, it would be expected that as the size of slugs increased, predation by *P. melanarius* would decrease. However, this argument in favour of predation over scavenging relies upon a constant mortality rate in slugs. South (1982) considered mortality in slugs to be reasonably consistent. Although, as time progressed the weather conditions generally became more favourable to slugs. Under such conditions their mortality rate might be expected to decline. Additionally, mortality rate in juveniles is greater than that in larger (older) slugs (South, 1982); therefore, reduced recruitment may also have led to a reduction in slug carrion availability. Conversely, a source of increased mortality would be from mature adults after egg laying.

The proportion of *Arion* spp.-positive beetles was, at all sampling time periods, greater than the proportion of *Deroceras* spp.-positive beetles. Additionally, at sampling time period two, the relative proportions of *Arion* spp.-positive and *Deroceras* spp.-positive beetles were significantly different to the

relative abundances of the two slug genera inside field-enclosures; the difference favouring feeding on *Arion* spp. Furthermore, the foregut mass of *Deroceras* spp.-positive beetles was greater than that of the *Arion* spp.-positive beetles (significantly in male beetles, but not in females). Since the median *Deroceras* spp. individual was at all times more massive than the median *Arion* spp. individual, the propensity of *P. melanarius* to consume *Arion* spp. to a greater extent than *Deroceras* spp., but for *Deroceras* spp.-positive beetles to have had greater foregut mass, both accord with the finding that *P. melanarius* feed to a higher degree on small slugs (McKemey *et al.*, 2001). It can be concluded from the foregut mass data that *P. melanarius* were feeding on the smaller individuals of the *Arion* spp. population, because were the *Arion* spp. to have been large individuals then the beetles should have eaten more, and their foregut masses should have been the same as those of the *Deroceras* spp.-positive beetles. At this stage it cannot be concluded that the *Deroceras* spp. individuals fed upon were small, because the maximum foregut mass of *P. melanarius* is not known. Therefore, it is unknown whether the mean foregut mass of the *Deroceras* spp.-positive beetles was at the level observed due to guts being at their maximum capacity, in which case it would be impossible to estimate whole slug mass because it would be impossible to know what proportion of the slug had been consumed; or alternatively, whether foregut mass represented sub-maximum capacities because the *Deroceras* spp. consumed were small.

The mechanisms underlying the increased median mass of slugs should be decreased frequencies of smaller slugs as a result of diminished recruitment, coupled with accumulated gains in mass of individuals, acquired through feeding. The most plausible explanation for a feeding preference on smaller slugs is that the efficacy of their defence mechanisms (Rollo and Wellington, 1979; Pakarinen, 1994a; Mair and Port, 2002; Foltan, 2004; Chapter 2) is lower than that of larger slugs. It is likely that increasing slug mass was an important factor in reducing the level of feeding on slug tissue, although, there may have been additional factors. The abundance of alternative prey generally increased with time: there was a negative relationship between slug-positive *P. melanarius* and abundance of alternative prey. As slugs became larger, and alternative prey became more abundant, it might be expected that there would be reduced

feeding on slugs and increased feeding on alternative prey. Additionally, mortality rate in larger (older) slugs is lower than that in juveniles (South, 1982), and may have led to a reduction in slug carrion availability.

Slug population size increased significantly between 15 July and 5 September (the dates of the first successful and final samples). The standard model for the dynamics of a population (section 1.3.4) considers the size of a population at a discrete point in time to be dependent upon the population size at a previous point in time, plus the numbers of immigrants and births, minus the numbers of emigrants and deaths that occurred during the interim period. In this experiment it was accepted that the polythene barriers would not prevent slugs climbing into or out of enclosures, but the assumptions were made that the flow of individuals in both directions would be minimal due to the limited dispersal of slugs (South, 1965, 1992; Fleming, 1989; Armsworth, 2005), and that the numbers entering and leaving would cancel-out. However, it is possible that the 30 cm-high barriers forming the enclosures had an effect on the microclimate inside. It is conceivable that reduced amounts of wind passing through enclosures facilitated higher humidity and soil moisture. These conditions are favourable for slugs and may have facilitated increased survival, and led to a positive flow of slugs into enclosures. Conversely, reduced wind passing through enclosures could have had the effect of increasing temperature, which may have been less conducive for slugs. Based on the assumptions of minimal and equal immigration and emigration of slugs, the growth in slug population size would be dependent upon only birth (egg hatching) and death events. Therefore, the slug population size increase was due to the recruitment rate being greater than the mortality rate.

Judging by the frequencies of slugs in the 0 – 9 mg mass-class, the rate of recruitment was not constant over the experimental period; there was a general decline. In *Deroceras* spp., recruitment decreased at each successive sampling time point. Recruitment to the *Deroceras* spp. population was lower than the increase in population size. Additionally, there were relatively large increases in frequencies of slugs in the larger mass-classes. Sampling bias for larger slugs, or changes in slug activity over the experimental period might account for the deficit between recruitment and increase in population size. All things being equal, it might be expected that sampling bias would remain equal through time,

thus, if there was an increasing bias in the sampling technique for large slugs, it should have varied with some other factor. Paulson-Lawrence and Bowers (2002) reported that there was no bias for or against juveniles when using the mustard technique to sample for earthworms. There was, however, variability in the effectiveness of the technique among species. If there had been a bias towards one of the slug genera, it seems more likely that it would have been towards *Deroceras* spp., since a greater proportion of their population is found nearer the soil surface (Hunter, 1966). Environmental conditions (South, 1989b; Young and Port, 1989; Choi *et al.*, 2004, 2006) should have been better for slugs after sampling time period three, because there was a decrease in the number of hours of sunlight each day, and an increase in rainfall. This could have facilitated behavioural changes in larger slugs, and increased the probability of them being collected. Interestingly, the reported autumn hatching peak in *D. reticulatum* (Hunter, 1966; South, 1992) had not occurred by 7 September, or it was not detected.

In the *Arion* spp. population there was also a general decline in recruitment. After an initial increase from sampling time period two to four, there was a decrease at each successive time point. Recruitment to the *Arion* spp. population was considerably greater than that in the *Deroceras* spp. population, and was sufficient to explain the increase in population size through time.

At the time the first sample was collected (time point two), the population density of *Deroceras* spp. was lower than that of *Arion* spp. The subsequent greater rate of growth in density of *Deroceras* spp. meant that by the time the final sample was collected (time point six), densities of the two genera were approximately equal. This was despite the *Arion* spp. population having had greater recruitment during each time period. Based on these observations of population dynamics, considered in conjunction with the higher frequencies of small slugs in the *Arion* spp. population, the propensity of *P. melanarius* to preferentially prey upon small slugs, and the greater proportions of *Arion* spp.-positive beetles, it can be hypothesized that the slower growth rate in the *Arion* spp. population was due to the increased predation pressure by *P. melanarius*.

Pitfall trap catches might be skewed towards higher frequencies of males searching for females with whom to mate. Symondson *et al.* (1996) reported catching significantly more male *P. melanarius* than females. The same authors

also suggested a greater role for female *P. melanarius* in slug pest control, because more females were slug-positive. In this study there was no difference in numbers of males and females caught, neither was there a difference among the proportions of males and females that were slug-positive.

The relatively high foregut mass of females at sampling time six might be a response to many females having recently laid their eggs, and subsequently seeking to accumulate fat reserves to survive the winter (Van Dijk, 1994). Sunderland (1975) reported that the proportion of beetle guts that were empty was related to sex, and whether females contained eggs: the proportion of female guts containing food was higher in those individuals without eggs.

In conclusion, *P. melanarius* fed upon slug tissue, but the proportion of feeding that was the result of predation, rather than scavenging, was not at a sufficiently high rate to bring about negative growth in the slug population size. Although, the slower rate of population growth in *Arion* spp. may have been due to predation pressure by *P. melanarius*. Slug-predation was visited upon the smaller individuals, consequently, as the mean mass of slugs increased over time, there was a decline in the rate of slug-predation. The presence of alternative prey was very important for *P. melanarius*; feeding upon alternatives reduced slug-predation rates.

Chapter 7 -

General discussion

7.1 Slug pest control

The opening chapter of this thesis described how slugs are thought to be major agricultural pests (Gould, 1961; Port and Port, 1986; Central Science Laboratory, 2008), and the principal pest in some crops (Glen, 1989). Agriculturalists can employ a tripartite integrated pest management approach to mitigate crop yield losses caused by slug damage: cultural control, chemical control, and natural enemies. Each of the three elements have been reported to have negative effects on slug populations, or facilitate reductions in crop damage (see reviews: Port and Port, 1986; Glen and Moens, 2002; Symondson *et al.*, 2002b). Additionally, there may be synergistic effects (Godan, 1983; Kelly and Martin, 1989; Langan *et al.*, 2004). Therefore, it may be in the interests of agriculturalists to make use of all three elements.

Effects caused by natural enemies can be obtained through augmentation, such as with the nematode, *Phasmarhabditis hermaphrodita* (Georgis *et al.*, 2006), or reliance upon naturally occurring pest control agents. Carabid beetles are considered one of the main predators of slugs (Tod, 1973; Godan, 1983; Symondson *et al.*, 1996; Bohan *et al.*, 2000a; Symondson *et al.*, 2002a). Within this taxon, specialist slug predators do exist (Pakarinen, 1994a; Wheater, 1989), but they are present in low densities. Therefore, predation pressure by this group is unlikely to have significant effects on slugs at the population level. Generalist predator carabids may be present at higher densities, and consume slug tissue (Symondson, 1989, 1994; Asteraki, 1993; Pakarinen, 1994a; Ayre, 1995; Symondson *et al.*, 1996, 2002a; Kromp, 1999; Langan *et al.*, 2001; Mair and Port, 2001). Research conducted on *Pterostichus melanarius* has led to this species being considered one of the main candidates capable of exerting significant control on slug pest populations (Symondson *et al.*, 1996, 2002a; Bohan *et al.*, 2000a).

Generalist predators can have strong trophic links to individual, or small groups of prey species (Villafuerte *et al.*, 1996; Angerbjorn *et al.*, 1999), and can be effective agents of pest control (Symondson *et al.*, 2002b). Nevertheless, their generalist, opportunistic habit may make them unreliable

agents of pest control. *Pterostichus melanarius* has been described as an ultra-generalist (Den Boer, 1982; Symondson *et al.*, 2002b), preying upon a wide range of animals (Pollet and Desender, 1986; Symondson *et al.*, 1996, 2000, 2002b; Kromp, 1999; Sunderland, 2002), eating seeds (Honek *et al.*, 2003; Koprdova *et al.*, 2008), and scavenging carrion (Calder *et al.*, 2005; Foltan *et al.*, 2005).

The overall picture of *P. melanarius*, generated by the research done to date, is one of an ultra-generalist predator that may at times prey heavily upon slugs (Symondson *et al.*, 1996, 2002a; Bohan *et al.*, 2000a), but at other times negligibly or not at all (Symondson *et al.*, 2006). When it occurs, predation may be limited to certain strata within slug populations (McKemey *et al.*, 2001). Beetles exhibit limited ability to sense slugs in the environment (McKemey *et al.*, 2004), but may be coincidently spatially aggregated with slugs (Bohan *et al.*, 2000a; Bell *et al.*, 2010). Scavenging of slug cadavers may be a significant confounding factor in the trophic interaction (Foltan *et al.*, 2005).

This project set out to investigate some key areas within the trophic interaction between *P. melanarius* and slugs. The main hypotheses tested were that: (1) the vital status of prey does not influence prey selection by *P. melanarius*, (2) the feeding history of *P. melanarius* does not affect prey selection, (3) *P. melanarius* are unable to detect *Deroceras reticulatum* by olfaction, and (4) *P. melanarius* do not exert a controlling effect on in-field slug populations. The following is a general discussion on the results obtained from the tests of those hypotheses, set in the wider context of the arable field environment.

7.2 The generalist and opportunistic habits of *Pterostichus melanarius*

Food for carabids is considered to be generally scarce (Sunderland, 1975; Van Dijk, 1982, 1996). This may to some degree sound rather counter intuitive given the array of pesticides commercially available, and the expenditure of time and money given over to pest management. However, prey availability can be lower than the actual abundance of organisms for many reasons: spatial

heterogeneity of prey distribution; temporal (daily or longer) separation of activity periods between predator and prey; disparity in relative sizes of predators and prey; efficacy of prey defence mechanisms; and differential use of the environmental matrix, e.g. earthworms are subterranean, whilst aphids dwell on plants. In the face of prey scarcity, it may be the optimum solution for *P. melanarius* to employ an ultra-generalist, opportunistic approach to feeding: taking any sources of energy and nutrients encountered. Slugs are a viable food resource in terms of quality (Chapter 5), therefore, it should be expected that in-field beetles would seek to exploit encounters with slugs. Indeed, it was suggested that slugs represent the greatest pest biomass in arable fields (Sunderland, 2002), therefore, slugs should provide a significant potential food resource, and encounters might be frequent.

Specialist predators exhibit adaptations that may increase their efficacy at finding and handling specific prey. Slug-specialist carabids (*Cychrus caraboides* and *Carabus violaceus*) target their attacks at specific locations on the bodies of their prey (Pakarinen, 1994a, Mair and Port, 2002). This action has the effect of neutralizing the mucus defence of the prey. Perhaps it should not be expected of generalists to possess traits that are prey-taxon specific. The feeding trials with *P. melanarius* and live *D. reticulatum* (Chapter 2) illustrated that *P. melanarius* does not target specific areas on the bodies of slugs when it makes attacks. Consequently, under the conditions prevailing in that experiment, where the mean slug mass was 157 mg and alternative prey were present, all attacks were unsuccessful. It was also suggested that *P. melanarius* are unable to detect *D. reticulatum* by olfaction (Chapter 4).

Although detection by olfaction was not displayed, *P. melanarius* can detect mucus trails (McKemey *et al.*, 2004). In response to the limited ability to detect slugs by chemoreception, a model for *P. melanarius* searching behaviour was proposed (Chapter 4). Essentially, it suggested that chemoreception in *P. melanarius* was a mechanism employed for detection over small distances. Based upon models of random walking (Reynolds and Rhodes, 2009), at some point in their random walk sequence, *P. melanarius* switches from rapid walking aimed at achieving large displacement, to slow tortuous walking (Mols, 1993), during which chemoreception might play an important role. Taking this further, it might be suggested that whether or not slugs are present in the area in which

beetles switch to tortuous walking is a stochastic event. Therefore, ultimately, this stochasticity may be an important factor in determining whether *P. melanarius* prey upon slugs. When slugs are present in the area, and have laid mucus trails, then this means of betraying their presence may confer increased probability on slugs being preyed upon in that area.

7.3 Slug tissue as a preferred food of *Pterostichus melanarius*

Pterostichus melanarius aptly demonstrated its preference for slug tissue in dead-prey bioassays (Chapter 2): it was consumed in preference to all of the alternatives available. Of particular significance was the preference over earthworm, which itself has been proposed as an important food for *P. melanarius* (Symondson *et al.*, 2000; King *et al.*, 2010). Furthermore, beetles that had a history of feeding solely on slug tissue for five weeks did not discriminate against further feeding on slug when presented with viable alternatives (Chapter 5).

Feeding history had a significant effect on prey selection (Chapter 5), but only within an overarching prey preference hierarchy. The prey preference hierarchy was: *Calliphora vomitoria* > *Aporrectodea caliginosa* > *D. reticulatum* > *Brassica napus*. Prey items presented were dead. The preference hierarchy exhibited by *P. melanarius* during the dead-prey bioassays in the experiment that tested the effects of prey vital status on prey selection (Chapter 2) was: *D. reticulatum* > *A. caliginosa* > *C. vomitoria* > *P. melanarius*. Interestingly, preference among the three prey types common to both experiments was reversed.

One of the main differences among beetles used in the two experiments was their feeding histories: the beetles used in the prey vital status experiment had been fed on cat food prior to bioassays; the beetles used in the feeding history experiment were split into five groups, each of which had received a single food type for five weeks. One of the diet-conditioned groups of beetles was fed cat food, and therefore direct comparison can be made between that group and those in the prey vital status experiment.

There were other differences between beetle capture and maintenance conditions prior to the experiments being conducted. Beetles were from different populations, caught in different fields on Rothamsted Farm, at different times over the adult activity period. They were maintained under controlled environment conditions for different durations, and under different containment conditions. Bioassays were conducted at different times during the adult activity period. Beetles used in the prey vital status experiment (Chapter 2) were caught from 08 August 2008, and maintained in mixed sex groups for 54 days before being bioassayed from 01 October 2008. Beetles used in the feeding history experiment (Chapter 5) were caught from 14 July 2009, and maintained individually for 34 days before being bioassayed from 17 August 2009. Additionally, prey were also different: purchased a year apart (*C. vomitoria*), or caught in different fields at different times (*D. reticulatum* and *A. caliginosa*).

Two of the potentially most important differences were that: first, males and females were mixed during the maintenance period of beetles used in the prey vital status experiment. Thus, there were higher probabilities of beetles having mated and females being gravid. The gravid stage of life-history in females may affect feeding behaviour (Sunderland, 1975; Van Dijk, 1994; Raubenheimer *et al.*, 2007). Second, the prey vital status experiment was conducted during October, which is outside the main activity period for adult *P. melanarius* (Ericson, 1977; Luff, 1982; Wallin, 1989; Symondson *et al.*, 1996; Thomas *et al.*, 2002a; Matalin, 2006).

Comparison of results from the two experiments serves to emphasize the myriad of factors that may influence feeding behaviour, and the problems associated with interpreting and extrapolating from laboratory based experiments (McKemey *et al.*, 2003). The pair of experiments also emphasize that prey preference is a highly dynamic process.

7.4 Importance of slug size on predation by *Pterostichus melanarius*

Slug size appears to be a critical factor in the predatory interaction between *P. melanarius* and slugs. All attacks by *P. melanarius* on the *D. reticulatum*

individuals presented as potential live-prey (Chapter 2) were successfully repelled, with the consequence that no feeding on live *D. reticulatum* occurred. It was further shown that attacked slugs do not suffer reduced survival (Chapter 3). Thus, such attacks would not have a positive effect on pest control. It is fortunate, therefore, that aborted attacks are not detected by PCR-based molecular analysis of foregut contents, and so do not introduce a confounding effect when interpreting slug-consumption rates from foregut content data.

The mucus defence of slugs appeared to be the principal mechanism responsible in deterring attacks (Chapter 2). Reduced efficacy of defence mechanisms may confer increased susceptibility to predation on very small slugs, such that *P. melanarius* can kill small slugs (McKemey *et al.*, 2001). However, small slugs may benefit from being more difficult to locate (McKemey *et al.*, 2003).

Data obtained from field enclosures (Chapter 6) on slug mass, and beetle foregut mass and contents, suggested that *P. melanarius* were consuming smaller slugs. As the size of slugs increased, so the rate of consumption decreased. If consumption was due to predation, rather than scavenging or secondary predation, then *P. melanarius* preying upon small slugs were making a positive contribution to slug pest control. Additionally, molluscicides may have reduced efficacy against small slugs (Godan, 1983; Kelly and Martin, 1989), therefore, predation by *P. melanarius* on the small slug class may be an advantage when an integrated approach to pest management is employed.

7.5 Slug population control by *Pterostichus melanarius*

Despite the presence of slug DNA in the guts of a comparatively large proportion of *P. melanarius* (Ayre and Port, 1996; Symondson *et al.*, 1996; Bohan *et al.*, 2000a; Dodd, 2004), these beetles were unable to enforce negative growth on slug population size (Chapter 6). Molecular analysis could not confirm that slug-positives were the result of predation rather than scavenging or secondary predation, however, some degree of predation is likely to occur. Analyses of the slug mass data (Chapter 6) and other research

(McKemey *et al.*, 2001) suggest that any effect of predation pressure on slug populations is likely to be exerted on the smallest slugs. The absence of control enclosures (with zero *P. melanarius*) meant that detecting the subtle effects of predation pressure by *P. melanarius* on slug populations was not possible.

The field in which the slug population control experiment (Chapter 6) was conducted received a molluscicide application on 4 October 2007, a few days after the crop had been sown. No further molluscicide was applied. Thus, the effect of *P. melanarius* on slug population size was tested in the absence of molluscicide. Acting unilaterally, *P. melanarius* did not exert negative population growth on slug populations, but complementarity between the effects of metaldehyde, and predation by *P. melanarius* on slugs that have consumed sub-lethal doses of metaldehyde (Godan, 1983; Kelly and Martin, 1989), might induce greater negative effects on slug populations than those by metaldehyde alone. Thus, the presence of this predator may still be of importance for slug pest control.

The period over which the experiment ran coincided with most of the *P. melanarius* imago activity period (Ericson, 1977; Luff, 1982; Wallin, 1989; Symondson *et al.*, 1996; Thomas *et al.*, 2002a; Matalin, 2006), but no effect on slug population growth was detected. Similarly, Symondson *et al.* (1996, 2002a) sampled only adult *P. melanarius* over their main activity period, and did not detect within year effects on slug population growth, but among year effects were detected (Symondson *et al.*, 2002a). In contrast to the seasonality of *P. melanarius* adults, slugs may be active throughout the year (South, 1982, 1989a; Godan, 1983). Additionally, the hatching peaks of *Arion* spp. and *D. reticulatum* occur in the spring (Hunter, 1966; South, 1992), before *P. melanarius* adults are active, and the autumnal secondary hatching peak of *D. reticulatum* occurs at a time when the density of adult *P. melanarius* may be in decline (Luff, 1982). Therefore, the role of *P. melanarius* larvae (Thomas *et al.*, 2009) preying upon slugs outside the period of adult activity might be of significant importance, and may have played a role in producing the between year effects observed by Symondson *et al.* (2002a). Very little research has been done on the larvae of *P. melanarius*; this is likely to be due to the difficulties involved in studying subterranean organisms. However, the densities of larvae should be greater than the densities of adults, and the sole function of

larvae is to feed in order to undergo metamorphosis. Therefore, the potential exists for them to make a positive contribution to slug pest control.

Adult *P. melanarius* consume slug eggs under laboratory conditions (Oberholzer and Frank, 2003; Hatteland *et al.*, 2010). Therefore, *P. melanarius* adults, and potentially larvae, may moderate slug population size by reducing recruitment through egg predation. Thus, the effects of *P. melanarius* on slug pest control may potentially take numerous forms: predation by adults on slugs and eggs, predation by larvae on slugs and eggs, and synergistic effects by adults and larvae with metaldehyde-based molluscicides. The field experiment (Chapter 6) was not designed, or able, to detect the effects of all mechanisms. Thus, the absence of negative growth in slug population size inside the field-enclosures does not rule-out the importance of *P. melanarius* to integrated pest management programmes against slugs.

7.6 Can and should agriculturalists do anything to encourage *Pterostichus melanarius*?

The research reported in this thesis set out to investigate elements of the trophic interaction between *P. melanarius* and slugs. The overall conclusion drawn from the data obtained during experimentation, particularly that obtained in the field experiment (Chapter 6), is that the generalist habit of *P. melanarius* is an impediment to the imago of this carabid exerting significant negative effects on slug population size. However, any amount of predation on slugs by adult *P. melanarius* is desirable for pest management. There is also the potential for predation on slug eggs (Oberholzer and Frank, 2003; Hatteland *et al.*, 2010), and predation by larvae (Thomas *et al.*, 2008, 2009). Therefore, it would be prudent for agriculturalists to make accommodations for *P. melanarius*. Additionally, as a consequence of its generalist habit, *P. melanarius* will also prey upon other agricultural pests, for example: aphids, wireworms, and leatherjackets (Pollet and Desender, 1986; Symondson *et al.*, 1996, 2000, 2002b; Kromp, 1999; Sunderland, 2002).

Cultural techniques and their timings affect carabids. Tillage generally has negative effects on *P. melanarius* (Symondson *et al.*, 1996). Therefore, direct-

drilling of seeds, and incorporation of straw by non-inversion tillage are preferred. Where tillage is employed, it may be most damaging during the spring (Purvis and Fadl, 1996). However, tillage also has negative effects on slugs (Glen, 1989; Symondson *et al.*, 1996, 2002a).

Management of beetle banks, field margins and hedgerows (Thomas *et al.*, 2002b; Collins *et al.*, 2003; MacLeod *et al.*, 2004; Pywell *et al.*, 2005) can affect the abundance and species composition inside arable fields. They can provide refugia for other beneficial arthropods, and alternative prey for *P. melanarius*.

The application of insecticides for control of insect pests have the potential to directly inflict mortality on *P. melanarius*, affect densities of alternative prey, and yield carrion (Gyldenkaerne *et al.*, 2000; Mauchline *et al.*, 2004; Navntoft *et al.*, 2006). These factors can all affect the trophic interaction between *P. melanarius* and slugs.

Molluscicide in the form of methiocarb is toxic to *P. melanarius* (Langan *et al.*, 2004), and application to fields can have negative impacts upon the *P. melanarius* population (Kennedy, 1990; Purvis and Bannon, 1992). Metaldehyde apparently does not affect beetle longevity (Langan *et al.*, 2004); additionally, efficacy of metaldehyde application may be increased through synergism with *P. melanarius* (Godan, 1983; Kelly and Martin, 1989; Langan *et al.*, 2004).

It can be seen that conflicts of interest may occur, where actions in favour of *P. melanarius* also favour slugs, or other arthropod pest species. Similarly, the provision of alternative prey for *P. melanarius* may help to sustain its population, and thus provide maximum impact on slug populations, but it may also divert beetles away from feeding on slugs. A balance needs to be found.

7.7 Future areas of research

The extent of the synergy between metaldehyde and *P. melanarius* is important to understand, because that may be one of the areas in which adult *P. melanarius* are most important to integrated pest management programmes against slugs. An enclosure experiment based upon the one described in Chapter 6 could be employed to test the synergistic effect. After removal of *P.*

melanarius from enclosures, the treatments then applied would be: (1) *P. melanarius*, (2) metaldehyde, (3) *P. melanarius* + metaldehyde, (4) control (no *P. melanarius* or metaldehyde). Analyses would focus upon slug population dynamics and mass-class frequencies, and gut contents of beetles.

The slug-conducive microclimate conditions that prevail under oilseed rape crops are suggested to enable strong growth in slug populations (Glen, 1989). The resulting large population then causes severe damage to winter wheat crops that follow oilseed rape in the crop-rotation. The conducive microclimate conditions that the dense canopies of rape crops provide may facilitate increased rates of slug activity, which in-turn may make them more susceptible to predation. Additionally, there may be high frequencies of small slugs in establishing populations. Both of these factors could mean that predation by *P. melanarius* might be at least as important in the oilseed rape crop as in the winter wheat crop. This could be tested with a repeat of the field experiment described in Chapter 6, but on this occasion performed in a crop of oilseed rape.

An in-field experiment to identify the scavengers of slug cadavers would be informative in helping to understand the extent to which scavenging may confound slug consumption-rate estimates. An array of infra-red video-recording cameras could be set-out, each trained on a slug cadaver. Analysis of the video recordings would identify the principal species in the slug cadaver-scavenging guild, and the extent to which slug cadavers are removed by *P. melanarius*. Data would provide an estimate of the proportion of cadavers removed by *P. melanarius*. Along with other parameter data, a simple modelling approach could be employed to estimate the proportion of slug-positive beetles that had scavenged. Other parameter data that would be necessary would include estimates of the proportions of cadavers that might be scavengable by *P. melanarius*, and the quantities of carrion available. Data exist on the above-, below-ground locations of slug cadavers killed by various means (Pechova and Foltan, 2008). However, estimates of the quantity of carrion available might be difficult to produce, since it is not know what proportion of slugs become carrion, rather than being preyed upon before reaching that state. Any estimates made would have to reflect spatial and temporal variability.

When attempts are made to quantify the contributions of predators to integrated pest management programmes, justifiably, attention is often given to the potentially confounding effects of carrion consumption. However, the process is yet more complex, because not all predation is equally beneficial. Predation on moribund individuals from the pest population may make no greater contribution to pest control than scavenging. Research has shown that *P. melanarius* feed to a greater extent on stressed (Mair and Port, 2002), and molluscicide-fed (Langan *et al.*, 2001) slugs. Thus, in order to more fully understand the contribution of *P. melanarius* to slug pest control, in addition to knowing which was predated and which was scavenged, it is necessary to know whether the predated slugs were healthy or moribund.

Finally, given the paucity of research on *P. melanarius* larvae, understanding of their trophic behaviour and potential contribution to slug pest control is extremely limited. Thus, this is an obvious area in which future research could be targeted, particularly experimentation at the (semi-) field scale. However, the difficulties associated with working on the larvae of *P. melanarius* (Thomas *et al.*, 2009) may render this an idealistic rather than realistic proposal.

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Abbreviations and symbols

General

bp	base pairs
DNA	deoxyribonucleic acid
ELISA	enzyme-linked immunosorbent assay
n	number
n_D	number of dead-prey bioassays
n_f	number of female
n_L	number of live-prey bioassays
n_m	number of male
NPI	non-prey-interaction
PCR	polymerase chain reaction
PIEF	prey-interaction excluding feeding
rRNA	ribosomal ribonucleic acid
UK	United Kingdom

Units

°C	degree Celsius
g	gram
h	hour
l	litre
m	metre
mol	mole
min	minute
s	second

Mathematical notations

%	percentage
Σ	summation
µ	10^{-6}
m	10^{-3}

c	10^{-2}
k	10^{3}

Statistical analysis

ANOVA	analysis of variance
CVA	canonical variates analysis
df	degrees of freedom
F	F-test
GLM	generalized linear model
H	Kruskal-Wallis test
LSD	least significant difference
MANOVA	multivariate ANOVA
P	probability value
Q	Friedman's non-parametric ANOVA
r^2	coefficient of determination
SEM	standard error of mean
t	t-test
U	Mann-Whitney U-test
χ^2	chi-square test

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