Evaluation Of Carabids As Predators Of Slugs In Arable Land

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Dedicated to Ruth

Abstract

An Enzyme-Linked Immunosorbent Assay (ELISA) was developed which detected slug antigens in postmortem gut analysis of carabid beetles. The ELISA was used to identify beetles which fed on slugs in three fields of oilseed rape and winter wheat in the Tyne valley, Northumberland.

Generalist species such as *Harpalus rufipes*, *Pterostichus melanarius*, *Pterostichus madidus*, *Amara similata* and *Nebria brevicollis* fed on slugs in the field. Molluscan specialists such as *Carabus violaceus* and *Cychrus caraboides* also fed on slugs in the field.

Laboratory studies indicated that many large and medium sized carabids were able to predate small slugs. Some beetle species did not eat slugs but exposure to the beetles increased slug mortality. Therefore, postmortem investigations may underestimate the impact that carabids exert on slugs as they do not measure the number of slugs killed.

Slug mucus affected the locomotory activity of generalist and specialist beetle species. Beetles foraged longer, covered greater distances, made more turns, walked slower and spent more time stationary on soil covered in slug mucus compared to control areas.

Abax parallelepipedus, *P.melanarius*, *Pterostichus niger* and *H.rufipes* all reduced slug damage to a chinese cabbage crop in a miniplot experiment compared with unprotected plots. However, these differences were not significant. *A.parallelepipedus* was most effective at reducing slug damage to the chinese cabbage but was rare in arable land. *H.rufipes* was least effective at reducing slug damage but was abundant in arable land in both years of the study. A high proportion of *H.rufipes* beetles fed on slugs in the field. None of these four species occurred at densities in the field which reduced slug damage in the miniplot experiment.

Contents

Chapter one

Pest Slugs and Their Natural Enemies

1.1 Pest status of slugs

Slugs attack a wide range of agricultural and horticultural crops throughout the world (Hunter and Runham, 1972) and are important agricultural pests in moist temperate climates (Stephenson and Bardner, 1976). The importance of slugs as pests has increased over recent years as the area of land under oilseed rape has increased. The area under brassica seed crops (including oilseed rape) increased from 5,127 ha in 1971 to 38,970 ha in 1975 (Stephenson and Bardner, 1976). In 1989, oilseed rape occupied 348,000 ha in the UK (Kelly and Martin, 1989). The dense cover of oilseed rape provides cool, moist conditions which are ideal for populations of slugs to increase (Martin and Kelly, 1986). This crop is normally followed by crops of winter wheat (Stephenson and Bardner, 1976; Glen, 1989) which are then attacked by the slugs.

Slugs attack oilseed rape seedlings (Port and Port, 1986; Glen *et al.*, 1990). However, in England, wheat and potatoes are the crops most likely to suffer from slug attack. Damage by hollowing of the grain is one of the most important causes of failure of winter wheat (Gould, 1961). All slug species associated with wheat grain damage in the field, produce a high degree of damage to seeds under laboratory conditions. In damaging the germ, certain slug species cause wheat and barley germination failures in the field (Duthoit, 1964).

Between 1966 and 1967 approximately 4,451 ha (11,000 acres) of wheat (one percent of the land under cereal) were treated with chemicals for slug control in England and Wales and 9,712 ha (24,000 acres) of wheat were redrilled. Over the same period, approximately 809 ha (2,000 acres) of potatoes were treated with chemicals and 39,000 tons of potatoes were reduced in value due to slug damage (Hunter, 1969). By 1982, the area of wheat treated with molluscicides had increased to thirty percent (Sly, 1984) and in 1992 over 292,000 ha were treated

with molluscicides (Davis *et al.*, 1992). Slugs are now perceived by cereal growers as the most troublesome pest of wheat and second to aphids as pests of barley (Glen, 1989).

Slug damage to the crop can be restricted to particular parts of a field, but modern methods of harvesting allow damaged potato crop to be mixed with undamaged crop. This can result in the entire crop being downgraded and losing market value. Only five percent of the crop damaged could mean the sample would not be suitable for packaging. Levels greater than 10 percent make the crop suitable only for processing (Beer, 1989).

Quick (1960) described 26 species of slug which are found in Britain, however only a few species act as pests. *Deroceras reticulatum* (Muller), *Arion fasciatus* (Nilsson), *Arion ater* (Linne) and *Arion hortensis* Ferussac, all damage wheat seeds in the laboratory (Duthoit, 1964). MAFF summaries of insect and allied pest reports show that *D.reticulatum*, *A.hortensis* and *Milax budapestensis* (Hazay) are the most important molluscan pests. Hunter (1978) recognised the importance of these latter three species on crops of wheat, potatoes, sugar beet and brassicas. In earlier studies, Hunter (1968a) found these three species were the dominant slugs on arable plots in Northumberland and ranked *D.reticulatum* as the most important pest of winter wheat (Hunter, 1969).

1.2 Review of slug control

Numerous methods of slug control have previously been attempted, including the use of physical barriers to prevent slugs invading land. The introduction of metaldehyde in 1936 allowed a more systematic approach to slug control (Stephenson and Bardner, 1976). A number of methods are currently used to control slugs. These include planting and harvesting dates, modification of habitat by additional cultivations, growing non-susceptible crop varieties and chemically by using poisoned baits or sprays (Hunter, 1978).

1.2.1 Cultural control

The distribution of slug damage in England can be quite localised and closely related to the general distribution of heavy soils. Slug damage can be quite specific to an area and can be quite localised, even within individual fields (Hunter, 1969).

Slug damage is often most severe in fields or parts of fields where the seedbed tilth is exceptionally rough and cloddy (Gould, 1961) and the level of slug damage to winter wheat can be inversely related to the proportion of fine soil in the seed bed (Glen *et al.*, 1989). Cultivation followed by compaction of the soil reduces the number of spaces and cracks in which slugs hide and limits the amount of vegetation cover. This reduces slug numbers as they cannot find shelter and are more vulnerable to the effects of drought, predation and frost (Hunter, 1967). Frost is an important mortality factor for immature *D.reticulatum* and *A.hortensis* slugs (Hunter, 1966). Increased numbers of cultivations expose slug populations to periods of high radiant temperature and predators. Cultivations by themselves may decrease slug populations (Glen *et al.*, 1988) by directly killing slugs with the mechanical action of the machinery (Hunter, 1967).

Straw burning can reduce slug populations (Martin and Kelly, 1986) and this has been shown to reduce slug damage to seeds and seedlings (Glen *et al.*, 1982). However, *D.reticulatum* is mainly a surface dwelling species and is adapted for adverse conditions. It has a high reproductive capacity and populations can survive when they cannot burrow underground for shelter. It is unrealistic for farmers to modify a chosen cropping system as there are often more powerful economic and cultural arguments for the regime adopted (Martin and Kelly, 1986).

1.2.2 The use of non-susceptible crop varieties

Slugs feed on a wide range of food materials but have very discerning feeding habits. They have distinct preferences for certain varieties of potatoes which leads to high levels of slug damage in the preferred variety (Gould, 1965; Hunter and Runham, 1972). Hunter *et al.*, (1968) found that cv maris piper suffered much more foliage damage than cv majestic in the field. However, damage to the tubers

is of more economic importance. Maris piper is the cv preferred by supermarkets and slug damage to this cv can be particularly severe (Beer, 1989). The mechanical damage of tubers (during harvesting) increases the chance of slug attack. Stephenson (1965) found tubers of five varieties were attacked by slugs when artificially damaged, but undamaged tubers were not attacked.

1.2.3 Chemical control

Metaldehyde was first used as a molluscicide in South Africa in 1934 (Kelly and Martin, 1989) and it was the first chemical to be used extensively for slug control (Hunter and Runham, 1972). Its primary molluscicidal action is the stimulation of excessive mucus secretions and dehydration. Methiocarb was first introduced into the UK as a pelleted molluscicide bait in 1968 (Kelly and Martin, 1989). Today, methiocarb and metaldehyde are the most extensively used molluscicides in Great Britain (Davis *et al.*, 1992).

Molluscicides are most commonly used in the form of poisoned baits and are most effective on warm humid nights (Webley, 1964). Their success relies on slugs actively foraging for the bait and consuming a lethal dose. However, even when conditions are suitable for feeding, not all of the slug population may be exposed to the bait. Hunter (1968b) found only a little over half the population was feeding with mean night temperatures of 12°C when the substrate was wet.

Weather can account for up to 80 percent of the variation from day to day in the number of slugs caught by baits (Webley, 1964). Air temperature, soil surface temperature, windspeed, soil moisture content and humidity are all correlated with slug activity (Young and Port, 1989). Slight increments in temperature (4 to 6°C) and relative humidity (90 to 100 percent) can result in a doubling of active slugs (Crawford-Sidebotham, 1972). Changes in light intensity, surface temperature, shelter temperature, temperature gradients age and hydration all affect the activity of *Limax maximus* Linne (Rollo, 1982). Juvenile slugs are less well controlled by molluscicide baits probably as a result of different activity and feeding patterns (Kelly and Martin, 1989).

Timing of the application of the control is critical (Crawford-Sidebotham, 1972) but difficult. Even when slugs do eat the bait, irritancy and lack of palatability due to the toxin often prevents the consumption of lethal doses and the ingestion of a sub-lethal dose can result in recovery. Bait pellets are still the most effective chemicals available to the farmer but their optimal use is difficult (Martin and Kelly, 1986), especially during moist weather (Crowell, 1967). To be effective poison baits must be applied over a considerable period of time (Hunter, 1968b), which is costly.

Natural enemies play an important role in regulating some cereal pests. There is concern that the adverse effects of pesticides on these predators may increase the need to carry out treatments against pests. Broad spectrum pesticide compounds often have detrimental effects on ground beetles (Thiele, 1977). Edwards and Thompson (1975) found insecticides such as fonofos, parathion and phorate were extremely toxic to predatory beetles. Wallin *et al.*, (1992) found *Pterostichus cupreus* (Linnaeus) beetles which fed on aphids contaminated with the aphicide pirimicarb were unable to build up fat reserves which affected their egg production. The molluscicide methiocarb has a 100 percent mortality rate on some carabid species under laboratory conditions although metaldehyde baits rarely affect them (Buchs *et al.*, 1989).

1.3 Biological control

Biological control has been defined as 'the destruction or suppression of pests by the introduction, encouragement or artificial increase of their natural enemies'. It is generally associated with the use of exotic species which have been introduced to control exotic pests. This is termed 'classical biological control'. The first major success of classical control occurred in the 1880's with the introduction of a ladybird *Rodolia cardinalis* (Mulsant) from Australia to California to control cottony cushion scale *Icerya purchasi* Maskell on citrus.

This type of biological control results in permanent regulation of the pest population by the predator which then exist together in an equilibrium below the economic threshold. In agricultural situations, this generally occurs in more stable

environments, e.g. the controlled environment of the glasshouse where whitefly have been controlled by the introduction of the parasitic wasp *Encarsia formosa* Gahan. The glasshouse environment is an important factor affecting the success of the wasp. At low temperatures the wasp is less effective. At high temperatures the wasp is too prolific as it eradicates the whitefly and then dies out itself.

Since agricultural land is annually disrupted there is little opportunity for an equilibrium between predator and prey. Crops are ephemeral and pests are often invasive. The control of pests is short term.

1.3.1 Invertebrates which attack molluscs

Several groups of invertebrate predators have been shown to attack molluscs. Stephenson and Knutson (1966) reviewed 42 papers from 1921 to 1965 and found protozoans, brachylaemid flatworms, lungworms, lampyrid beetles and sciomyzid fly larvae were the most important natural enemies. More recently, Quicke (1987) observed orbweb weaving spiders feeding on slugs caught in their webs. The predatory gastropod *Zonitoides nitidus* (Muller) will feed on the snail *Limnaea truncatula* (Muller) (Moens, 1982) and on the eggs of *D.reticulatum* (Moens and Vase, 1986).

Since the 1960's, much work has revolved around the impact of parasites and polyphagous predators (including carabids) on populations of cereal aphids. These two groups are also well documented natural enemies of slugs. Some workers have described dipteran larvae to be parasitic on molluscs. Stephenson (1965) found larvae of the slug killing fly *Tetanocera elata* (Meigen) infected 14 percent of *D.reticulatum* slugs in an abandoned allotment. Trelka and Berg (1977) gave a more detailed account of *T.elata* and *Tetanocera plebeia* Loew. The two species initially live as parasites intimately associated with a particular slug for several days, before changing to a predatory mode of life after the last larval moult and killing several additional slugs. Both species feed on *Deroceras laeve* (Muller) and *D.reticulatum* as parasites but utilise slugs of different genera as predators.

Stephenson (1965) reported Knutson raising phorid larvae through to pupae on eggs of *D.reticulatum*. Phorid larvae also feed on eggs of *D.laeve* (Robinson, 1965). Robinson and Foote (1968) detailed the mode of attack of the phorid *Megaselia aequalis* (Wood) on *D.laeve* eggs. First and second stage larvae are confined to feeding in a single egg but third stage larvae leave the egg to assume a more predatory role, usually destroying an additional four eggs. The larvae of the Scatopsid, *Coboldia fuscipes* Meigen also attack eggs of *D.reticulatum* and *A.hortensis* (Ayre, 1995).

1.3.2 Carabid predators

Several workers have described a number of interactions between carabid predators and molluscs. *Scaphinotus interruptus* (Menetries) will feed on *D.reticulatum* in laboratory terraria (Ingram, 1946). Larochelle (1972), reviewed nineteen papers and found the Cychrini tribe, including the *Cychrus*, *Scaphinotus* and *Sphaeroderus* genera, fed principally on snails and slugs. Slugs and snails dominate the diets of both *Cychrus* and *Carabus* species (Gruntal and Sergeyeva, 1989) and the *Cychrus* genera are specialised mollusc feeders (Evans and Forsythe, 1985). *Cychrus attenuatus* Fabricius is a specialist mollusc predator (Loreau, 1984).

Carabus violaceus Linnaeus will kill the slugs *A.hortensis* and *Milax gagates* (Draparnaud) in the field (Tomlin, 1935) and *D.reticulatum* in the laboratory (Stephenson, 1965). Tod (1973) observed *Carabus arvensis* Herbst eating slugs in laboratory studies and *Carabus problematicus* Herbst took *Arion subfuscus* (Draparnaud), *Arion intermedius* Normand, *Arion circumscriptus* Johnston, *Arion rufus* (Linne) and *Limax tenellus* Muller in laboratory feeding trials (Bless, 1977).

Other carabid genera are known to feed on slugs including a number of Pterostichini which Evans (1967) described as general scavengers and predators. *Pterostichus melanarius* (Illiger), *Abax parallelepipedus* (Piller and Mitterpacher), *Pterostichus niger* (Schaller) and *Pterostichus madidus* (Fabricius) will eat *D.reticulatum* in the laboratory (Stephenson, 1965). *Abax ater* (Villers) will predate *A.subfuscus*, *A.intermedius*, *A.circumscriptus*, *A.rufus* and *L.tenellus* in the laboratory

(Bless, 1977).

Carabids which predate slugs in laboratory investigations may not predate slugs in the field. Differences in daily activity cycles or a greater choice of food for the predator may prevent predators attacking slugs. Postmortem gut dissections have therefore been used as a method of assessing the food of predators in the field (Sunderland, 1975; Vickerman and Sunderland, 1975; Sunderland and Vickerman, 1980). Postmortem investigations have the advantage that predation can occur at any time and does not need to be directly observed.

Postmortem investigations have been used to identify mollusc remains in *P.melanarius* and *Harpalus rufipes* (Degeer) (Cornic, 1973), *A.parallelepipedus* (Davies, 1953), *P.madidus* (Davies, 1953; Luff, 1974), *Agonum viduum* (Panzer), *Agonum obscurum* (Herbst), *Agonum thoreyi* Dejean, *Agonum fuliginosum* (Panzer), *Pterostichus minor* (Gyllenhal), *Pterostichus diligens* Sturm, *Pterostichus strenuus* (Panzer) and *Pterostichus vernalis* (Panzer)(Dawson, 1965).

However, the extent of slug predation is likely to be underestimated as slugs are composed primarily of soft tissues and their remains can only be identified when the shell or radula is ingested. If the shell or radula is not ingested or the beetle is a liquid feeder, then slug predation will not always be identified. Many Agonini, Carabini and Harpalini beetles caught in the field contain unidentifiable liquid food (Davies, 1953). Hengeveld (1980a) thought the red liquid found in the gut of *H.rufipes* in Davies (1953) study was the remains of earthworms, caterpillars and molluscs.

In recent years serological techniques have been used in postmortem investigations. A serological technique has been used to identify molluscan remains in the gut contents of *Pterostichus niger* (Schaller), *Pterostichus anthracinus* (Illiger), *P.madidus*, *P.melanarius*, *Carabus catenulatus* (=*problematicus*) Fabricius, *Carabus nemoralis* Mueller, *C.violaceus*, *Cychrus caraboides* (Linnaeus), *Nebria brevicollis* (Fabricius), *Nebria gyllenhalli* (Schoenherr), *Calathus piceus* (Marsham), *Calathus fucipes*

(Goeze), *Calathus melanocephalus* (Linnaeus) and *Calathus micropterus* (Duftchmid) (Tod, 1973).

1.3.3 Advantages of carabid predators

Unlike parasitoids where food is supplied to the developing larvae, polyphagous predators (such as carabids) must search for food at all active stages of their life cycle. It is advantageous for them to be able to withstand periods of food shortages either by being tolerant of shortages or by utilising alternative foods. This enables the predator to persist when prey are scarce and therefore prevents predator extinction. Sunderland (1975) concluded that non-specialist feeders have the ability to persist in crops during periods of low prey density. They are therefore present during periods of pest immigration and increase and are a useful component in resisting pest outbreak (Luff, 1980).

Many agricultural ecosystems are disrupted annually and it is important to have predators which are efficient colonisers, have a high immigration value and a rapid rate of increase as prey density increases. These predators are more likely to be polyphagous predators. *P.melanarius* immigrates into agricultural ground to exploit the periodic abundance of food (Wallin, 1985). Other species such as *Agonum dorsale* (Pontoppidan), *Amara plebeja* (Gyllenhal), *Bembidion lampros* (Herbst) and *Bembidion obtusum* Serville aggregate at patches of high aphid density (Bryan and Wratten, 1984).

Carabids are already known to attack a number of crop pests in agroecosystems including aphids (Vickerman and Sunderland, 1975; Sunderland, 1975; Sunderland and Vickerman, 1980; Griffiths, 1982; Sunderland *et al.*, 1987; Sopp, 1987; Sopp *et al.*, 1992; Holopaninen and Helenius, 1992), wheat bulb flies (Jones, 1975; Burn, 1982) and cabbage root fly (Coaker and Williams, 1963).

Carabids are thought to regulate aphid populations from year to year. Dunning *et al.*, (1975) found aphid numbers rapidly declined as carabid numbers and activity peaked. Edwards *et al.*, (1978) and Edwards *et al.*, (1979) found inverse

correlations between beetle and aphid numbers in wheat. *A.dorsale*, *P.melanarius* and *H.rufipes* were the most important carabid species and *P.melanarius* depressed aphid populations by 20 percent in spring wheat. Edwards *et al.*, (1979) concluded polyphagous predators (including carabids) were better at reducing aphid numbers than aphid specific predators.

1.4 Carabids in agriculture

Carabids are found in many terrestrial ecosystems including moorlands (Pearson and White, 1964), scrub (Williams, 1959), fens (Dawson, 1965), marshes (Murdoch, 1966), woodlands (Williams, 1959) and agroecosystems (Jones, 1976 and 1979). Agricultural land is an unstable habitat which annually undergo changes such as harvesting, ploughing, sowing and crop growth. However, agricultural land provides stable conditions for species dependant on bare soil and provides an artificial environment for carabids whose habitats are otherwise ephemeral (den Boer, 1977).

Most carabids have distinct habitat preferences. In a study in Ontario, Rivard (1964) found 30 of 159 species were restricted to wooded areas. Other species are found in several habitats. *P.madidus* is not restricted to wooded or open areas (Williams, 1959) and has a continuous distribution over different vegetative types (Greenslade, 1964a). *P.niger* has no preference for cereal fields or woods and moves between both habitats. Other species such as *H.rufipes* prefer cereal fields (e.g. Luff, 1980; Wallin, 1986). Pollard (1968a) classified *C.violaceus*, *P.melanarius*, *P.madidus* and *H.rufipes* as field species. Carabids are found in a number of crops including sugar beet (Dunning *et al.* 1975; Baker and Dunning, 1975), barley and wheat (Sunderland, 1975; Sunderland and Vickerman, 1980), oilseed rape (Attah, 1986) and corn (Whitford and Showers, 1987).

In contrast to other ecosystems, cereal fields offer periodic sources of abundant food during the summer when pest outbreaks occur. Alate aphids migrate to emerging crops and their high reproductive capacity enables them to build up to high densities. Consequently, it is advantageous for carabids to emigrate from uncultivated areas where competition for food is high (Wallin, 1986). *P.melanarius*

utilizes cereal fields for reproduction and larval development and large numbers of tenerals emerge in this habitat (Wallin, 1987).

Over recent years, much research has attempted to assess the impact of carabids on aphid pests. Investigations into carabid predation of slugs have generally been laboratory based. However, these investigations have identified a number of carabid species which kill and eat slugs.

The impact of the indigenous carabid fauna on slugs in arable land has received little attention. Larochelle (1972) concluded Cychrini beetles may be useful in keeping down numbers of harmful molluscs and Poulin and O'Neil (1969) found predation by *Calosoma frigidum* Kirby contributed to the decline of field populations of *A.ater*. Altieri *et al.*, (1982) released the molluscan specialist *Scaphinotus striatopunctatus* (Chandoir) into a field of commercial grown daisy-flowers in California and found the beetle reduced populations of *L.maximus* and *Helix aspersa* Muller. Symondson (1992) investigated *A.parallelepipedus* and *P.madidus* as control agents of *D.reticulatum* in polythene tunnels and Stephenson (1965), found *P.madidus* and *P.melanarius* eliminated *D.reticulatum* from soil filled arenas. Burn (1992) created beetle exclusion plots at Boxworth and found that in over half the trials more slugs were trapped in plots where beetles had been excluded. This suggested that predators had reduced slug numbers. However, the beetles responsible for reducing slug numbers were not identified.

There is little information on which species predate slugs in agricultural land and to what extent they utilise molluscs as food. Key species need to be identified which have an abundance in agroecosystems and feed on slugs. Luff (1980) concluded the widespread distribution and frequent abundance of *H.rufipes* gave this species considerable potential as a general predator. *H.rufipes* also feeds on slugs in commercial orchards (Cornic, 1973). Slug predation in arable land by the indigenous carabid fauna would enhance the status of this already beneficial group.

A need for Biological control ?

When considering the need for biological control of slugs the success of existing control strategies needs to be assessed. At present, the success of poison chemical baits depends on slugs actively foraging for the bait and consuming a lethal dose. Slug activity is affected by a number of factors including temperature and humidity. Sub-lethal doses are often consumed which can lead to recovery of the slug under the right conditions. These factors collectively hinder the optimal use of chemical control.

The use of broad spectrum pesticides could exacerbate pest problems in cereals and other crops (Vickerman and Sunderland, 1977; Vickerman, 1992). There is concern that the adverse effects of pesticides on predators may increase the need to carry out treatments against pests (Burn, 1992). The application of smaller amounts of selective pesticides is now being demanded on crops grown for certain supermarket chains (Finch, 1993). There is a general trend towards the co-ordination of all cultural, biological and chemical control methods in Integrated Pest Management (IPM) programmes.

Beneficial invertebrates which inhabit the crop are thought to play a role in reducing the numbers of pests, particularly cereal aphids. Although carabids are frequently cited as slug predators in the literature, little of the work is quantitative and the role of natural enemies in the population dynamics of arable slug populations is poorly understood (Burn, 1992). Work is needed to identify key slug eating species, subsequently to determine the elements essential for maintaining optimum levels of these key species (Finch, 1993) and to assess the impact of agricultural chemicals on these species. This area is now gaining momentum with the activity of a number of groups including the International Organisation for Biological Control (IOBC) and the Beneficial Arthropod Regulatory Testing Group (BART). At a recent workshop at the Brighton Crop Protection Conference, Barrett *et al.*, (1994) developed a guidance document for the testing of the effects of pesticides on non-target arthropods for regulatory purposes with respect to the EC directive for the sale of plant protection products.

1.5 Project outline

Aims and objectives

The aim of the work in this project was to identify carabid predators of slugs in arable land and assess their relative importance. The work embraces a number of methods described by Sunderland (1987) and Wratten (1982) to assess the role of and the diets of natural enemies. The overall aim could be visualised as a number of objective studies which assessed various aspects of carabid predators of slugs. These objectives are set out below.

1.5.1 Predator size and slug predation

The objective of this study was to initially identify which carabid species occurring in agricultural land fed on slugs in the laboratory. Many of the larger predators have been documented in the literature. This study investigated the role of small and medium sized predators.

1.5.2 Activity cycles, predation rates and orientation to prey

The first objective of this study was to identify compatible activity cycles between slugs and five model carabid predators. The second objective of this study was to assess the predatory activity of five model predators and searching behaviour of seven model predators. This would enable the determination of predation rates, capture efficiencies, handling time and orientation to slug prey.

1.5.3 Identification of predators in the field

The objective of this study was to identify the extent to which carabids fed on slugs in arable fields. A serological technique (Enzyme-linked immunosorbent assay, ELISA) was to be developed which identified slug proteins in the gut of carabid beetles.

1.5.4 Abundance of slug eating predators

The objective of this study was to estimate the population density of slug eating carabids and investigate the distribution, abundance and population stability of

these predators.

1.5.5 Predator density manipulation and slug damage

The objective of this study was to determine the density of beetles needed to reduce slug damage to a chinese cabbage plot and to compare these densities with populations of beetles occurring in the field.

Chapter two

Laboratory Investigations of Slug Predation and Beetle Behaviour

2.1 Introduction

Three studies were made which investigated the interactions of a number of carabid species with *D.reticulatum*. The first study was made in 1991 and investigated the predation of small *D.reticulatum* slugs by a range of carabid beetle species which occurred in three arable fields in the Tyne valley. This was an essential first step upon which the rest of the project was based.

In the same year, a laboratory based time-lapse video tape behavioural study was made investigating the interactions between five large carabid species and *D.reticulatum* slugs. A further behavioural study was made in 1992 which investigated the orientation of seven carabid species to mucus trails of *D.reticulatum*.

2.2 Predator size and slug predation

2.2.1 Introduction

Twenty four carabid species of various sizes were investigated to determine which beetle species predated slugs and the effect of beetle size on slug predation. Beetle species have characteristic annual activity cycles, therefore these investigations were made at a number of temperatures to simulate field temperatures at which slug and beetle populations are active.

The food of carabids has been investigated by a number of authors (see chapter three, section 3.8.2.2). The aim has been to identify carabid predators of crop pests such as aphids (e.g. Edwards *et al.*, 1979; Sunderland and Vickerman, 1980; Holopainen and Helenius, 1992) and wheat bulb fly (e.g. Jones, 1975). Aphids and wheat bulb fly are both very small organisms compared to slugs, which may be

difficult prey for beetles to overcome. The main obstacle preventing slug predation by carabids is the tough mucus covered skin of slugs which generalist beetles find hard to penetrate. Pakarinen (1994) found *P.niger* was unable to overcome and kill *A.fasciatus* due to the slugs tough skin and mucus defence. The mucus exuded by the slug fouls the mouthparts of predator species which have not evolved successful attack strategies. The mucus covered skin does not protect the slug from specialist carabid predators, but the difficulties caused by the mucus maybe the reason for the small number of specialist species (Pakarinen, 1994).

Molluscan specialists have evolved anatomical, behavioural, physiological and external morphological features which enable them to predate molluscs (Hengeveld, 1980b). *Cychrus* is a specialised snail and slug feeder (Evans and Forsythe, 1985) and slugs dominate the diet of *Cychrus* and *Carabus* species (Gruntal and Sergeyeva, 1989). Specialists are less limited by the size of the prey (Hengeveld, 1980a) but even specialist species can have problems in overcoming molluscs (e.g. Ingram, 1946).

The majority of beetles occurring in arable sites in this project were less specialised predators (chapter five). The Pterostichini were well represented and this group is considered to consist of generalist predators. Evans (1967) described *A.parallelepipedus*, *P.madidus* and *P.niger* as generalist predators and scavengers. Evans and Forsythe (1985) found *A.parallelepipedus* could deal with a variety of arthropod prey. Davies (1953) concluded *Pterostichus* species were scavengers of any organic material fresh or decaying. However, Digweed (1993a) found four *Pterostichus* species were able to crush the shell and eat two small terrestrial snails and mollusc remains have been recovered from the gut of a number of generalist carabid species caught in the field (Davies, 1953; Dawson, 1965; Cornic, 1973; Luff, 1974).

As well as the tough mucus covered skin, the large size of adult slugs protects them from predation by smaller carabids. Tod (1973) found a significant correlation between beetle size and mollusc predation and concluded that smaller carabids

were scavengers of dead slugs. Wheater (1988) found a positive correlation between the size of prey and the mandible gape of five Pterostichini species. The ease of capture restricts the prey taken by smaller predators, therefore larger predators have a greater range of prey. Smaller carabids such as *Notiophilus biguttatus* (Fabricius) have a higher success rate when attacking smaller Collembola (Ernsting and Van der Werf, 1988).

Previous studies on mollusc predation by carabids have generally involved large carabid predators and large slugs. Symondson (1989) used *A.parallelepipedus* to control large *D.reticulatum* slugs weighing between 0.25-0.525g (2.5-5.25g in Symondson's paper). The age structure of field populations of *D.reticulatum* can be more variable. Hunter (1968a) found small slugs dominated the populations of *D.reticulatum* in arable land over the summer months. Many small carabid species which feed on slugs are active during this time. Dawson (1965) found mollusc remains in the guts of three *Agonum* species and three small *Pterostichus* species. When small slugs are active, there clearly exists an opportunity for smaller carabids to exert an impact on slug populations.

2.2.2 Methods

D.reticulatum slugs were collected from Close House field station and cultured in plant propagators in a controlled environment room at 13°C. The base of the propagators were covered with a layer of moist paper towelling and the slugs were fed on a diet of bran, carrots and chinese cabbage. Slug eggs were regularly collected from the propagators and washed in distilled water. The developmental stage of the slug eggs were carefully monitored and the eggs were maintained at either 8, 12 or 16°C, to synchronise hatching.

Hatched slugs were kept in petri dishes lined with filter paper at a density of ten per dish. They were maintained between 4 to 8°C and fed on a diet of chinese cabbage. Preliminary investigations into slug weight indicated that there was no significant increase in weight (size) up to three days after hatching. Where possible, one day old slugs were used in these studies but on occasions it was necessary to

use two and three day old slugs.

Adult and larval carabid predators were collected from arable fields and woodland sites around Close House field station, kept in plastic trays containing moist filter paper and maintained between 4 to 16°C. Predators were fed on a diet of blow fly larvae, earthworms, grass seeds and bran. Beetles from several tribes were collected, including large species such as *P.madidus* and small species such as *Trechus quadristriatus* (Schrank). The beetles chosen for investigation represented the carabid community of the three field sites. A complete list of all of the beetle species used in this study are presented in the results section (Table 2.2.7).

When sufficient numbers of a particular species had been collected for an experiment, they were removed from culture and placed into separate petri dishes containing moist filter paper. Where possible, 25 replicates were used for each beetle species. However, availability of predators in the field and the length of time predators had spent in culture meant fewer replicates were made of some species.

Predators selected for the investigation were maintained at either 4, 8, 12, 16 or 20°C in their petri dishes to reflect the variety of temperatures occurring in the field. A light to dark regime of 16:8 hours was provided in all of the incubators. The predators were left to acclimatise for one night without food to heightened their feeding motivation. Predators which died during this period were not replaced.

The following day, a single hatched *D.reticulatum* slug was carefully added to each petri dish with a wet brush and exposed to the predator overnight. The petri dishes were checked the following day and the slugs were recorded as either alive, dead or consumed. Controls were made at each experimental temperature and consisted of thirty petri dishes each containing a single slug.

2.2.3 Results

Slug mortality included all slugs which were killed by a predator. Slug mortality in the controls was compared with slug mortality with exposure to the various beetle species using two x two contingency tables. When the minimum expected frequency of a test was less than five, Fisher's Exact test was used as it is more reliable with low frequencies (only a P-value is returned). When the minimum expected frequency exceeded five, Pearsons value was calculated. Full data sets at each experimental temperature, were only available for *P.madidus*, *H.rufipes*, *Harpalus aeneus* (Fabricius) and *N.brevicollis* adults and larvae. Any replicates in which predators died when exposed to the slugs were omitted from the analysis. Data on slug survival in the controls is presented in Appendix 2.1.

2.2.3.1 Temperature and slug mortality

Slugs were killed and eaten at each temperature when exposed to *H.rufipes* (Fig. 2.2.1). Slug mortality increased with increasing temperature and slug mortality was significantly greater than slug mortality in the controls at 8, 12, 16 and 20°C (Table 2.2.1).

Slugs were killed and eaten at each temperature when exposed to *P.madidus* (Fig. 2.2.2). Slug mortality increased with increasing temperature and slug mortality was significantly greater than slug mortality in the controls at 12, 16 and 20°C (Table 2.2.2).

Slugs were killed at each temperature except 4°C when exposed to *N.brevicollis* adults and slugs were eaten at 8, 12 and 16°C. Slug mortality was greatest at 8°C where more than 80 percent of slugs were killed (Fig. 2.2.3). At 20°C, slugs were killed but not eaten. Slug mortality was significantly greater than the controls at 8, 16 and 20°C (Table 2.2.3). Slugs were killed and eaten at each temperature when exposed to *N.brevicollis* larvae (Fig. 2.2.4). A high slug mortality occurred at all five temperatures and slug mortality was significantly greater than the controls at all five temperatures (Table 2.2.4).

Fig. 2.2.1 The mortality and consumption of one day old slugs when exposed to Harpalus rufipes adults at five temperatures. The histograms show slug consumption as a percentage of slug mortality at each temperature.

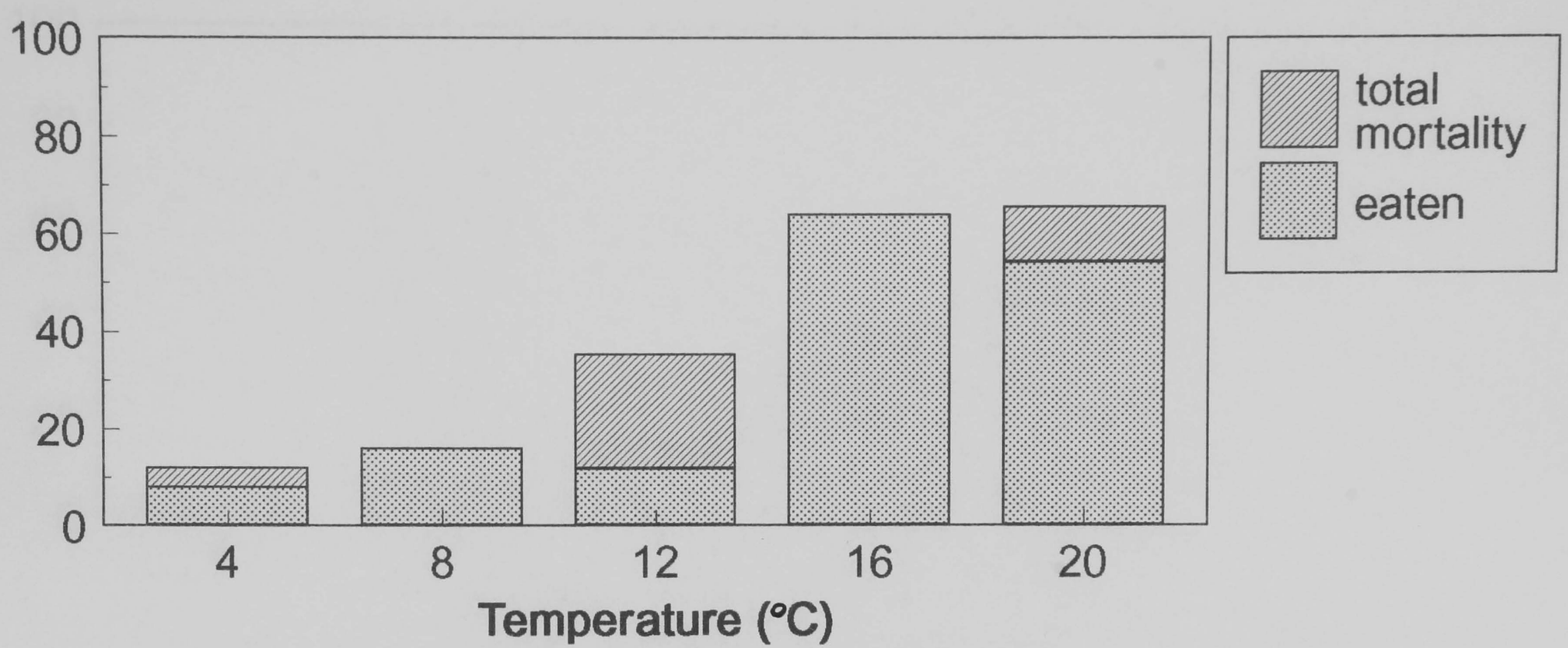


Fig. 2.2.2 The mortality and consumption of one day old slugs when exposed to Pterostichus madidus adults at five temperatures. The histograms show slug consumption as a percentage of slug mortality at each temperature.

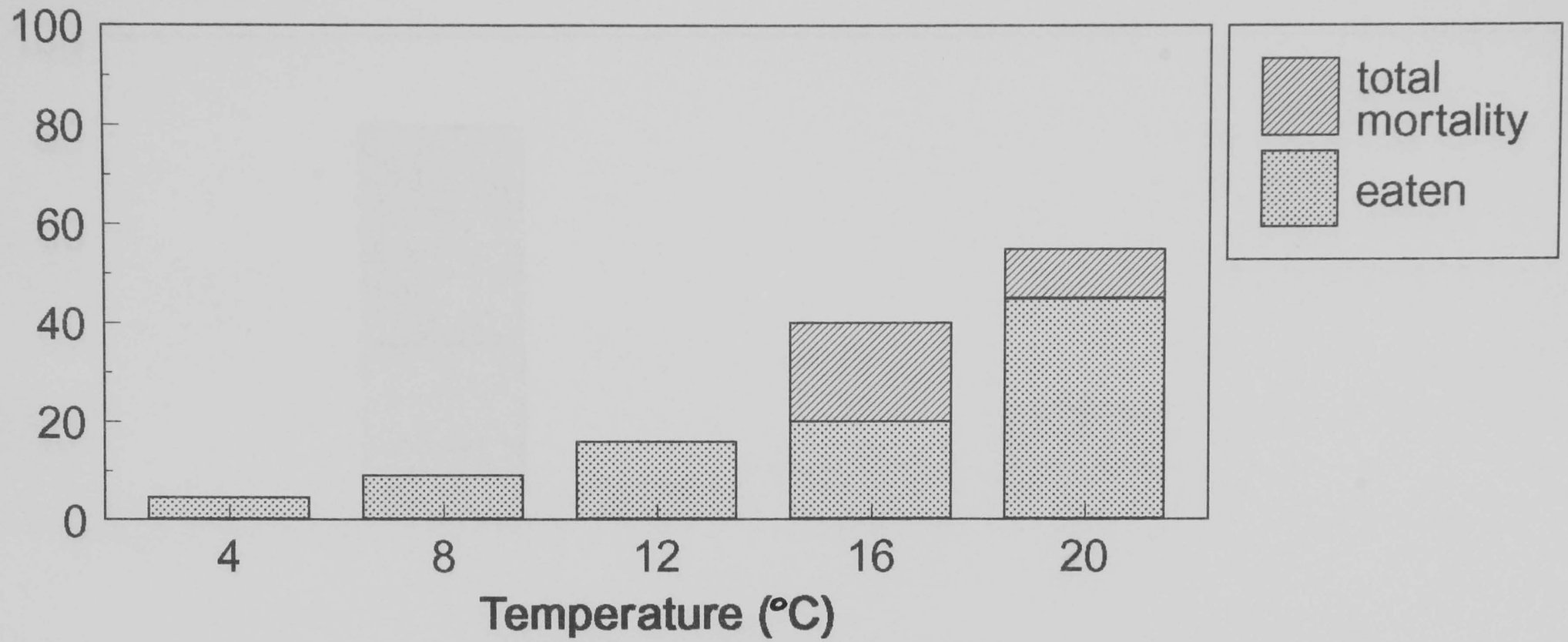


Fig. 2.2.3 The mortality and consumption of one day old slugs when exposed to Nebria brevicollis adults at five temperatures. The histograms show slug consumption as a percentage of slug mortality at each temperature.

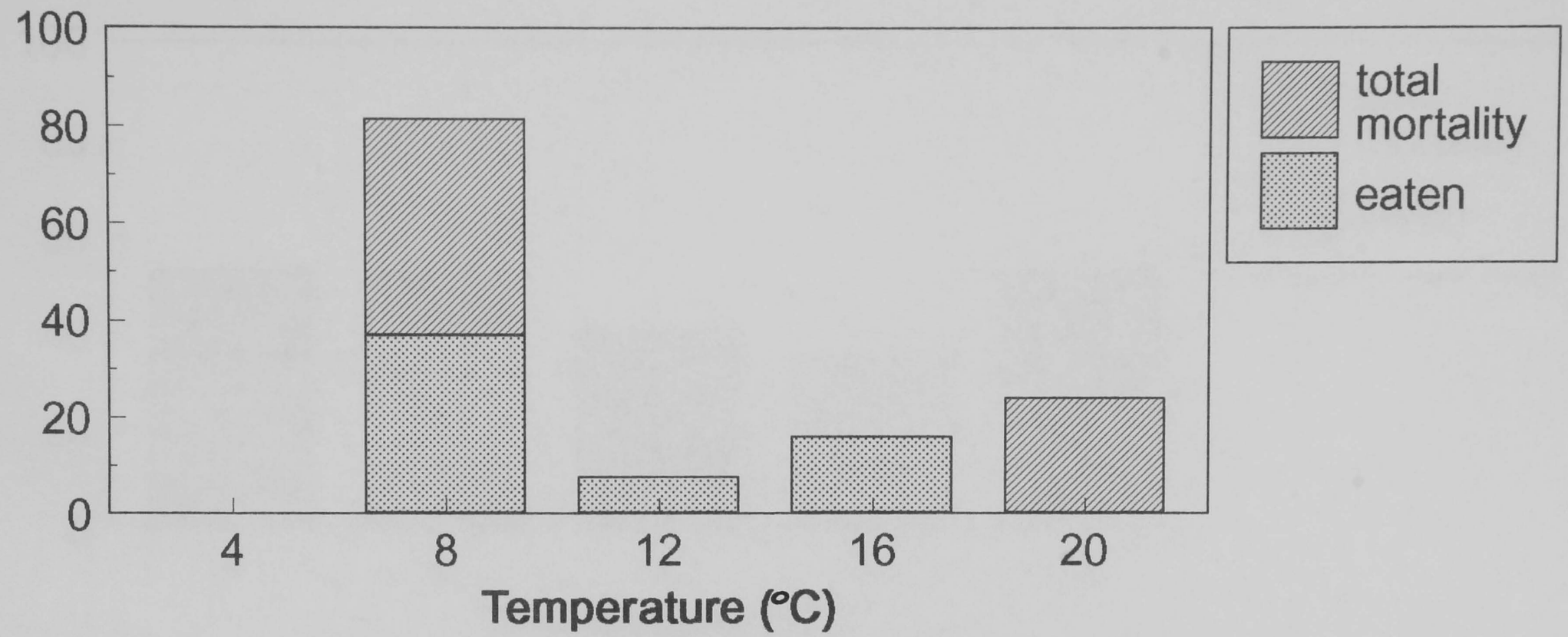


Fig. 2.2.4 The mortality and consumption of one day old slugs when exposed to Nebria brevicollis larva at five temperatures. Histograms show slug consumption as a percentage of slug mortality at each temperature.

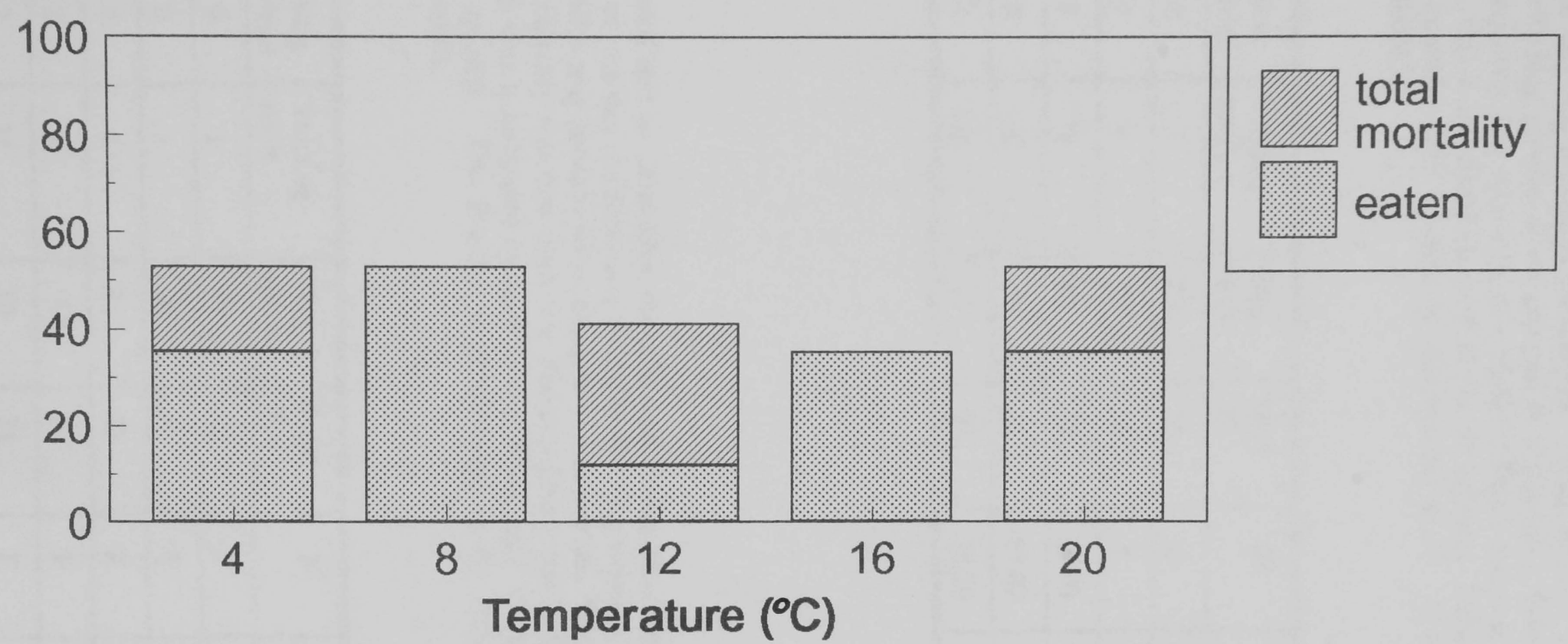


Table 2.2.1 Chi-squared test to determine the effect of *Harpalus rufipes* adults on the survival of one day old *Deroceras reticulatum*. Slug survival in the controls is compared with slug survival when exposed to the beetles. When the minimum expected frequency was less then five Fisher's Exact test was used (only returns P-value), this is indicated by an F in the X^2 column. Otherwise Pearson's statistic is quoted. The P-value is abbreviated to P. *P<0.05, **P<0.01 and ***P<0.001.

Temp (°C)	Slug eaten	Slug dead	Tot.slug mort.	Slug alive	Pred. No.	X^2	P
4	2	1	3	22	25	F	-
8	4	0	4	21	25	F	*
12	4	8	12	23	34	13.03	***
16	16	0	16	9	25	27.07	***
20	19	4	23	14	37	28.39	***

Table 2.2.2 Chi-squared test to determine the effect of *Pterostichus madidus* adults on the survival of one day old *Deroceras reticulatum*. Slug survival in the controls is compared with slug survival when exposed to the beetles. When the minimum expected frequency was less then five Fisher's Exact test was used (only returns P-value), this is indicated by an F in the X^2 column. Otherwise Pearson's statistic is quoted. The P-value is abbreviated to P. *P<0.05, **P<0.01 and ***P<0.001.

Temp (°C)	Slug eaten	Slug dead	Tot.slug mort.	Slug alive	Pred. No.	X^2	P
4	1	0	1	21	22	F	-
8	1	0	1	10	11	F	-
12	4	0	4	21	21	F	*
16	2	2	4	7	11	F	**
20	9	2	11	10	21	F	***

Table 2.2.3 Chi-squared test to determine the effect of *Nebria brevicollis* adults on the survival of one day old *Deroceras reticulatum*. Slug survival in the controls is compared with slug survival when exposed to the beetles. When the minimum expected frequency was less then five Fisher's Exact test was used (only returns P-value), this is indicated by an F in the X^2 column. Otherwise Pearson's statistic is quoted. The P-value is abbreviated to P. *P<0.05, **P<0.01, ***P<0.001. '-' indicates that the statistic could not be computed.

Temp (°C)	Slug eaten	Slug dead	Tot.slug mort.	Slug alive	Pred. No.	X^2	P
4	0	0	0	26	26	-	-
8	10	12	22	5	27	39.80	***
12	2	0	2	24	26	F	-
16	4	0	4	21	25	F	*
20	0	6	6	19	25	F	**

Table 2.2.4 Chi-squared test to determine the effect of *Nebria brevicollis* larvae on the survival of one day old *Deroceras reticulatum*. Slug survival in the controls is compared with slug survival when exposed to the beetles. When the minimum expected frequency was less then five Fisher's Exact test was used (only returns P-value), this is indicated by an F in the X^2 column. Otherwise Pearson's statistic is quoted. The P-value is abbreviated to P. *P<0.05, **P<0.01 and ***P<0.001.

Temp (°C)	Slug eaten	Slug dead	Tot.slug mort.	Slug alive	Pred. No.	X^2	P
4	6	3	9	8	17	F	***
8	9	0	9	8	17	F	***
12	2	5	7	10	17	F	***
16	6	0	6	11	17	F	**
20	6	3	9	8	17	F	***

Slugs were killed and eaten at a range of temperatures when exposed to *H.aeneus* (Fig. 2.2.5) and slug mortality was significantly greater than the controls at 20°C (Table 2.2.5). Slugs were killed at 12 and 20°C but not at 4°C when exposed to *Amara aulica* (Panzer) (Fig. 2.2.6). Slugs were only eaten at 12°C and slug mortality was significantly greater than the controls at 12 and 20°C (Table 2.2.6).

2.2.3.2 Beetle size and slug mortality

The beetle species were divided into three groups according to their maximum body length (after Lindroth, 1974). The three groups represented small (up to 7mm), medium (7.1 - 9.9mm) and large (10mm and above) species (Table 2.2.7).

More large and medium sized beetles ate slugs and caused slug mortality when compared to the small sized beetles. Of the seven small species, only *B.lampros* killed slugs. Six of the eight medium sized species ate slugs or caused slug mortality. These included *Pterostichus*, *Amara* and *Agonum* species. *Loricera pilicornis* (Fabricius) killed but did not eat slugs and eight of the nine large sized species caused slug mortality.

2.2.4 Discussion

2.2.4.1 Effect of temperature and slug mortality

Carabids are mainly active from May to September in arable land (Jones, 1979) but each carabid has a characteristic annual activity cycle (Luff, 1987). *H.aeneus* was the first large carabid recovered from arable fields in the spring of this project (chapter five) and slugs were predated at 8°C by this species, indicating that this is an early season predator which will attack small slugs.

H.rufipes and *P.madidus* consumed most slugs at 20°C, however predation also occurred at 4°C when most invertebrates are considered to be inactive (e.g. Mellanby, 1961). *H.rufipes* has an annual activity period in northern England from April to November (Luff, 1980) and *P.madidus* has an activity period from March to November (Luff, 1973). The range of field temperatures which occur during the

Fig. 2.2.5 The mortality and consumption of one day old slugs when exposed to Harpalus aeneus adults at five temperatures. The histograms show slug consumption as a percentage of slug mortality at each temperature.

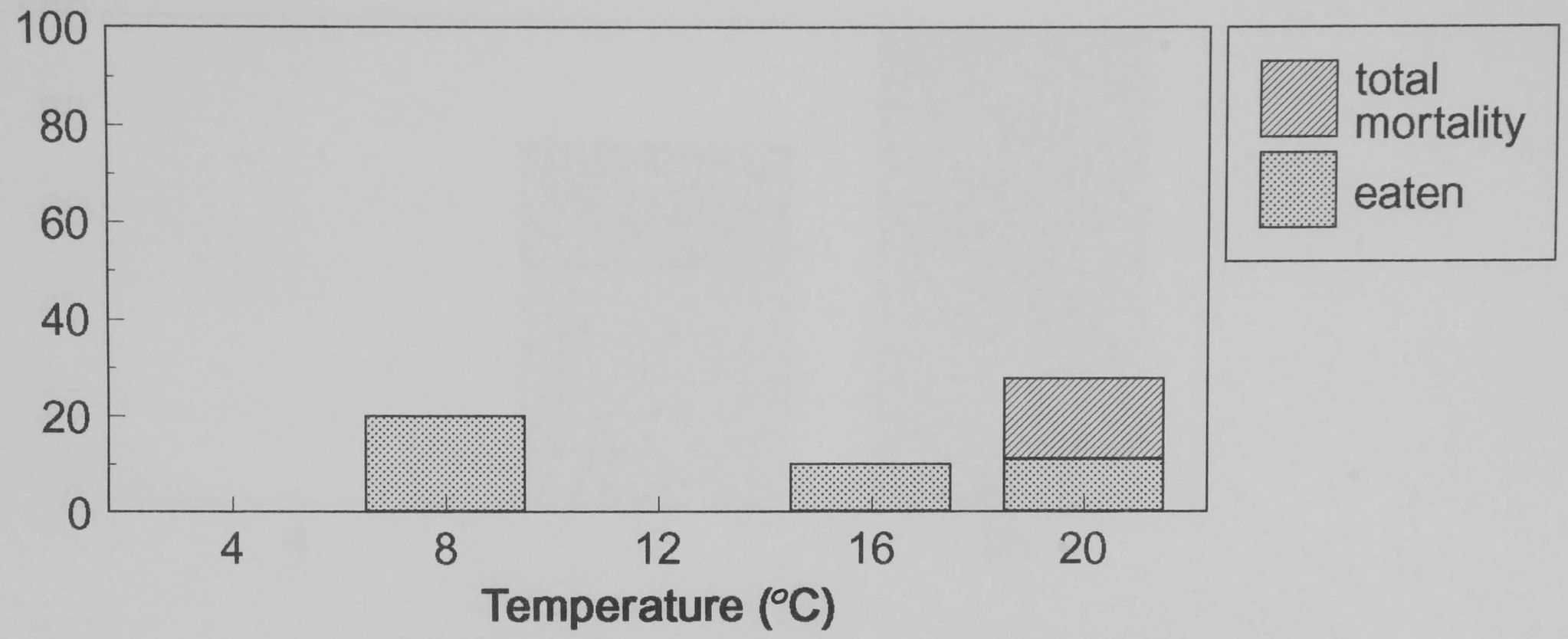


Fig. 2.2.6 The mortality and consumption of one day old slugs when exposed to Amara aulica adults at three temperatures. The histograms show slug consumption as a percentage of slug mortality at each temperature.

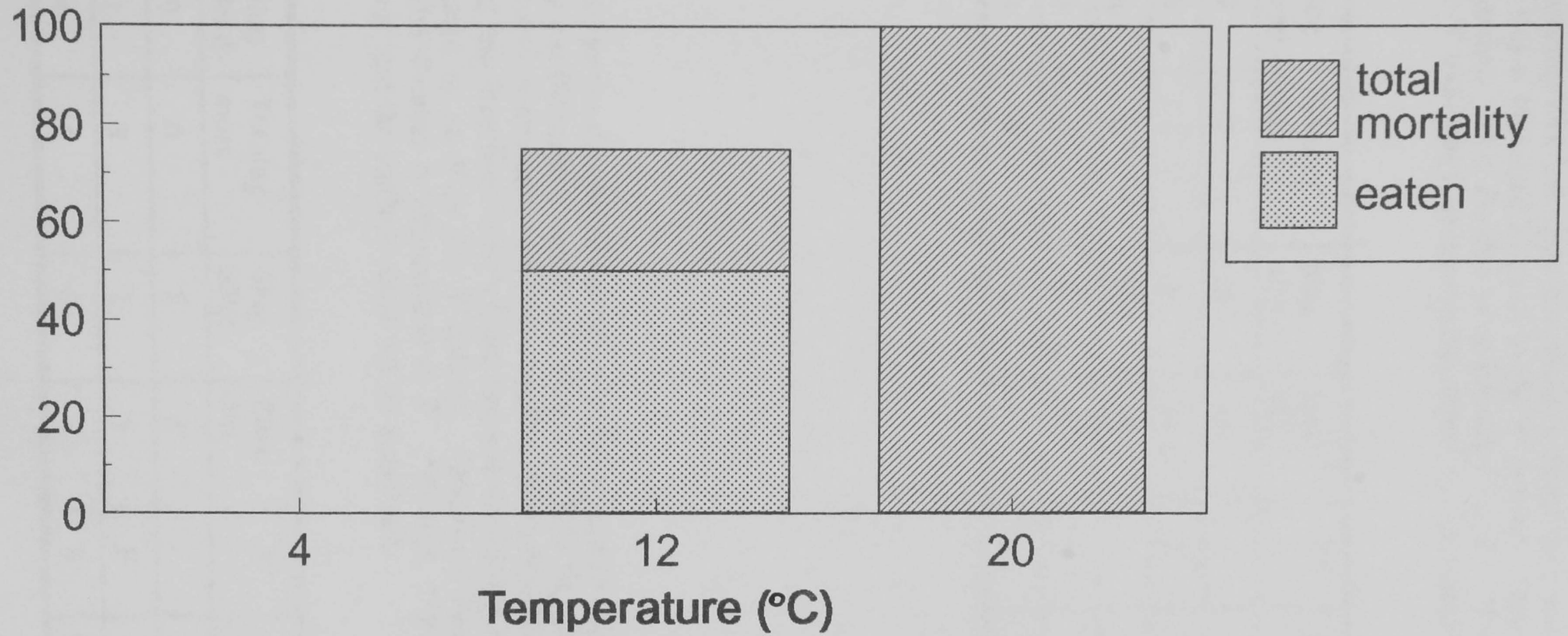


Table 2.2.5 Chi-squared test to determine the effect of *Harpalus aeneus* adults on the survival of one day old *Deroceras reticulatum*. Slug survival in the controls is compared with slug survival when exposed to the beetles. When the minimum expected frequency was less then five Fisher's Exact test was used (only returns P-value), this is indicated by an F in the X^2 column. Otherwise Pearson's statistic is quoted. The P-value is abbreviated to P. *P<0.05, **P<0.01, ***P<0.001. '-' indicates that the statistic could not be computed.

Temp (°C)	Slug eaten	Slug dead	Tot.slug mort.	Slug alive	Pred. No.	X^2	P
4	0	0	0	18	18	-	-
8	2	0	2	8	10	F	-
12	0	0	0	18	18	-	-
16	1	0	1	9	10	F	-
20	2	3	5	13	18	F	**

Table 2.2.6 Chi-squared test to determine the effect of *Amara aulica* adults on the survival of one day old *Deroceras reticulatum*. Slug survival in the controls is compared with slug survival when exposed to the beetles. When the minimum expected frequency was less then five Fisher's Exact test was used (only returns P-value), this is indicated by an F in the X^2 column. Otherwise Pearson's statistic is quoted. The P-value is abbreviated to P. *P<0.05, **P<0.01, ***P<0.001. '-' indicates that the statistic could not be computed.

Temp (°C)	Slug eaten	Slug dead	Tot.slug mort.	Slug alive	Pred. No.	X^2	P
4	0	0	0	5	5	-	-
12	2	1	3	2	5	F	**
20	0	4	4	0	4	F	***

Table 2.2.7 Predation of one day old *D.reticulatum* by 24 carabid species. Species are arranged according to the size categories in section 2.2.4.2. The number next to each species represents the number of replicates. In the temperature column, C1 = combined data presented in graphical form elsewhere. C2 = combined data from beetles tested at 8, 12 and 20°C. C3 = combined data from beetles tested at 12 and 20°C. The percentage mortality in the final column includes all slugs which died when exposed to the predator. Mortality is abbreviated to Mort.

Species		Size range (mm)	Temp (°C)	% Eaten	% Mort.
A.obscurum	(13)	5.0 - 6.6	20	0	0
C.fossor	(11)	5.5 - 6.5	20	0	0
B.tetracolum	(05)	4.9 - 6.1	20	0	0
N.biguttatus	(10)	5.0 - 6.0	20	0	0
A.flavipes	(01)	3.9 - 4.7	20	0	0
B.lampros	(01)	3.0 - 4.4	20	100	100
T.quadristriatus	(33)	3.5 - 4.0	C2	0	0
A.apricaria	(03)	6.5 - 9.0	C2	66	66
C.melanocephalus	(06)	6.0 - 8.8	20	0	0
L.pilicornis	(03)	6.0 - 8.5	04	0	33
	(20)		12	0	0
	(20)		16	0	0
	(18)		20	0	11
S.nivalis	(01)	6.0 - 8.5	20	100	100
A.dorsale	(03)	6.0 - 8.2	C3	12	37
A.plebeja	(16)	6.3 - 7.8	C3	0	0
A.fuliginosum	(04)	5.5 - 7.8	20	25	25
P.strenuus	(11)	6.0 - 7.2	20	27	36
P.melanarius	(09)	15.0 - 17.0	20	66	66
P.madidus	(88)	13.0 - 17.0	C1	17	21
H.rufipes	(144)	10.0 - 16.7	C1	31	40
A.aulica	(13)	11.0 - 14.3	C2	15	53
N.brevicollis	(129)	10.0 - 14.0	C1	12	26
P.nigrita	(07)	8.8 - 12.8	20	57	75
H.aeneus	(74)	8.5 - 12.0	C1	6	10
A.similata	(06)	7.8 - 10.0	20	0	10
P.atrorufus	(04)	7.4 - 10.0	20	0	0

activity cycles of *H.rufipes* and *P.madidus* may account for their predation of slugs over the experimental temperatures.

N.brevicollis has two activity peaks in a year, in June and in the autumn (e.g. Penney, 1966). *N.brevicollis* was one of the last large carabid species caught in arable sites in the autumn of this project (chapter five). It attacked slugs at four temperatures and the highest slug mortality occurred at 8°C. The high levels of predation this predator inflicts at low temperatures indicate that it will predate slugs late in the season. *T.quadristriatus* was the most abundant carabid caught during the autumn period of this project (chapter five). Unfortunately this species did not kill or eat slugs in this study.

N.brevicollis larvae ate most slugs at 8°C, although a high level of slug mortality occurred at all five temperatures. Carabid larvae are often voracious predators, Mitchell (1963a) found *B.lampros* and *T.quadristriatus* larvae attached themselves to large earthworms which wriggled vigorously as the larvae fed on them. The role of carabid larvae as soil predators of pests is largely unknown (Luff, 1987). Snails are the main food of larvae of *Licinus* species (Lindroth, 1974) and the larvae of *P.madidus* occasionally eat *Limax* species (Luff, 1974). *N.brevicollis* larvae are active throughout the entire winter period, from September until May (Williams, 1959). Other species such as *P.melanarius*, *T.quadristriatus* and *H.rufipes* overwinter as larvae, although they may have a winter diapause (Luff, 1987). Some beetles therefore have the potential to extend their predatory activity into the winter period when *D.reticulatum* is still active.

2.2.4.2 Beetle size and slug mortality

The results show that many large and medium sized carabids can overcome the slug's mucus defence and attack and kill small slugs. However, only one of the small carabid species (*B.lampros*) killed slugs. A newly hatched slug is the smallest slug that a predator can attack and overcome. As the slug grows, an upper size threshold will be reached which effectively protects the slug against generalist predators (Hengeveld, 1980a). Size thresholds have been found in other studies.

Amara aenea (Degeer) is unable to handle large prey items such as fourth instar codling moth larvae, but it has a strong feeding preference for first instar larvae (Hagley *et al.*, 1982).

Some predators may prefer larger prey. *P.melanarius* is a large predator and has a strong preference for larger prey (Hagley *et al.*, 1982) which also eats slugs in the field (Tod, 1973). Tod (1973) found a positive correlation between the size of a beetle species and the proportion of beetles which contained slug remains. In this study, a higher proportion of medium and large carabid species fed on slugs compared to small species. Therefore, large and medium sized species are of greater importance than smaller species.

P.madidus eats slugs in the laboratory (Stephenson, 1965) and molluscan remains have been recovered from *P.madidus* beetles caught in the field (Davies, 1953; Luff, 1974). This study confirmed that *P.madidus* will kill small slugs. Other smaller Pterostichini, such as *P.strenuus* feed on molluscs (Dawson, 1965). In this study, *P.strenuus* and *Pterostichus nigrita* (Paykull) also ate small slugs.

Liquid food has been found in the gut of *H.rufipes* (e.g. Davies, 1953; Sunderland, 1975). Davies thought *H.rufipes* belonged to a predominantly herbivorous group but Sunderland suggested the liquid food was from Tenthredinidae larvae. Hengeveld (1980a) argued the liquid food in Davies study represented the remains of earthworms, snails and caterpillars that had been pre-orally digested. Although Stephenson (1965) found *H.rufipes* did not eat slugs of an unspecified size in laboratory trials, it does eat slugs in the field (Cornic, 1973). Results from this study and chapter four support the argument of Hengeveld and confirm that *H.rufipes* will kill small slugs.

However, even *H.rufipes* beetles will be limited in the size of slug they can tackle. Dempster (1967) found that predation of *Pieris rapae* (Linnaeus) by *H.rufipes* was most important during the caterpillar's first two larval stages. As the caterpillar grew, arthropod predation became less important. Conversely, larger carabid

species might ignore small slugs altogether. The low rate of consumption of small prey by *P.melanarius* may be due to difficulties in detection and manipulation of the prey (Hagley *et al.*, 1982). However, in this study *P.melanarius* ate small slugs.

Most of the carabid tribes investigated contained a species which ate slugs. Notable exceptions were *L.pilicornis* and *N.biguttatus* which belong to the Loricerini and Notiophilini tribes. Crowson (1955) thought that these two tribes and the Nebriini (including *N.brevicollis*) were specialist Collembola feeders. Hengeveld (1980a) argued *N.brevicollis* was either a generalist with a preference for Collembola or a specialist that will eat other foods. *N.brevicollis* will eat a variety of prey (Penney, 1966). Although *N.brevicollis* did not attack slugs of an unspecified size in laboratory trials (Stephenson, 1965), in this study *N.brevicollis* frequently ate small slugs.

Three of the four *Amara* species killed slugs. *Amara similata* (Gyllenhal) killed slugs and *Amara apricaria* (Paykull) and *A.aulica* killed and ate slugs. This group is generally considered to be phytophagous and Davies (1953) thought the *Amara* were exclusively vegetarian. However, Dennison and Hodkinson (1983) found *A.plebeja* fed on diptera larvae and Allen (1953) reported *Amara convexior* Stephens killed and fed on a staphylinid. It seems likely that the full extent of the *Amara* diet is not understood. The *Agonum* species *A.dorsale* is an important aphid predator (Edwards *et al.*, 1978; Edwards *et al.*, 1979; Sunderland and Vickerman, 1980), *A.obscurum* and *A.fuliginosum* have also been found with mollusc remains in their guts (Dawson, 1965). Results from this study confirm that *A.fuliginosum* and *A.dorsale* will predate slugs.

2.2.4.3 Slug mortality in the field

Some predators killed but did not eat their slug prey, this may be due to the low motivational feeding state of these predators and/or due to the wasteful killing of prey. Wasteful killing has been reported in other carabids such as *P.melanarius*, which will kill prey when satiated (Hagley *et al.*, 1982).

Contacts between predators and prey could lead to rejection but mortality of the slug prey (personal observations). This may be due to the way in which prey are assessed by predators. Frank (1967) found three Pterostichini moved at random until their mouthparts touched winter moth pupae, then the pupae were nearly always attacked. The antenna of *L.pilicornis* have enlarged setae and the beetle uses an antennal strike to capture its Collembola prey (Bauer, 1982). Newly hatched slugs appear to be quite vulnerable to physical stress and are often killed by physical encounters with predators which lead to their rejection as prey. This information is very useful when interpreting serological data which only measures mass of prey consumed and not the number of prey attacked, injured or killed. The wasteful killing or accidental killing of slugs by predators indicates that carabids may have a larger impact on populations of slugs than realised by serological or other techniques.

When slug predation does not occur, it should be possible to conclude that the beetles would never predate slugs. However, the reverse scenario has been recorded; Luff (1974) found *P.madidus* never fed on the snail *Cochlicopa minima* (Porro) (=*C.lubricella*) in the laboratory, but the snails remains were found in over 30 percent of field caught specimens. Beetles used in this investigation were starved prior to exposing them to slug prey. This may have heightened their motivational feeding state and made them less selective in their choice of food. Slugs may then have been predated when they would normally be rejected. However, Luff (1974) argued that prey which are readily accepted in the laboratory are more likely to be utilized in the field.

2.2.5 Conclusions

Many of the carabids used in this study induced slug mortality, often at a range of experimental temperatures. Some of the small predators did not eat slugs but exposure to the beetles sometimes increased slug mortality. Slug survival in the controls was always greater than slugs which were exposed to beetles, even when predation was eliminated. Serological data may not reveal the full extent of slug mortality by carabid beetles.

The carabid predators used in this investigation were by no means a comprehensive list of all the carabids occurring in arable situations. Many other smaller predators may occur in the field which may exert an impact on populations of slugs. However, in the field a wide variety of alternative foods are available which may affect the extent to which these beetles predate slugs.

2.3 Behavioural studies of predator-slug interactions

2.3.1 Introduction

The interactions between five large carabid beetles and *D.reticulatum* slugs were investigated, to assess the activity cycle, foraging time, predation rate, capture efficiency and handling time of each predator (Wratten, 1982).

2.3.1.1 Activity, slug predation, foraging and handling time

D.reticulatum is largely nocturnal (e.g. Barnes and Weil, 1945; Dainton, 1954) therefore nocturnal predators have the greatest chance of interacting with the prey. Time-lapse video tape recording techniques were used to confirm that slug and beetle activity coincided.

Luff (1983) described a useful predator as one which is voracious, continually feeding and with a small prey handling time. Wratten (1982) identified the need to assess attack rates and handling time of prey by potential predators. Although carabids are voracious predators (Hagley *et al.*, 1982), large slugs are difficult prey for carabids to handle (Pakarinen, 1994). In this project, *D.reticulatum* slugs found on arable land ranged from 0.04-1.49g (chapter four). These are large prey and a predator eating such a large meal may be satiated at each feed (Mills, 1982). This would reduce the number of prey killed by each predator. There is little information in the literature concerning the kill rate of slugs by carabids. Stephenson (1965) assessed the kill rates of a number of carabids on *D.reticulatum* and found only *C.violaceus* killed at a rate of more than one slug per day, however slug size was not specified.

2.3.2 Species chosen for investigation

Two generalist predators and three mollusc specialist predators were selected as model predators. The two large generalists were *P.niger* and *A.parallelepipedus.* Evans and Forsythe (1985) described the former species as an opportunistic feeder and the latter species as scavengers capable of dealing with many types of prey.

Slugs are important components of the diet of *Carabus* and *Cychrus* species (e.g. Gruntal and Sergeyeva, 1989). *C.violaceus, C.caraboides* and *C.nemoralis* were selected as model molluscan specialist predators.

2.3.3 Methods

A 27 x 51 x 15 cm plastic arena was prepared. It was filled to a depth of 10 cm with soil collected from Close House. The soil was broken up to give a fine tilth and large organisms such as earthworms and slugs were removed.

An electric fence ran around the inside walls of the arena. This consisted of two parallel stainless steel wires spaced one centimetre apart. The wires were secured against the walls of the arena and a potential of 9 volts ac passed through the wires. Slugs crawling over the two wires, connected the two circuits and received a small electric shock which prevented them crossing the electric fence and leaving the arena.

Day lighting was provided by a 250w metal halide lamp and night light from two boxes each containing a 25w tungsten filament bulb behind a Wratten 87c filter. The filter absorbed wavelengths of less than 750nm. The two boxes were angled into the arenas and provided sufficient light for the video recordings but did not interfere with beetle or slug behaviour. The activity in the arena was recorded with a National Panasonic wv1850 VCR which is sensitive to very low light levels and infra-red light. The camera recorded on VHS format video tapes, 24 times slower than normal and was replayed at normal speed.

The experiments were conducted in a controlled environment room, which was

maintained at 13°C with a light:dark regime of 16:8 hours. Greenslade (1963) concluded a beetle's habitat dictated whether it was diurnal or nocturnal. Since this project was concerned with the impact of beetles on slug populations in arable land, the beetles were collected from arable or open land. The predators were used as soon as possible after capture. Prior to experimentation, predators were placed into petri dishes with damp filter paper and kept without food for 48 hours in the controlled environment room. Slugs were cultured in the controlled environment room in the plant propagators (described in section 2.2.2).

Six slugs weighing between 0.1 - 0.7g were added to each arena and allowed to settle in for one hour before a single predator was added. A video tape recorder was used to record the predator's activity for a 48 hour period which included nocturnal and diurnal activity. The number of experimental runs made for each predator species was limited by their availability in the field. Beetles were used for one run only and were then discarded.

The first five runs were made in full sized arenas, runs six to fifteen were made in half sized arenas. The remaining runs (16-51) were made in quarter sized arenas (see Appendix 2.2). The half sized arenas were constructed by dividing the full sized arena horizontally with a 15cm deep sheet of zinc (plate 2.1). Earlier studies at Close House indicated that slugs avoided crawling over the metal surface. These sheets were used to prevent slugs from moving between arenas. The zinc barrier was abandoned in favour of glass sheets, which were used to divide the arenas into quarters. The tops of the glass sheets were lined with double sided sticky tape which was coated in salt. This repelled the slugs and proved to be 100 percent successful in preventing slug movement between the arenas.

2.3.4 Results

2.3.4.1 Activity, slug predation, foraging and handling time

Observations were made on the behaviour of forty eight beetles, covering ninety six nights. The predators were mainly active during the night, generally emerging

Plate 2.1 Experimental arena used in section 2.3 and 2.4.

within an hour of sunset and foraging until sunrise. When the day-light came on, the predators moved quickly to seek out shelter and hide.

Griffiths (1982) and Griffiths *et al.*, (1985) broke the activity of *A.dorsale* into; no activity, running, searching and eating. In this study, only the active components of beetle behaviour were assessed, therefore beetle behaviour was broken down into; time spent active on the soil surface and time spent attacking or feeding on prey before resuming activity. The number of prey contacts made by the predator before an attack which resulted in a 'kill' was noted along with the total number of slugs killed.

As the investigation was concerned with foraging and handling, only activity leading up to the death of a slug was evaluated (see Appendix 2.3 for the number of observations). Therefore only those specimens which predated a slug were included in the analysis. As the predators foraged during the night, only the night time activity was assessed. Only the activity leading up to the first kill was considered. If a specimen made its first kill on the second night, then only the second nights foraging activity was considered.

The number of contacts between the beetle and any slug before a slug was predated, the time elapsed between the start of foraging and an attack on a slug which resulted in a kill and the time taken to kill and consume the slug (handling time) were recorded. The results are shown in Figures 2.3.1 - 2.3.4 and Table 2.3.1.

The number of contacts that each beetle species made with slugs before making a kill was investigated. Treatments (beetle species) were assessed using a one way ANOVA. There were no significant differences in the number of contacts ($F=2.73$, d.f.$=4,21$, $P<0.05$). This was due mainly to the large standard errors shown by *A.parallelepipedus*, *C.violaceus* and *P.niger* (see Fig. 2.3.1). However, the generalist beetles made more contacts before predating a slug than the specialist beetles (Fig. 2.3.1). The mollusc specialists *C.nemoralis, C.caraboides* and *C.violaceus* generally predated a slug within the first few contacts, although one *C.violaceus* beetle made

Fig. 2.3.1 Prey contacts and slug predation in five beetle species

Histograms show the mean number of contacts between beetle and slugs before a slug is predated. I=standard error. Number of samples in brackets ().

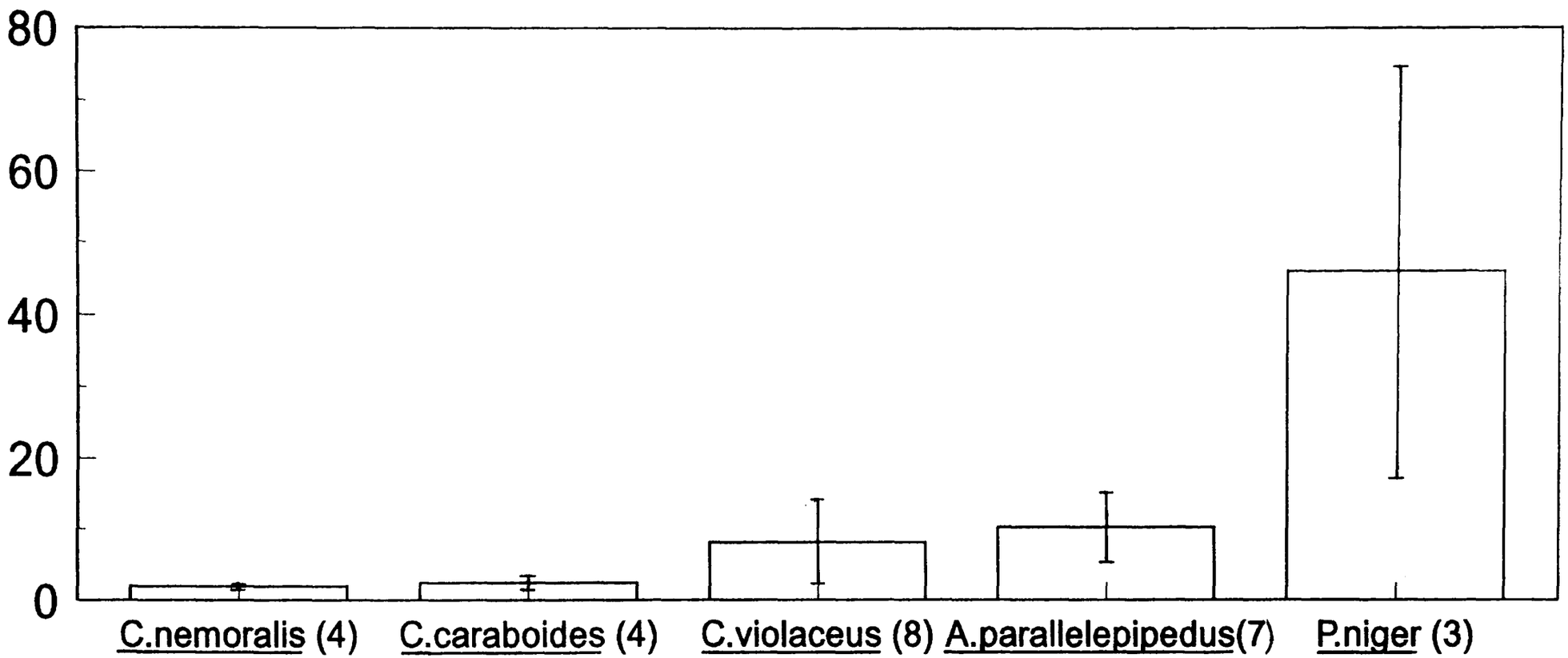


Fig. 2.3.2 Foraging time and slug predation in five beetle species
Histograms show the mean time each species foraged before predating a slug. I=standard error. Number of samles shown in brackets ( ).

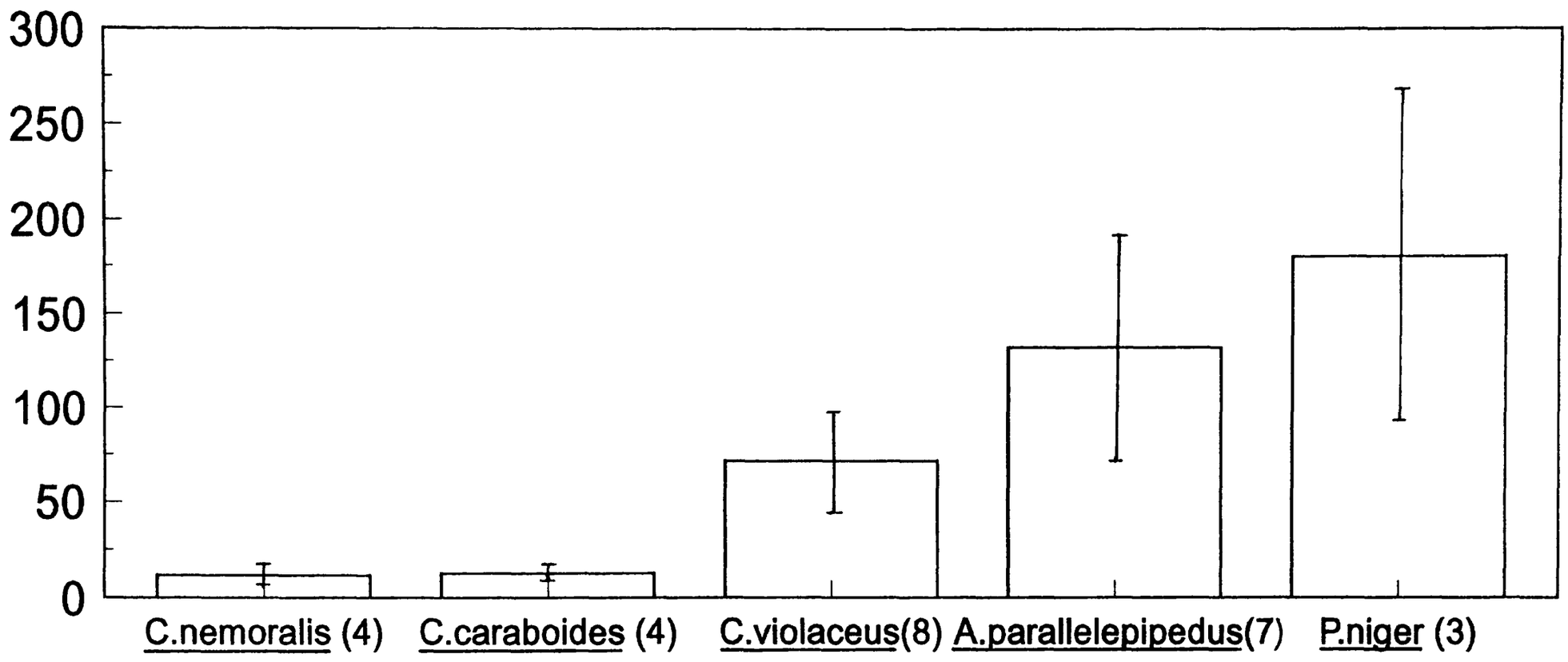


Fig. 2.3.3 Prey discovery by five beetle species

The relationship between foraging time and prey contacts. Data for each species presented. Number of samples shown in brackets ().

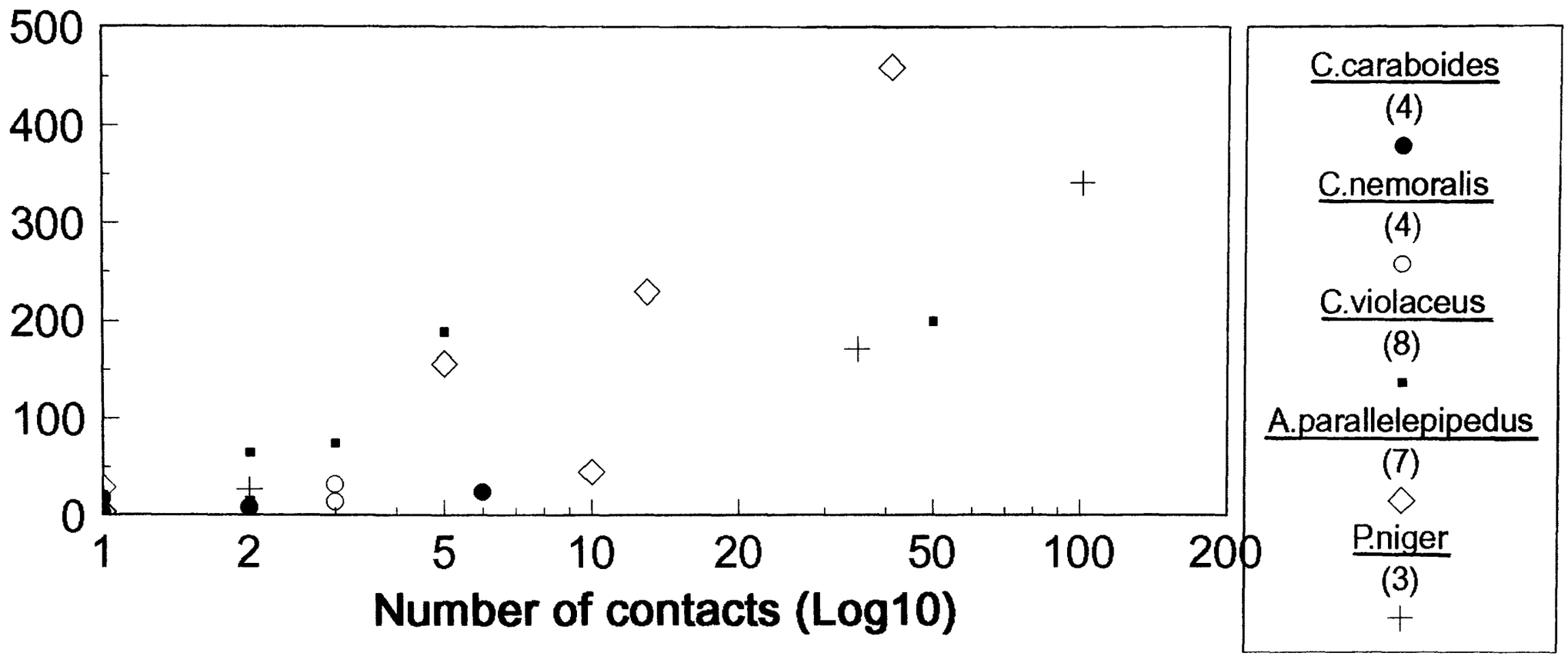


Fig. 2.3.4 Prey handling times in five beetle species

Histograms show the mean time each species spent killing and eating a slug prey. I=standard error. Number of samples are shown in brackets ().

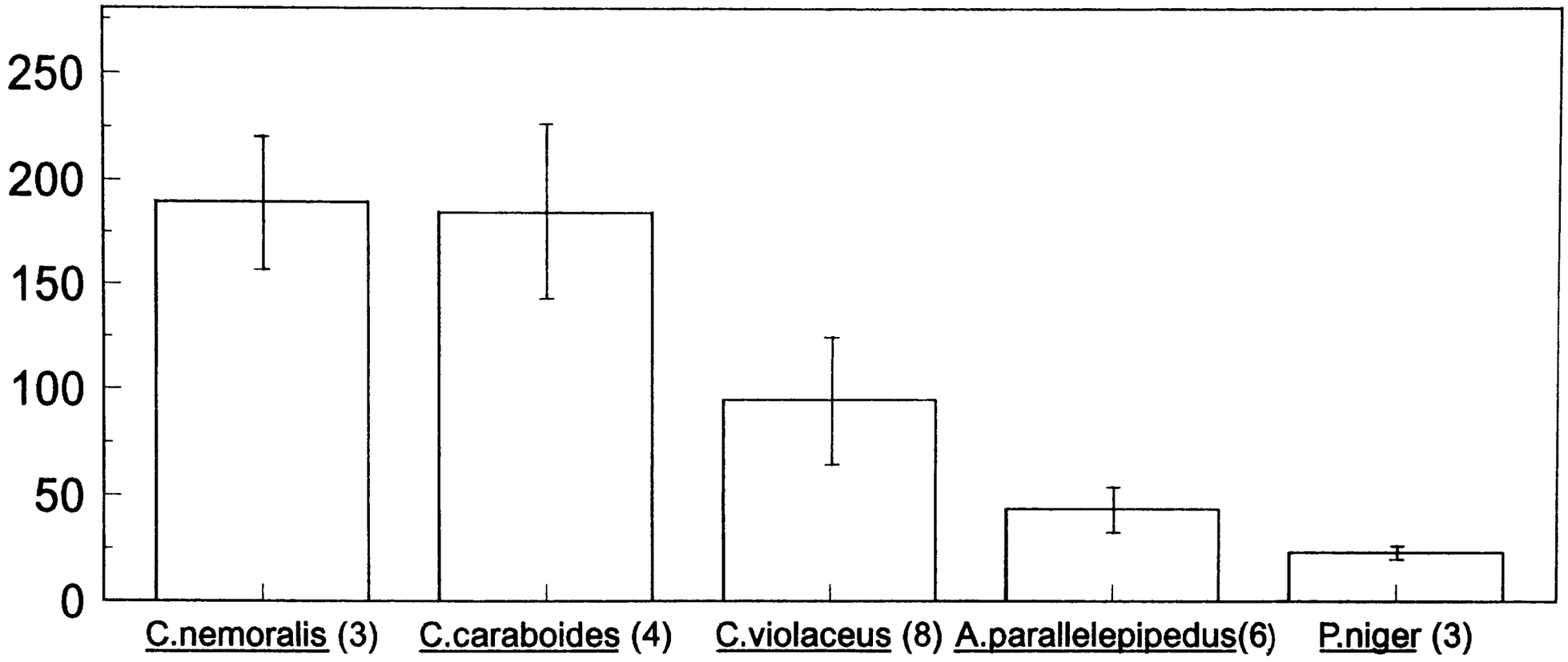


Table 2.3.1 The number of contacts between predators and slugs before a slug was predated is shown in column two. The time elapsed between the start of foraging and an attack on a slug which resulted in a kill is shown in column three. The time taken to consume the first slug killed (handling time) is shown in column four. The dashes (-) in column four indicate that the handling time is unknown, as the slug was carried underground to be consumed. Only data concerning those predators which predated a slug are presented.

Species	No. of contacts before kill	Time elapsed before first kill (minutes)	Time spent feeding on first kill (minutes)
C.nemoralis			
run number 01	3	14	-
run number 22	3	32	208
run number 37	1	0	234
run number 48	1	1	126
C.violaceus			
run number 10	5	189	9
run number 14	50	200	83
run number 30	2	65	162
run number 34	1	1	35
run number 35	3	75	119
run number 39	1	21	85
run number 46	2	15	261
run number 47	1	7	7
C.caraboides			
run number 11	1	2	51
run number 15	2	8	143
run number 32	6	24	10
run number 40	1	17	226
A.parallelepipedus			
run number 04	13	230	-
run number 12	5	156	17
run number 17	1	3	37
run number 18	10	45	51
run number 42	1	4	74
run number 44	1	29	69
run number 51	41	460	15
P.niger			
run number 03	2	27	-
run number 20	35	172	21
run number 21	101	342	25

fifty contacts (Table 2.3.1). The two generalist predators *A.parallelepipedus* and *P.niger* made more contacts than the mollusc specialists, although three *A.parallelepipedus* beetles predated a slug on their first contact. On average, *P.niger* made many more contacts before predating a slug than the other four species.

The length of time that each beetle species spent foraging before making a kill was investigated. Treatments (beetle species) were assessed using a one way ANOVA. There were no significant differences in the time spent foraging ($F = 1.71$, d.f. $= 4,21$, $P < 0.05$). This was due to the large standard errors between runs (see Fig. 2.3.2). There was a trend of longer time spent foraging before predation by the generalist predators. *C.nemoralis* and *C.caraboides* beetles generally spent a few minutes foraging before making a kill (Fig. 2.3.2). On average *C.violaceus* foraged for about an hour and the two generalists spent the longest periods of time foraging before making a kill. *A.parallelepipedus* foraged for an average of over two hours and *P.niger* for an average of three hours before making a kill.

The smaller arenas used in replicates 6-51 effectively increased the slug density and therefore the likelihood of a predator encountering a slug in any given time period. There was a positive correlation between the length of time a predator spent foraging and the number of contacts made with slugs (Fig. 2.3.3). As the predators generally didn't make a kill on the first contact, the size of the arena and therefore slug density was not considered to be affecting the results.

Prey handling covered the period of time from a predator contacting the slug it killed (on the occasion it was killed) to the predator commencing foraging again after consuming that slug meal. The mollusc specialist predators spent longer handling (feeding) on the slugs and the two generalist species spent least time handling the slugs (Fig 2.3.4). *P.niger* spent the least time (23 minutes) handling prey. The three mollusc specialists spent the longest time handling prey. *C.violaceus* spent 90 minutes handling prey. *C.nemoralis* and *C.caraboides* spend over three hours handling prey.

The length of time that each beetle species spent killing and consuming a slug prey (handling time) was investigated. Treatments (beetle species) were assessed using a one way ANOVA. There were no significant differences in the time spent handling prey ($F=2.66$, d.f.$=4,18$, $P<0.05$). This was due to the large standard errors between runs (see Fig. 2.3.4).

A capture efficiency (C.Ef) was calculated for each predator species. Several beetles made multiple kills, but only the number of contacts leading up to the first kill were considered. If a beetle made its first kill on the second night, then only those contacts made with the prey on the second night were considered. The C.Ef was calculated by dividing the number of beetles making a kill, by the number of contacts made with the prey before the kill was made (Halsall, 1990). As the C.Ef is influenced by hunger level of a predator (Mols, 1979) only those predators which eventually killed a slug were included in the calculation. A species which always kills on its first contact has a C.Ef of one.

If a single *C.violaceus* specimen is ignored, the three mollusc specialists have high, similar C.Ef (Table 2.3.2) and half of the encounters with slugs resulted in the slug being killed. The two generalist species have much lower C.Ef and the C.Ef for *P.niger* is particularly low. One in ten encounters resulted in the slug being killed by *A.parallelepipedus* beetles and approximately one in fifty encounters between slug and *P.niger* resulted in the slug being killed.

All of the replicates were used to calculate a predation rate for each beetle species (Table 2.3.3). *C.violaceus* had the highest predation rate (0.43 slugs/beetle/night). *A.parallelepipedus* had the lowest predation rate (0.26 slugs/beetle/night).

2.3.5 Discussion

2.3.5.1 Activity, slug predation, foraging and handling time

Slugs are mainly nocturnal and in order to predate slugs, beetles must be active at night when slugs are active. The nocturnal or diurnal activity of a beetle species

Table 2.3.2 The capture efficiency of five carabid predators of *Deroceras reticulatum*. Two capture efficiencies are shown for *C.violaceus*, the value in brackets corresponds to run number 14 being omitted from the calculation.

	No. of Replicates	Capture efficiency
C.caraboides	4	0.4
C.nemoralis	4	0.5
C.violaceus	8(7)	0.12 (0.5)
A.parallelepipedus	7	0.09
P.niger	3	0.02

Table 2.3.3 The total number of slugs killed by each predator species over the experimental period (two nights). The rate of slug 'kills' per night is given in the final column. Data for those beetles which failed to kill a slug over the two nights are included in the calculation of the kill rate.

Species	No.of runs	Slugs killed		Slugs killed/ beetle/night.
		Night1	Night2	
C.caraboides	6	5	0	0.41
C.nemoralis	8	6	0	0.37
C.violaceus	15	4	9	0.43
A.parallelepipedus	15	6	2	0.26
P.niger	4	2	1	0.37

can depend on its geographic location or habitat. *C.nemoralis* has a plastic activity cycle, it is nocturnal in the north and east of Europe and diurnal in the south (Greenslade, 1963). In the same geographic area, activity can depend on habitat. Greenslade (1963) found that *P.madidus* beetles caught in woodland were nocturnal and beetles caught in grasslands were diurnal. This would reduce the potential role of *P.madidus* as a slug predator in agriculture as agricultural lands are effectively grasslands.

Luff (1978) found a large amount of nocturnal activity in the field habitat and several beetles which killed slugs in the previous study (section 2.2) were nocturnal. These included: *A.dorsale* (Luff, 1978; Griffiths, 1982), *N.brevicollis* and *H.rufipes* (Greenslade, 1963; Luff, 1978), *Synuchus nivalis* (Panzer), *P.madidus*, *P.niger*, *A.aulica* and *H.aeneus* (Luff, 1978). Of the beetles used in this study, Greenslade (1963) reported *C.violaceus*, *C.caraboides*, *P.niger* and *A.parallelepipedus* were nocturnal and *C.nemoralis* had a plastic activity cycle. In this study, the beetles assessed were active mainly during the night, including *C.nemoralis*. Therefore these beetle species and the species listed above are all active when slugs are active and have the potential to predate slugs in arable fields.

All of the beetle species investigated in this study, killed and ate large *D.reticulatum* slugs, including the two generalists *P.niger* and *A.parallelepipedus* which Evans (1967) considered to be general predators and scavengers. There were differences between the species in capture efficiencies, predation rates, foraging and handling times.

Slugs respond to tactile stimulation by exuding large amounts of fluid from the body surface (Deyrup-Olsen and Martin, 1982). This can deter slug predators by fouling their mouthparts and forelegs. Pakarinen (1994) found the mucus exuded by slugs protects them from attack by the generalist *P.niger* but not from the specialists *C.caraboides* and *C.violaceus*. The success of the two mollusc specialists was in their ability to kill the slug before too much mucus was exuded. Both specialists were able to kill quicker than *P.niger* which was not able to kill slugs quickly and often

had its forelegs and mouthparts fouled with mucus. In this study, some *C.violaceus* beetles disengaged from their attacks on slugs and engaged in extended mouth cleaning activities to clean themselves of slug mucus.

From this evidence it appeared that *C.violaceus* beetles occasionally experienced difficulties in overcoming the mucus defence system of the slug and lacked the 'quick kill' strategy of *C.caraboides* and *C.nemoralis* described by Pakarinen. However, *C.violaceus* expressed the highest predation rate which suggests it is able to overcome occasional difficulties in tackling slugs and all three mollusc specialists expressed similar capture efficiencies.

The number of contacts each beetle made with a slug before making a kill was quite variable both between species and within some species. At least one beetle of each species attacked and killed a slug within the first two contacts. This occurred most frequently with the three mollusc specialists *C.caraboides, C.nemoralis* and *C.violaceus* which generally predated a slug within the first few contacts. This reflects the specialisation of *Carabus* and *Cychrus* species on molluscs and indicates that they spend short periods of time foraging before predating a slug. The third molluscan specialist, *C.violaceus* spent more time foraging than the other two specialists, but less time than the two generalists.

The variations in foraging times within a species are possibly due to an individual's hunger and motivational feeding state (Ernsting and Van der Werf, 1988) or the reluctance in feeding on slugs. Parameters such as beetle sex may be influencing the results, e.g. male beetles may be searching for female beetles to mate with and in this study caution should be taken with the small sample sizes. However, the mean time spent foraging before making a kill was three hours for *P.niger* and over two hours for *A.parallelepipedus*. Such long periods of time spent foraging in the field would bring the predator into contact with other prey species which it may find more attractive and predate at the expense of predating slugs.

This may be particularly true of generalist predators which Pakarinen (1994) argued

would leave slugs untouched if other prey were available. This argument is supported by the capture efficiencies of the five species. If a single *C.violaceus* replicate is omitted, all three specialist species had similar, high C.Ef. The two generalists expressed capture efficiencies much lower than the three specialists. Pakarinen (1994) found *C.caraboides* and *C.violaceus* were significantly more successful than *P.niger* in hunting slugs. In this study, *P.niger* spent long periods of time foraging before making a kill. The evidence from these two studies suggests that *P.niger* may only kill slugs when no other prey are available.

The predation rate can be used to predict the number of slugs killed by each predator per night. *C.violaceus* had the highest predation rate and killed 0.43 slugs per night. Stephenson (1965) also found *C.violaceus* was the most voracious of seven carabids tested and ate five slugs in four days (or 1.25 slugs per night). Of the other species which killed slugs, he found *P.niger* had the lowest predation rate (0.06) and *A.parallelepipedus* had a predation rate of 0.4. In this project, the predation rate of *P.niger* was higher (0.37) and *A.parallelepipedus* was lower (0.26). This may limit the usefulness of carabids as predators of slugs in arable land, as the carabid fauna consists mainly of generalist predators (chapter five). However, in the field slugs exist in mixed age groups and small slugs can dominate the slug population (e.g. Hunter, 1968a). As generalist predators readily kill small slugs (section 2.2), predation rates in the field may be much higher.

Sandness and McMurtry (1972) found the hunger level of predatory mites directly affected the frequency of captures and length of digestive pause. Mills (1982) argued that for large prey, satiation occurred after every meal. The slugs used in this investigation were large prey which accounts for the low predation rate. Despite the large size of the experimental slugs, some individuals of all three mollusc specialists species made more than one kill and one *C.violaceus* beetle made three kills on one night.

Prey handling is an important component of any predator-prey system. Extended

prey handling times reduces the time a predator is foraging for food. Slug consumption was the major component of prey handling. The five predator species varied in the length of time they spent eating a slug but all spent relatively long periods of time feeding. The specialists spent longer periods feeding than the generalists. This was particularly true of *C.nemoralis* and *C.caraboides* which spent long periods feeding due to pre-oral digestion of the slug before ingestion.

Luff (1983) described a useful predator as one which is voracious, continually feeding with a small prey handling time. Some of the predators exhibited some of the qualities but none exhibited all of these qualities. However, predators cannot realistically be expected to fulfil such criteria when dealing with large prey such as slugs. The slugs used in this study were large specimens and it is conceivable that if smaller slugs were used, predation rates would have been higher and handling times lower. Similar relationships have been found between the weight of Diptera prey and feeding duration of wolf spiders (Edgar, 1970).

2.3.6 Conclusions

All five model predators have compatible activity cycles with the main pest slug *D.reticulatum* and all were found on arable land in this project, with the exception of *C.nemoralis* (see chapter five). The predators did not fulfil all of Luff's criteria in describing a useful predator. i.e. predation rates were low and handling times were relatively long. However, slugs are extremely large prey when compared to other agricultural pests such as aphids and cabbage root fly. For such large prey, multiple kills and short handling times cannot realistically be expected. However, all of the beetle species killed large slugs and some made multiple kills in one night.

The predation rates exhibited by the five species ranged between 0.26-0.43 slugs killed per night and the mollusc specialists nearly always had better predation rates than the generalist predators. The capture efficiencies of the specialists was much better than the generalists and reflects the degree of specialisation of *Carabus* and *Cychrus* species on mollusc. The abundance of alternative prey and the relatively

low capture efficiencies of slugs by the two generalist species may affect the degree to which generalists attack and feed on large slugs in the field.

2.4 Orientation of beetles to slug mucus

2.4.1 Introduction

This behavioural study investigated orientation to slug mucus in seven carabid species. Searching behaviour which increases the likelihood of a predator finding the target prey is a desirable characteristic and increases the predators effectiveness as a natural enemy of that prey. Wratten (1982) identified the importance of searching behaviour in the assessment of the role of natural enemies.

In this study orientation to slug mucus is defined as changes in a predators locomotion after contacting slug mucus. Changes in locomotion can take many forms. Hassell and May (1974) described a model typical of many invertebrate predators and parasites which change their turning locomotion after eating or parasitising a prey. Their pre-feeding locomotion changes to one of tight turning after consuming or parasitising a prey. As many prey are aggregated, this locomotory activity keeps the animal in the vicinity of the prey and increases the likelihood of the predator encountering another prey.

This type of behaviour has most frequently been reported in parasitic wasps (Hymenoptera) (e.g. Spradbery, 1970; Waage, 1977, 1978 and 1979). Laing (1937 and 1938) described the movement of the Chalcid parasite *Trichogramma evanescens* Westwood after parasitising a host egg. Its movement took the form of a twisted track, winding around and away from the host. This greatly increased the frequency of contacting another host egg. This behaviour has also been reported in carabid beetles. Halsall (1990) found four species of carabid reduced their speed of movement (orthokinetic response) and increased their turning rate (klinokinetic response) after feeding on aphids.

Many parasitoids are thought to respond to chemical volatiles associated with or

secreted by the host. The Hymenoptera parasitoid *Goniozus natalensis* Gordh decelerates, walks slower and makes more turns when passing areas of filter paper impregnated with larval frass of its host *Eldana saccharina* Walker (Smith *et al.*, 1994). Carabids also respond to the previous presence of prey on the substrate surface. Ernsting *et al.*, (1985) found the locomotory activity of *N.biguttatus* was affected by Collembola previously occupying the substratum. The change in behaviour enabled it to increase the number of prey encountered. Wheater (1989) found visual, gustatory and olfactory signals were used by large carabids to orientate to prey of various kinds, including slugs. The molluscan specialists *C.caraboides*, *C.violaceus* and *C.problematicus* all orientated to slug mucus using gustatory senses but four Pterostichini, *P.madidus*, *P.melanarius*, *P.niger* and *A.parallelepipedus*, did not orientate to slug mucus.

2.4.2 Species chosen for investigation

Seven model predators were investigated in this study which represented a mixture of mollusc specialist and generalist carabids found in arable land under crops of winter wheat and oilseed rape (chapter five).

The generalist predators were *P.madidus*, *P.niger* and *H.rufipes*. *P.madidus* was present at all three field sites and was relatively abundant in both years of the study (chapter five). In addition, *P.madidus* beetles collected from the field sites had fed on slugs (chapter four). *H.rufipes* was also one of the more abundant carabids at one of the field sites in both years of the study and large numbers of specimens collected from the field had fed on slugs. *P.niger* beetles collected from field sites had also fed on slugs and it kills large *D.reticulatum* slugs (section 2.3).

The mollusc specialist predators were *C.nemoralis*, *C.violaceus*, *C.problematicus* and *C.caraboides*. *C.nemoralis* was not found in the field sites in this project but showed orientation to slugs in study 2.3. *C.violaceus* was the most abundant *Carabus* in field sites in 1991 and 1992 in this project and is a resident of arable land (e.g. Pollard, 1968a). *C.problematicus* and *C.caraboides* were also found at the field sites.

2.4.3 Methods

This study commenced in the autumn of 1992. As the age of the beetle may affect its behaviour, only newly emerged and/or active beetles were used in the experiments. The arenas and video equipment used in section 2.3 were used in this study. All experiments were conducted in the controlled environment room at 13°C with a dark:light regime of 12:12 hours.

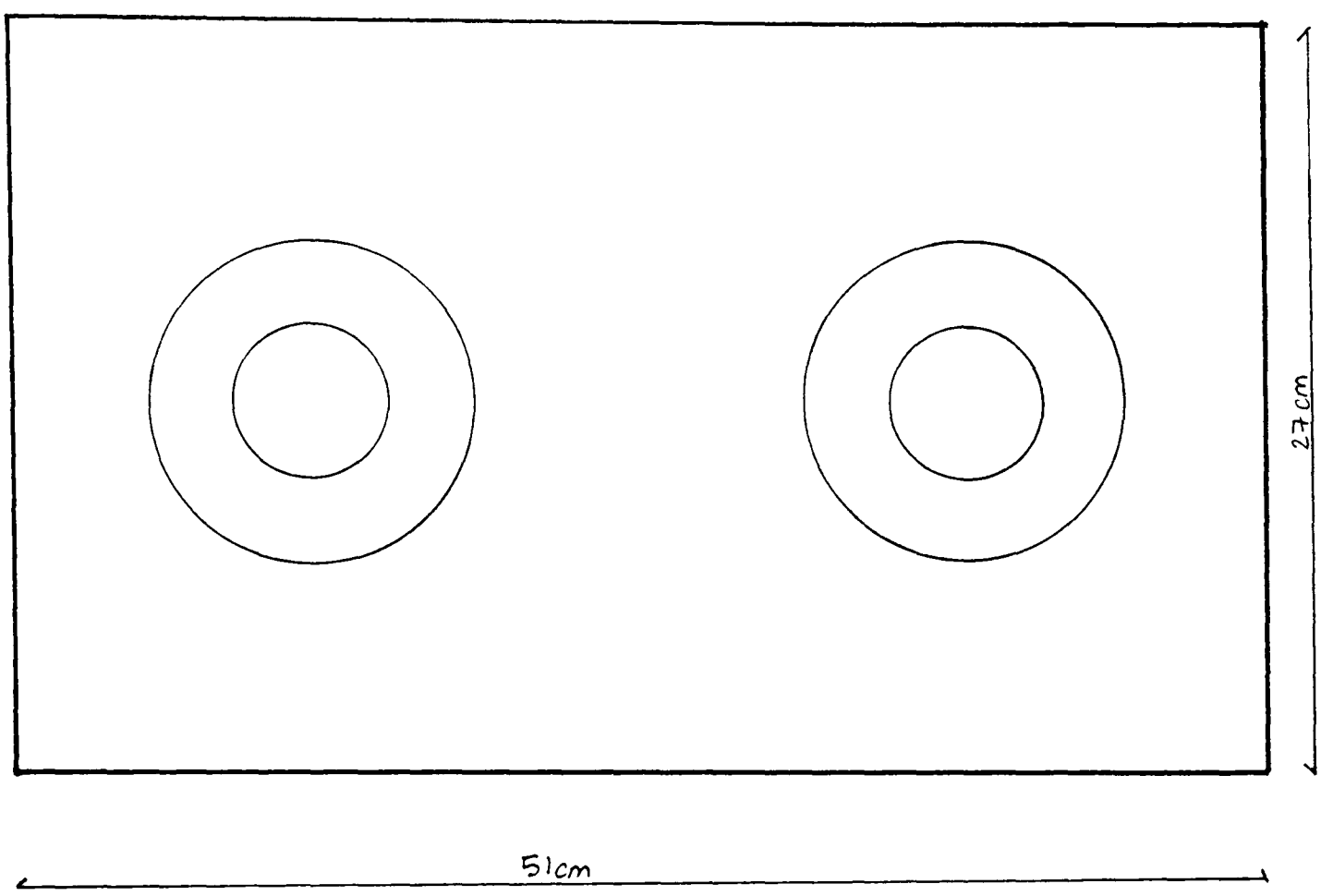
The soil of the arenas was firmly compacted and two adjacent circular zones (12.5cm in diameter) were marked on the soil surface using an inverted tin can. Within each circle an inner zone (6cm in diameter) was similarly marked. One set of markings was designated the inner and outer control zones (i.e. no slugs) and the second set of markings was designated the inner and outer slug zones. The two sets of zones were separated by 20cm and as the walls of arenas are known to affect beetle activity (Griffiths *et al.*, 1985), the zones were defined away from the arena walls (Fig. 2.4.1).

Twenty *D.reticulatum* slugs were placed in the inner slug zone and the 6cm diameter tin can was inverted over the slugs and firmly pushed into the ground. This prevented the slugs from escaping. The slugs were left for five hours to lay down a mucus layer on the soil surface.

After this period the can was carefully removed and the slugs were gently lifted from the soil surface using forceps. Extreme care was taken not to damage the mucus layer on the soil surface. Faecal remains and slug eggs were removed with a pair of Jewellers forceps. The quality of mucus layer was solely dependant on the activity of the slugs. However, a good covering of over 60 percent of the designated slug zone was always achieved. The edges of the zones were firmed down and the arena was ready for the introduction of the beetles.

The beetles used in this investigation were starved separately in petri dishes in the controlled environment room for 24 hours prior to the experiment. This enhanced their foraging activity and allowed them to acclimatise to conditions in the

Fig. 2.4.1 Layout of the experimental arenas used to investigate the effect of slug mucus on the locomotion of carabid beetles (see section 2.4).



controlled environment room.

The availability of beetles determined the number of replicates made of the larger species. The number of beetles used in each replicate was determined by the time constraints of the project. Initially beetles were introduced separately for one night's recording. However due to time limitations, five beetles per arena had to be used in the later experiments (Table 2.4.1). No apparent interference occurred between the beetles when five beetles were used together. The purpose of the experiment was to record and interpret beetle activity in the slug and control zones. Beetle activity was concentrated around the walls of the arenas and beetles met only occasionally in the centre of arenas. Meetings in the slug and control zones were extremely rare and were not included in the analysis.

The starved beetles were introduced into the arenas. A time lapse video recorder (section 2.3.3) was used to tape the night time activity of the beetles in the arenas. The beetles were removed from the arenas the following morning and the movement during each entry into the inner zones was analyzed.

2.4.4 Analysis of the tapes

A video tape recorder was connected to a VDU and the video tapes were played onto the VDU through an Amiga PC. All the data were collected from the image of the arena on the VDU using the Micromeasure programme. The time/date display on the video tape was calibrated to the time facility in the Micromeasure programme. The length of the arena wall (a constant length) was used to calibrate length in the Micromeasure programme. To calculate extent of turning, the number of degrees turned per one second interval was selected.

From the video image on the VDU, the four zones in the arena were defined using the area define facility of the Micromeasure programme. A beetles movement through the zones could then be traced using a computer mouse. The Micromeasure programme generated data for several parameters of the beetles movement in the inner zones. Data were recorded for the six defined categories

Table 2.4.1 The number of beetles used in the orientation to slug mucus experiment. Column two indicates the number of experimental runs made of each beetle species and column three indicates the number of beetles used in each experimental run. The figures in columns four and five indicate the number of entries made by each beetle species into the inner slug and inner control zones. These entries were used in statistical tests to compare locomotory behaviour in the inner slug zone with the inner control zone.

Species	No.of runs	Beetles per rep.	No. of entries analyzed	
			Inner slug	Inner control
C.violaceus	4	1	4	5
C.problematicus	5	1	16	18
C.nemoralis	4	1	39	9
P.niger	4	1	25	9
P.madidus	1	5	43	29
C.caraboides	1	5	11	8
H.rufipes	5	1	12	11

of behaviour (section 2.4.5).

These data were collected on every occasion a beetle entered an inner zone. The parameters were measured from the beetle first entering the inner zone and stopped after the beetle exited the inner zone.

2.4.5 Information retrieved from the tapes

The following information was extracted from each entry into the inner slug and control zones:

1. Time spent moving in inner zones.

2. Time spent stationary in inner zones.

3. Speed of movement (total and net) in inner zones.

4. Distance covered in inner zones.

5. Degrees turned per second in inner zones.

6. Number of re-entries into inner zones.
   (re-entry defined as beetle leaving inner zone and re-entering the inner zone before leaving the outer zone).

7. Number of loops
   (loop defined as a 180° change in direction of beetle leaving the inner and outer zone which brought it back into the inner zone).

2.4.6 Results

Preliminary investigations of the tapes indicated that the beetles were nocturnal, therefore only data from night-time activity were used in the analysis. Within a species, some beetles were very active and showed multiple entries into the zones

whilst other beetles were not active at all during the experimental period. Only data from a single *C.violaceus* specimen were available.

The mean of all the defined categories were used to construct histograms. Where possible t-tests were made to compare the data between inner slug and control zones. Data for each entry into the inner slug and inner control zones are presented in Appendix 2.4 and 2.5. The number of observations used to assess beetle locomotion in the following categories are presented in Table 2.4.1.

2.4.6.1 Time spent moving, time spent stationary and the distance moved in inner zones

If the slug mucus was acting as an arrestant then beetles showing an interest in the mucus were expected to respond to the mucus in the following three categories: Spend more time moving in the inner slug zone, looking for a meal. Spend more time stationary in the slug zone, investigating the soil surface. Walk greater distances in slug zone compared to the control zone, foraging for a slug meal.

Time spent moving

Comparisons were made between the time spent moving in the inner slug zone with the inner control zone for each beetle species. All seven beetle species spent more time moving in the inner slug zone when compared to the inner control zone (Fig 2.4.2). *C.problematicus* ($t=3.85$, d.f.$=32$, $P<0.001$), *C.nemoralis* ($t=2.75$, d.f.$=43$, $P<0.01$), *C.caraboides* ($t=2.63$, d.f.$=17$, $P<0.05$), *P.niger* ($t=3.16$, d.f.$=32$, $P<0.01$) and *P.madidus* ($t=2.1$, d.f.$=70$, $P<0.05$) spent significantly more time moving in the inner slug zone.

Distance moved

Comparisons were made between the distance moved in the inner slug zone with the inner control zone for each beetle species. All of the species except *H.rufipes* covered a greater distance in the inner slug zone compared to the inner control zone (Fig. 2.4.3). *C.nemoralis* ($t=2.48$, d.f.$=43$, $P<0.05$), *P.niger* ($t=3.85$, d.f.$=32$, $P<0.001$) and *C.caraboides* ($t=2.44$, d.f.$=17$, $P<0.05$) covered significantly greater

Fig. 2.4.2 The mean amount of time spent moving
by seven beetle species per entry into the inner slug and control zones.
I = standard error.

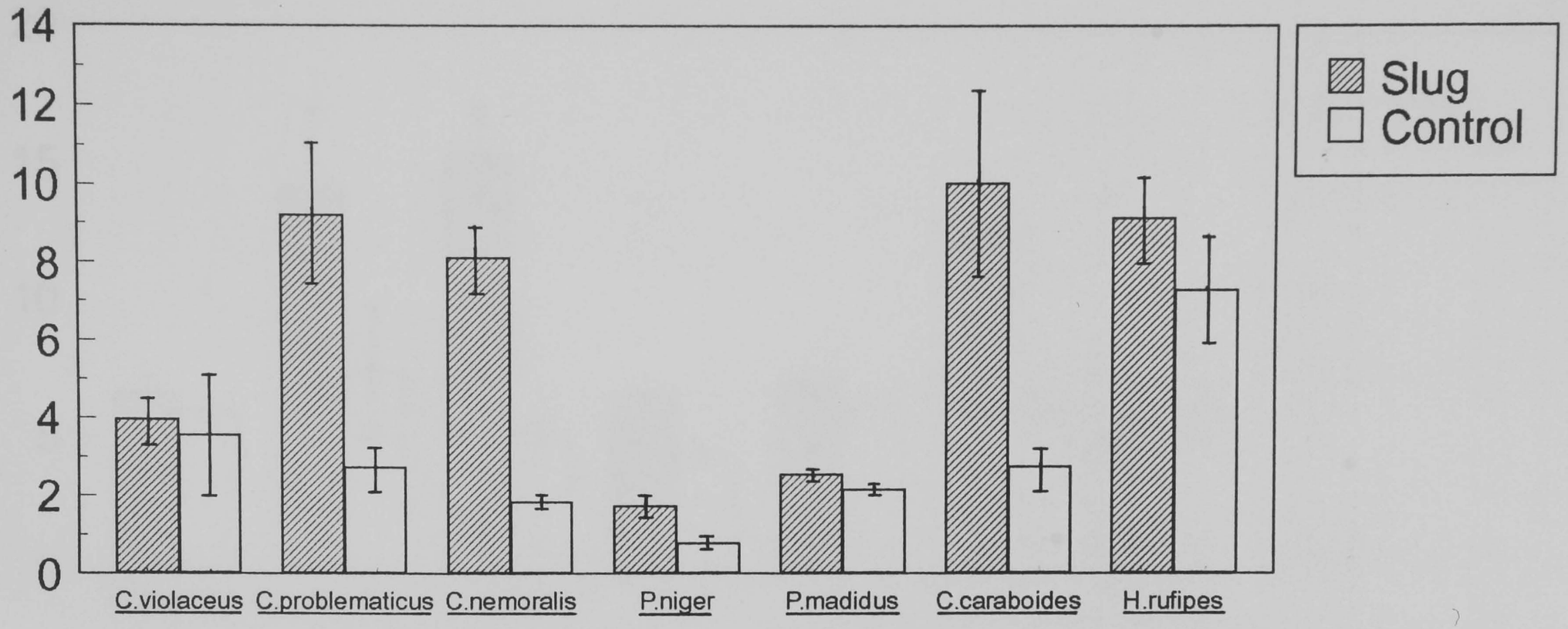
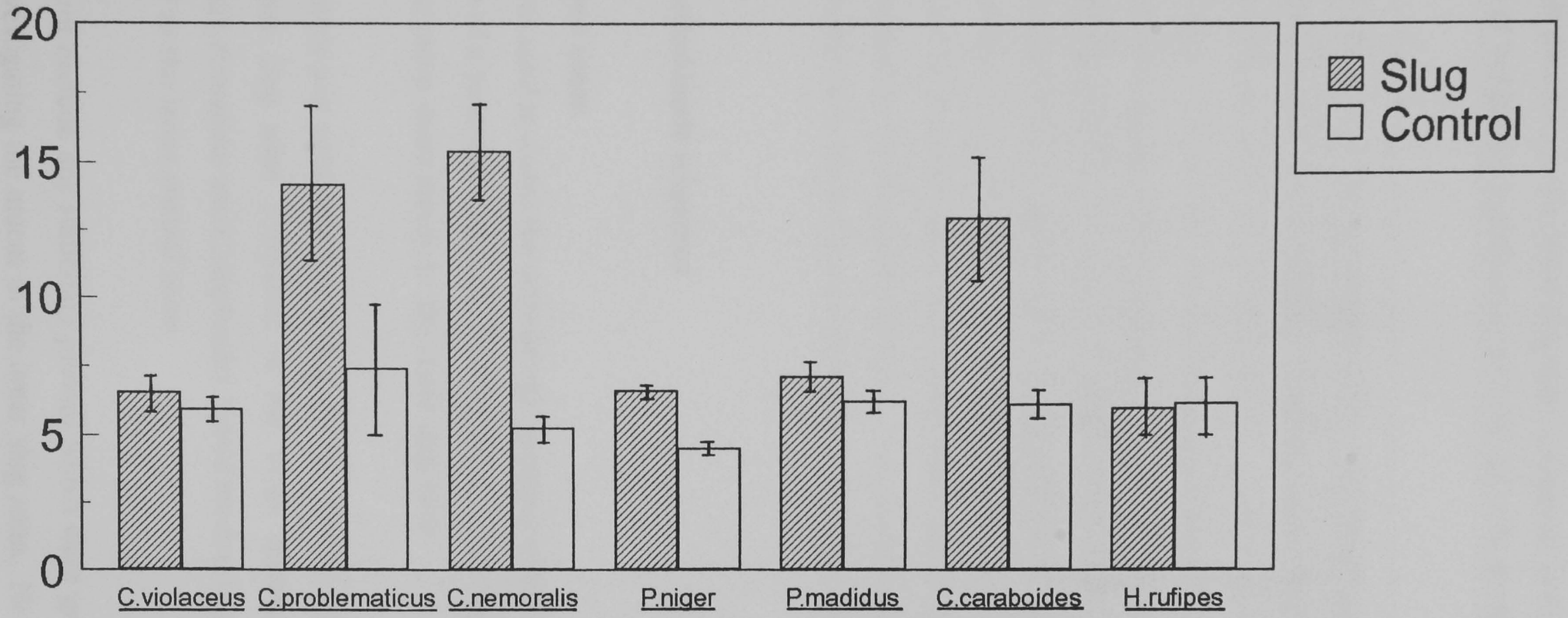


Fig 2.4.3 The mean distance covered
by seven beetle species per entry into the inner slug and control zones.
I = standard error.



distances in the inner slug zone compared to the inner control zone. *P.madidus* beetles also covered greater distances in the inner slug zone compared to the inner control zone, but this was of borderline significance ($t=1.95$, d.f.$=70$, $P<0.054$).

Time spent stationary

Comparisons were made between the time spent stationary in the inner slug zone with the inner control zone for each beetle species. The slug mucus had a very strong effect on the amount of time which five of the beetles spent stationary in the inner zones (Fig. 2.4.4). *P.niger*, *C.caraboides* and *C.problematicus* beetles stopped moving in the inner slug zone but never in the inner control zone. *H.rufipes* spent the greatest amount of time stationary in the inner slug zone but the result was not significant. *C.problematicus* ($t=2.71$, d.f.$=32$, $P<0.05$), *C.nemoralis* ($t=2.43$, d.f.$=43$, $P<0.05$), *C.caraboides* ($t=3.29$, d.f.$=17$, $P<0.01$), *P.niger* ($t=2.17$, d.f.$=32$, $P<0.05$) and *P.madidus* ($t=2.82$, d.f.$=70$, $P<0.01$) spent significantly more time stationary in the inner slug zone compared to the inner control zone. Only *C.violaceus* spent longer periods stationary in the inner control zone compared to the inner slug zone.

2.4.6.2 Klinokinetic and orthokinetic responses

Speed of movement in inner zones

The speed of movement was used to assess the orthokinetic response of the beetles after contacting the mucus of a potential slug prey. Beetles which were looking for slug prey were expected to move more slowly in the inner slug zone.

The total speed of movement was considered first (Fig. 2.4.5). Only *C.violaceus* moved faster in the inner slug zone compared to the inner control zone. *C.problematicus*, *C.nemoralis*, *P.madidus* and *C.caraboides* moved much slower in the inner slug zone compared to the inner control zone.

The net speed of movement exclude any stationary periods which were generally associated with beetles investigating the mucus in the inner slug zone. Net speed

Fig 2.4.4 The mean time spent stationary
by seven beetle species per entry into the inner slug and control zones.
I = standard error.

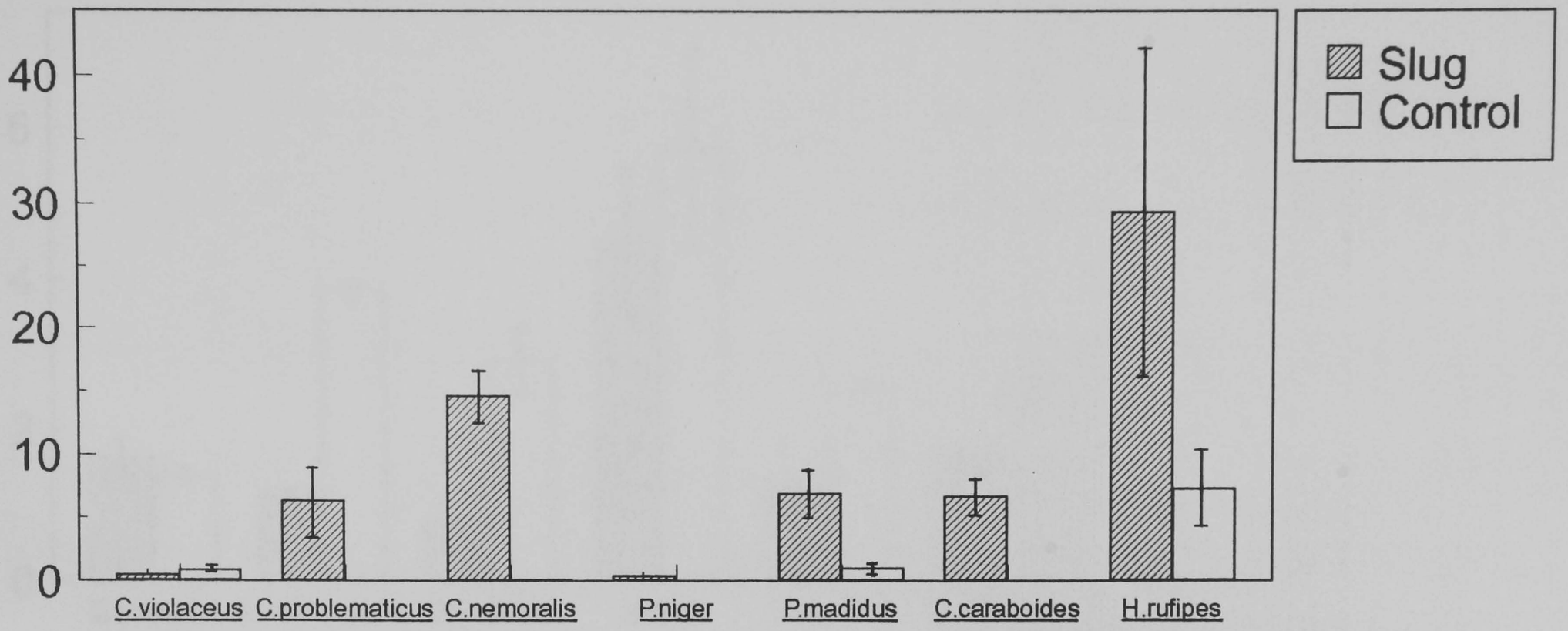
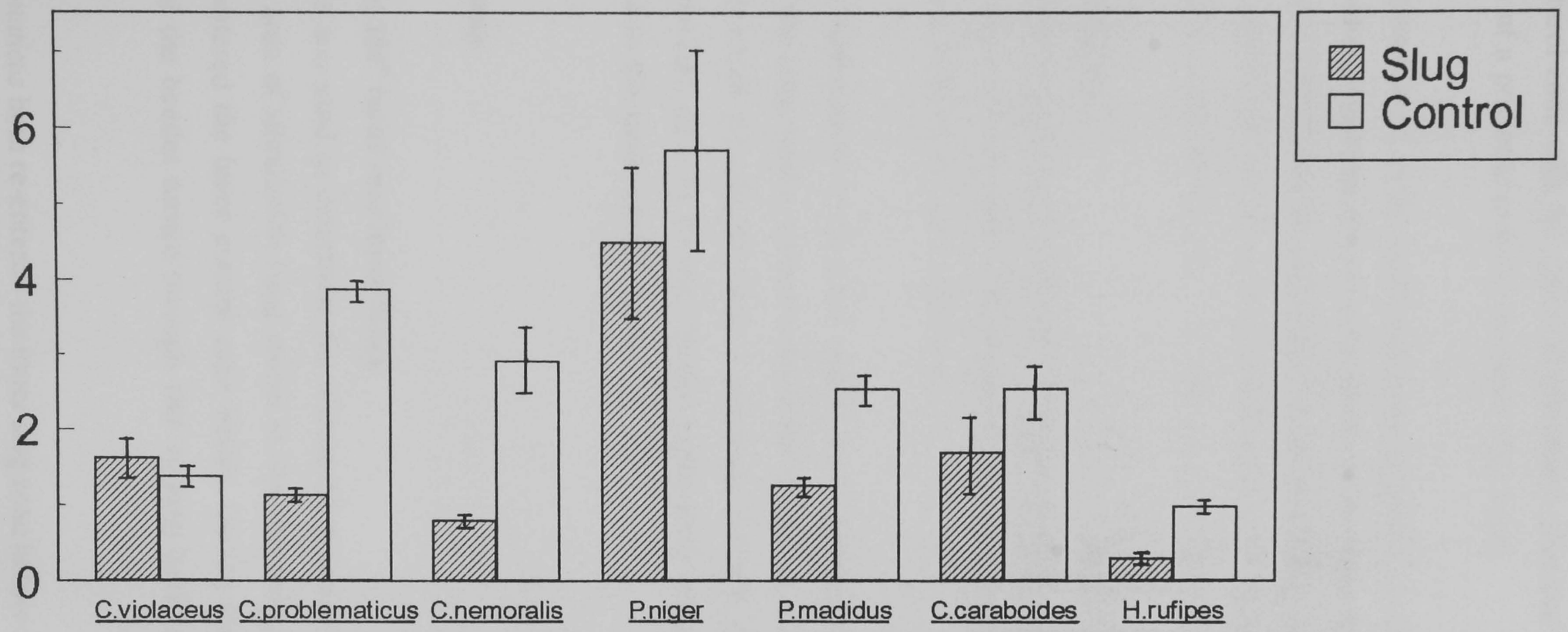


Fig 2.4.5 The mean total speed of movement
of seven beetle species per entry into the inner slug and control zones.
I = standard error.



of movement was used to directly compare the 'normal' speed of movement which occurred in the inner control zone with the speed of movement after the beetles had contacted the mucus of a potential prey (in the inner slug zone).

Again only *C.violaceus* moved faster in the inner slug zone compared to the inner control zone. All of the other six beetles moved more slowly in the inner slug zone (Fig. 2.4.6). *C.problematicus* moved considerably slower in the inner slug zone, but this was not significant. Significant reductions in speed of movement in the inner slug zone were found with *C.caraboides* beetles ($t=-3.30$, d.f.$=17$, $P<0.01$).

Degree of turning in inner zones

The degree of turn was used to assess the klinokinetic response of the beetles after contacting the mucus of a potential slug prey. Beetles which were looking for slug prey were expected to turn more in the inner slug zone.

C.violaceus and *H.rufipes* turned more in the inner control zone, compared to the inner slug zone but all of the other beetles turned more in the inner slug zone (Fig. 2.4.7). *C.problematicus* ($t=2.29$, d.f.$=32$, $P<0.05$), *C.nemoralis* ($t=3.09$, d.f.$=43$, $P<0.01$) and *P.madidus* ($t=2.31$, d.f.$=70$, $P<0.05$) turned significantly more in the inner slug zone compared to the inner control zone.

2.4.6.3 Klinotactic responses

Number of re-entries and 180° turns into inner zones

The number of re-entries was used to determine the klinotactic response of the beetles after leaving the area of stimulation (slug mucus in the inner slug zone). None of the beetles re-entered the inner control zone before leaving the outer control zone and none of the beetles turned through 180° to loop back into the inner control zone.

C.nemoralis and *C.problematicus* both re-entered the inner slug zone before leaving

Fig. 2.4.6 The mean net speed of movement (orthokinetic response) of seven beetle species per entry into the inner slug and control zones.
I = standard error.

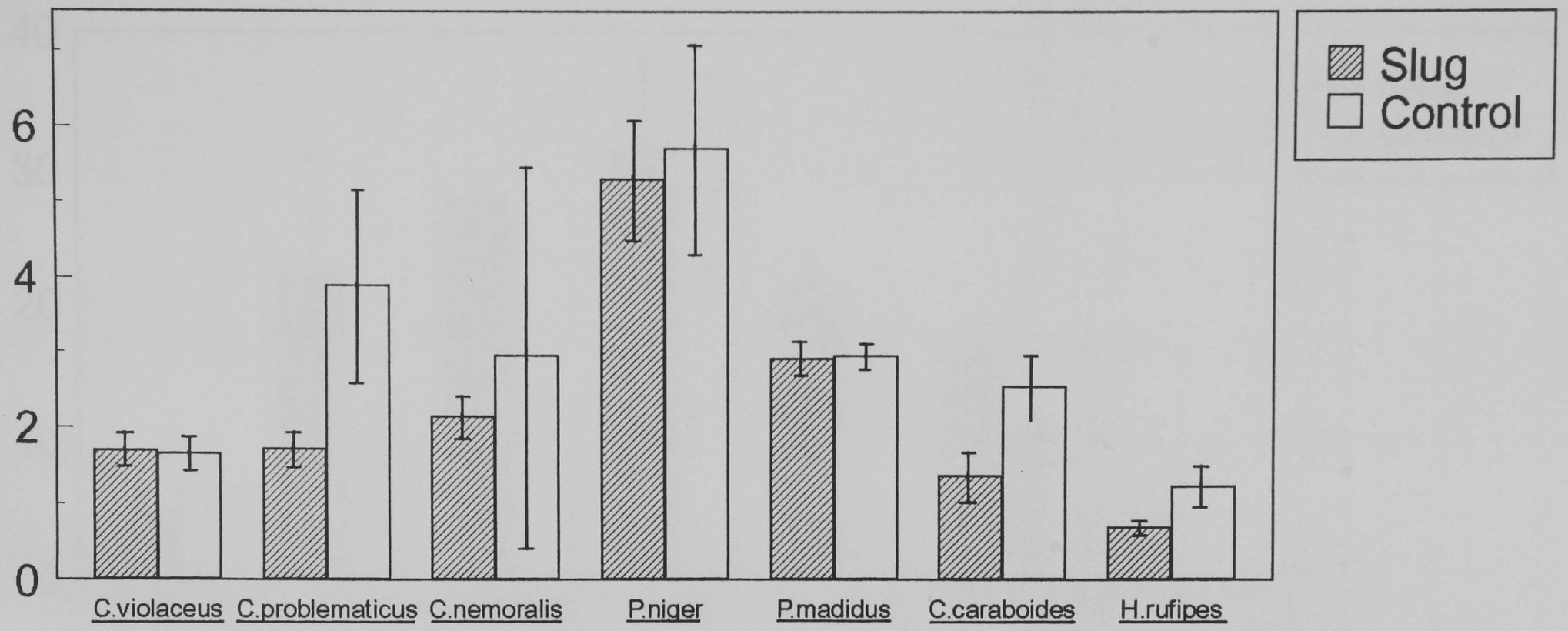
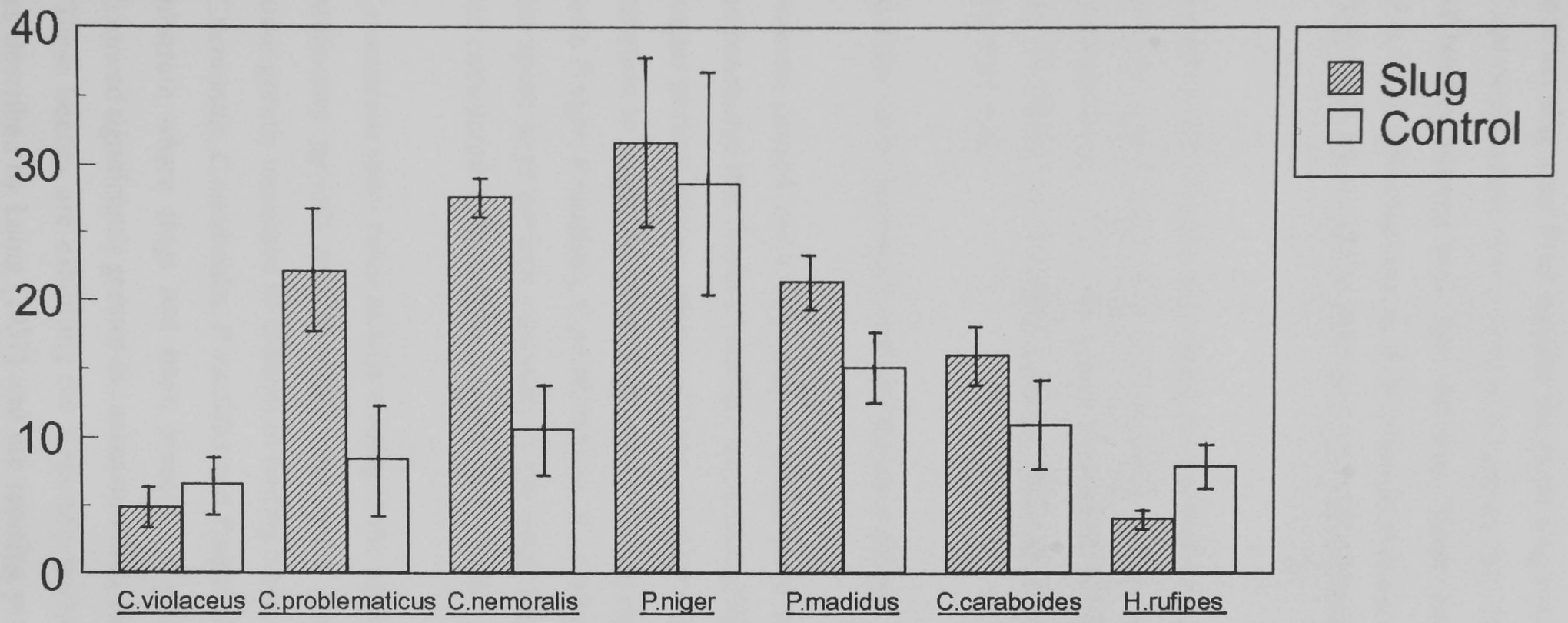


Fig 2.4.7 The mean degree of turn (klinokinetic response)
Degree of turn measured as degrees of turn per second of time per entry into the inner slug and control zones. I = standard error.



the outer slug zone and *C.nemoralis*, *P.madidus* and *C.caraboides* exhibited 180° turns to loop back into the inner slug zone from outside the outer slug zone. This was most pronounced in *C.nemoralis* where over a third of all entries into the inner slug zone were a result of beetles looping back into the area (Table 2.4.2). A typical *C.nemoralis* trace (Fig 2.4.8) demonstrates the differences in walking pattern between the two zones. Only these three species exhibited klinotactic movements.

2.4.7 Discussion

The movement categories used to assess beetle locomotion in this study are derived from examples in the literature which have been used to show orientation to a particular prey by a parasite/predator. The time spent stationary, moving and distance moved were used to assess the foraging activity of seven beetles on substrate previously occupied by slugs.

2.4.7.1 Time spent moving, time spent stationary and the distance moved in the inner zones

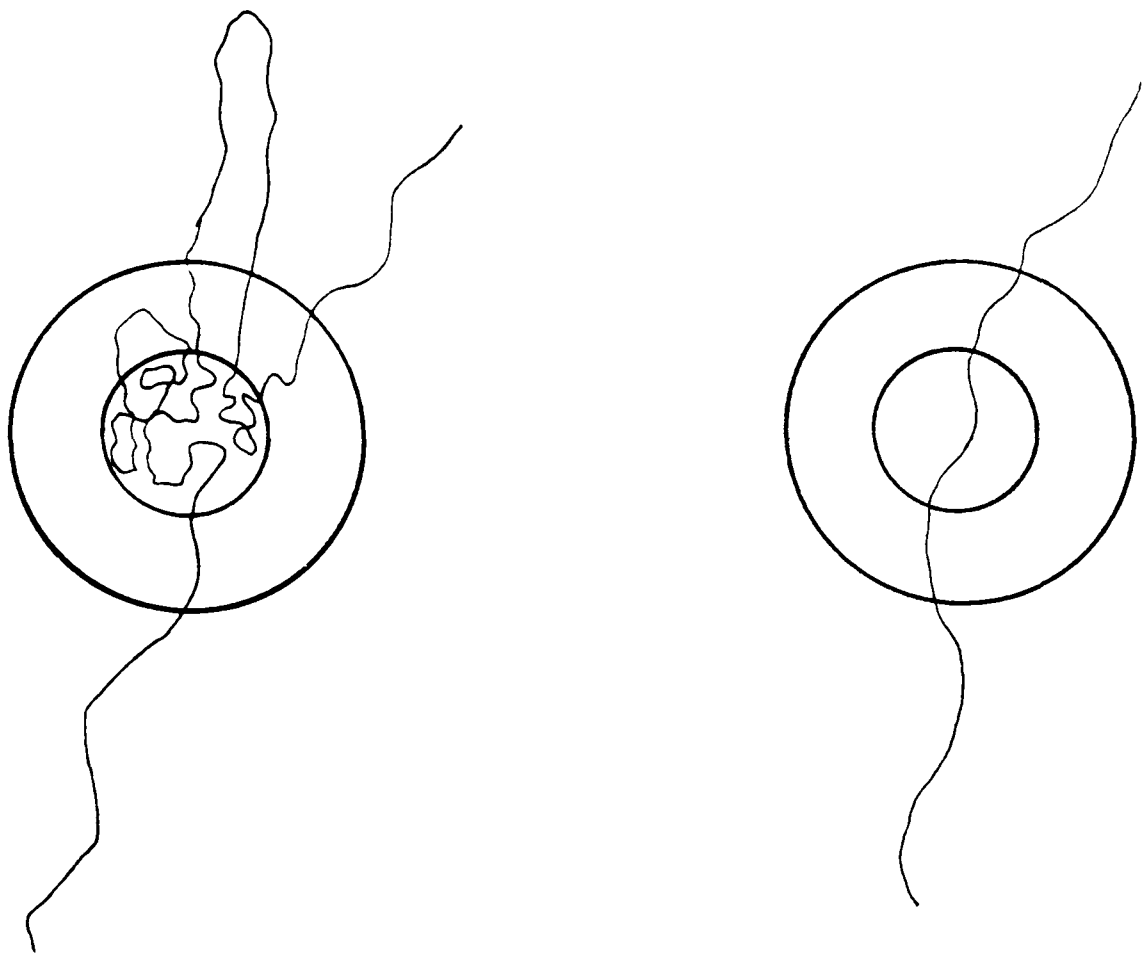
Laing (1937) found *T.evanescens* paused more frequently in areas where its moth host had been confined and concluded this demonstrated the attraction of the moth area. In this project the longer periods of time spent stationary in inner slug zone were used to identify orientation to slugs. Significant responses to slug mucus in this category were found with *P.niger*, *P.madidus*, *C.problematicus*, *C.nemoralis* and *C.caraboides*. *H.rufipes* also spent large periods stationary in the inner slug zone. These beetles are therefore considered to be arrested by the slug mucus.

Laing (1937) also found *T.evanescens* spent twice as long moving in the moth areas. He concluded that the stationary periods and extended movement phases of *T.evanescens* in the moth areas greatly increased its chances of finding hosts. In this project, *C.problematicus*, *C.nemoralis*, *C.caraboides*, *P.madidus* and *P.niger* all spent more time moving on substrate where slugs had been present. *C.nemoralis*, *C.caraboides* and *P.niger* all moved significantly greater distances on substrate where slugs had been present. These beetles are exhibiting the stationary periods and extended movement phases described by Laing (1937) and are reacting positively

Table 2.4.2 Data used to assess the klinotactic behaviour of each beetle species. The frequency of re-entries and 180° changes of direction (looping) which brought beetles back into the inner slug and inner control zones are presented as a proportion of total entries into the respective zones (see text for details). A value of zero indicates that beetles never re-entered or looped into that zone and a value of 1 indicates that beetles always re-entered or looped back into that zone.

Species	slug zone		control zone	
	re-entry	loop	re-entry	loop
C.violaceus	0	0	0	0
C.problematicus	0.12	0	0	0
C.nemoralis	0.33	0.33	0	0
P.niger	0	0	0	0
P.madidus	0	0.11	0	0
C.caraboides	0	0.18	0	0
H.rufipes	0	0	0	0

Fig. 2.4.8 Two typical tracks made by *Carabus nemoralis* beetles moving through the slug and control zones. The slug zones are represented by the two circles on the left.



to slug mucus.

This change in movement may be a general behavioural response which carabids exhibit to a number of chemical clues from various potential prey. *P.melanarius* spends significantly longer periods of time on sand on which leatherjackets have been raised, compared to clean sand (Chapman, 1994) and *N.biguttatus* changes its behaviour when moving across substratum previously occupied by Collembola (Ernsting *et al.*, 1985).

In the field, concentrated slug mucus patches are associated with slugs which circle each other before mating (personal observation). The low frequency of these patches in natural situations means there is little adaptive advantage in orientating to such patches. However, a number of carabid species do orientate to mucus trails laid down by a single slug (Wheater, 1989). Therefore the orientation to slug mucus shown by beetles in this study is considered to be a positive reaction and increases the chance of a beetle locating a slug prey.

2.4.7.2 Klinokinetic and orthokinetic responses

Responses before prey are encountered

Klinokinetic and orthokinetic changes in movement are more specialised responses associated with host searching behaviour and responses to aggregated prey. Hassell and May (1974) described a klinokinetic response of tight turning behaviour by invertebrate predators/parasites after encountering a prey which kept them in the vicinity of the prey. This type of behaviour increases the chance of the predator encountering another prey in a prey patch.

Rhyssa persuasoria (L.) exhibits a klinokinetic response in its exploratory movement over wood containing its host (Spradbery, 1970), suggesting the wasp is orientating to a diffuse stimulus associated with the host. Similarly, the locomotory activity of *N.biguttatus* is affected by its (Collembola) prey previously occupying the substratum (Ernsting *et al.*, 1985), implying non-visual clues are used by this carabid.

In this study, *C.caraboides* beetles significantly reduced their speed of movement in the inner slug zone and *C.nemoralis*, *C.problematicus* and *P.madidus* significantly increased their turning rate in the inner slug zone. These carabids are orientating to slug mucus and adopting a foraging strategy which increases their likelihood of contacting a slug. This work agrees with Wheater (1989) who found *C.caraboides* and *C.problematicus* orientated to slug mucus. Digweed (1993b) also found *C.nemoralis* females orientated to slug mucus, although Pakarinen (1994) found *C.caraboides* didn't follow mucus trails.

Responses after feeding / parasitising prey

Mitchell (1963a) found *B.lampros* and *T.quadristriatus* moved slowly in small closed turns for several seconds after feeding and Halsall (1990), found *A.dorsale*, *H.rufipes*, *N.brevicollis* and *P.madidus* reduced their speed of movement and increased their angle of turn after feeding on an aphid. This response was not investigated in this study as prey were not offered to the beetles. In section 2.3, *C.nemoralis* moved away from a slug on which it had been feeding and within a few seconds turned 180° and walked back to the dead slug to recommence feeding.

2.4.7.3 Klinotactic (looping / re-entry) responses

A klinotactic response is a more specialised form of klinokinetic response. A klinotactic response occurs after the predator/parasite has left the area of stimulation and involves a change in direction which causes it to move back to the area of stimulation (e.g. prey patch/slug mucus). It is generally associated with parasitoids which have an intimate relationship with their hosts (e.g. Smith *et al.*, 1994). It is particularly useful when prey are aggregated and causes the predator/parasite to orientate back to the prey patch. Two types of klinotactic responses were observed in this experiment, these were defined as 're-entry' movements and 'looping' movements.

Re-entry into inner zones

The parasitic wasp *Nemeritis canescens* (Gravenhorst) elicits a klinotactic response when leaving patches of substrate where its host had been confined (Waage, 1977).

When the wasp crosses the host patch edge it turns sharply to bring itself back into the patch. Waage (1978) calculated the wasps klinotactic response and found a turning angle of 157° relative to the orientation at the moment of stimulation. This behaviour greatly prolonged the time the wasp spent in the patch.

In this study, *C.problematicus* and *C.nemoralis* were the only beetle species which re-entered the inner slug zone before leaving the outer slug zone. They are therefore orientating back to the area of stimulation i.e. the slug mucus. Since *Carabus* and *Cychrus* species are specialised mollusc feeders (e.g. Evans and Forsythe, 1985; Gruntal and Sergeyeva, 1989) a strong orientation to slug mucus has an adaptive advantage which enables these predators to hunt for their preferred food.

Looping movement

The Hymenoptera *G.natalensis* turns through 180° to walk back to an area of filter paper impregnated with host frass (Smith *et al.*, 1994). The change of movement occurs within one centimetre of the wasp leaving the area of stimulation. In this study two mollusc specialists, *C.caraboides* and *C.nemoralis* and the generalist *P.madidus* exhibited this movement. Beetles left the inner slug zone and moved straight out of the outer slug zone and continued walking for several centimetres before turning through 180° and walking back into the inner slug zone. The Pterostichini *P.madidus* is considered to be a generalist predator (e.g. Evans, 1967) and Wheater (1989) found *P.madidus* did not orientate to slug mucus. In this project *P.madidus* exhibited the same looping (klinotactic) movement as the two mollusc specialists *C.caraboides* and *C.nemoralis*.

Some polyphagous predators can learn to respond to chemical clues from a number of prey species. The polyphagous parasitoid *Bracon mellitor* Say orientates to a number of hosts. It learns to respond to chemical cues from particular species and becomes more selective and efficient in locating that species (Vinson *et al.*, 1977). This may be particularly important when potential hosts are abundant for short periods. In carabids, klinotactic locomotion is probably a general response to a

number of prey stimuli. *P.madidus*, *A.dorsale*, *H.rufipes* and *N.brevicollis* all exhibit klinotactic responses to patches of aphids (Halsall, 1990). The klinotactic orientation to slug mucus by *P.madidus* in this project may be a general behavioural response, but it is a useful response by a generalist predator to slugs.

2.4.7.4 Mechanism of orientation

The orientation of carabids to prey has received little attention. Many carabid species are very active in the field and may locate prey by random encounters. Frank (1967) found *P.madidus* moved at random until its mouthparts touched Lepidoptera prey and *B.lampros* and *T.quadristriatus* find immobile food by chance physical encounters (Mitchell, 1963a). However, some carabids are thought to use chemical cues to detect prey. *L.pilicornis* aggregates to its Collembola prey using Collembola pheromones (Bauer, 1982). Chemical cues left by Collembola and leatherjackets on substrate affect the locomotory activity and foraging behaviour of *N.biguttatus* and *P.melanarius* (Ernsting *et al.*, 1985; Chapman, 1994).

Transpecific chemical messengers which are of benefit to the receiver are termed kairomones and are significant initiators of behaviour in a number of beetle species (Borden, 1977). The mechanism causing the beetles orientation to the slug mucus was not investigated in this project. However, results from this and other studies indicate that chemical cues from a number of prey species cause changes in movement in carabid beetles.

The orientation to prey via prey kairomones is generally associated with parasitoids. A substance from the body and salivary secretions of the tobacco budworm *Heliothus virescens* (F.) elicit a host-seeking response in its parasitoid *Cardiochiles nigriceps* Viereck (Vinson and Lewis, 1965). The aphid parasitoid *Praon volucre* (Haliday) uses the sex pheromone of *Sitobion fragariae* (Walker) as a host location clue (Lilley *et al.*, 1994). A symbiotic fungus associated with the host larvae is involved with attracting the parasitoid *R.persuasoria* (Spradbery, 1970), *Ibalia leucospoides* (Hochenw) and *Megarhyssa nortoni nortoni* (Cresson)(Madden, 1968). A volatile in the frass of *E.saccharina* larvae may initiate a walking and turning

response in the parasitoid *G.natalensis* (Smith *et al.*, 1994).

There are probably such volatiles in slug mucus. Newell (1966) recorded a *D.reticulatum* slug crossing the trail of another slug which it followed for nearly two metres, until it caught up and mated with the slug making the trail. Newell thought this indicated an attractant was secreted in the mucus from the pedal mucous gland. The high nitrogen content of the frass of the wood-wasp larvae *Sirex noctilio* Fabricius may be used in host location (Madden, 1968). In a review, Vinson (1976) concluded that contact chemicals were particularly important for host location by parasitoids. These included hydrocarbons, sugars, proteins and magnesium chloride. Slug mucus also contains complex molecules. *H.aspersa* has eight glands on its foot which secrete four kinds of mucus, a protein, calcium carbonate granules, a pigmented secretion and fat-globules (Campion, 1961).

Wheater (1989) concluded that some species responded to prey using only contact senses and *C.caraboides*, *C.problematicus* and *C.violaceus* responded to slug mucus via tactile or gustatory chemoreceptors on the ends of their palps. Other species including *P.madidus* and *P.niger* used olfaction for prey detection and did not respond to slug mucus. In section 2.3, there was some evidence that *C.nemoralis* orientated to slugs via non-tactile clues. One beetle emerged from its burrow and orientated straight to a slug which it attacked and killed. In this project *P.madidus* responded strongly to slug mucus after contacting it, indicating a tactile or gustatory stimulation.

The difference between Wheater's study and this study may be the strength of the mucus trail laid down. Wheater used a mucus trail laid down by a single *A.subfuscus* slug. In this project, twenty slugs were used to lay down a mucus layer over several hours which may have increased the concentration of any chemicals which arrested beetle movement. The strength of the attractant will diminish as the trail ages. Vinson and Lewis (1965) found the trail laid down by a *H.virescens* larvae was attractive to its host *C.nigriceps* for about three hours. Some species, such as *N.biguttatus*, have the ability to discriminate between concentrations of prey

product stimulus which enable it to estimate prey density and so adopt a foraging strategy to maximise its efficiency (Ernsting *et al.*, 1985).

Polyphagous carabids probably perceive a range of kairomone messages from a number of prey which cause the beetles to forage for food in the area from which the kairomones are emitted (e.g. Bauer, 1982; Ernsting *et al.*, 1985; Chapman, 1994 and this study).

2.4.8 Conclusions

Locomotory categories identified in the literature were used to investigate the reaction of seven beetle species to slug mucus. Five of the seven beetle species used in this study had their locomotory activity significantly altered by the presence of slug mucus on the soil surface. The slug mucus acted as an arrestant to beetle movement. Beetles spent more time stationary, foraged for longer and moved for greater distances when slug mucus was present on the soil.

C.caraboides showed significant orthokinetic response to slugs mucus and *C.nemoralis, C.problematicus and P.madidus* showed significant klinokinetic response to slug mucus. These three specialist mollusc species and the Pterostichini generalist *P.madidus* exhibited klinotactic (looping) response to slug mucus. This type of behaviour is considered to increase the likelihood of locating a slug prey and promotes the role of generalist beetles as slug predators.

2.5 General conclusions

Carabids can be regarded as specialist or generalist predators. Results from these studies have shown that many generalist carabid species, including large and medium sized species, can overcome the slug's mucus defence system and exploit slugs as a food source. Many species found in arable crops have compatible daily activity cycles to slugs and predate slugs at low temperatures.

Encounters between beetles and slugs may not always result in the slug being consumed, but can sometimes result in slug mortality. ELISA data may not fully

describe the number of slugs, attacked, injured or killed by many carabid beetles.

The size of the slug is probably limiting to many generalist predators, but is probably not limiting to slug specialists species. As individual slugs grow, they will eventually reach a size above which they cannot be predated by many generalist predators. Two Pterostichini *P.niger* and *A.parallelepipedus*, spent longer periods of time foraging in the presence of large slugs before predating a slug than did the three slug specialists. They also had a low capture efficiency compared to three mollusc specialists. In the field, this behaviour would tend to bring them into contact with other types of prey which may be eaten in preference to slugs. Any impact a carabid exerts on populations of slugs will be affected by the abundance of alternative prey.

Molluscs are preferred food for several species of *Carabus* and *Cychrus* beetles. Both genera increase the likelihood of locating slugs by adopting klinokinetic and orthokinetic walking patterns after contacting slug mucus. The generalist predators also changed their locomotory activity after contacting slug mucus and one generalist, *P.madidus* exhibited klinotactic orientation to slug mucus. This is possibly a general response to a wide range of prey stimuli. However, such changes of locomotory behaviour are considered useful in the pursuit of slug prey.

Chapter three

Development of a Serological Technique

3.1 Introduction

Early investigations into the food of carabid beetles generally involved the dissection of predators guts and identification of ingested prey remains (Davies, 1953; Luff, 1974; Vickerman and Sunderland, 1975; Sunderland, 1975; Sunderland and Vickerman, 1980; Holopainen and Helenius, 1992). Prey species can only be determined by gut dissections when a hard part, usually the arthropod exoskeleton, can be identified (Davies, 1953). Although this technique has been used to identify molluscs in the guts of predators (e.g. Luff, 1974), identification relies on the ingestion of the molluscan radulae which will not always be eaten by the predator (Davies, 1953).

The difficulties involved in identifying invertebrate material in the guts of predators has prompted a number of authors to develop serological techniques. The use of serology in the analysis of predator gut contents has been reviewed by a number of authors (Pickavance, 1970; Boreham and Ohiagu, 1978; Calver, 1984; Sunderland, 1987 and 1988) and has been used in assessing a number of predator-prey interactions. Serology is particularly useful for predators which suck the juices of prey and where there are no particulate remains to be identified.

Serology has been widely used in virology and medicine and was first used in entomology to identify the blood meals of mosquitos (e.g. Weitz, 1956). Each arthropod prey is composed of many chemical substances (antigens), some of which are unique to that species. Mammals injected with antigens produce specific antibodies which bind to them. These antibodies can be extracted from the blood and used to identify antigens similar in structure to those that stimulated their production (Calver, 1984). Immunological tests can then be devised which cause interactions between these antibodies and antigens in the gut contents of predators (Boreham and Ohiagu, 1978).

Numerous serological methods have been described. Precipitin tests have frequently been used by ecologists because they are technically simple (Dempster *et al.*, 1959; Frank, 1967; Tod, 1973; Dennison and Hodkinson, 1983). However they are the least sensitive. The assay is generally performed in tubes or gels on a glass slide or petri dish. The test solution is placed in a central well, the antisera containing the antibodies placed in the periphery wells and the two solutions diffuse towards each other. If antibodies in the antisera are specific to antigens in the test solution a precipitate is formed which can be seen as a line in the gel. Precipitin tests have been used to identify the prey of woodland carabids (Dennison and Hodkinson, 1983) and to identify molluscan remains in the gut of carabid and staphylinid predators (Tod, 1973).

Immunoassays are techniques used for the detection and quantification of antigens or antibodies. Immunoassays were first introduced into agriculture by Clarke and Adams (1977) who used a labelled antibody which detected antigens. Labels can be radioisotopes, florescent or bioluminescent materials or enzymes which catalyse colour reactions e.g. Enzyme-linked immunosorbent assays (ELISA). The main advantages of immunoassays are that they are quantitative rather than qualitative and have greater specificity and sensitivity. ELISA's have been developed which can detect less than one percent of a homogenised aphid (Crook and Sunderland, 1984).

Immunoelectrophoresis has been used to assess predation of lepidopteran by Coleoptera species (Allen and Hagley, 1982). Precipitin tests have been developed which have identified carabid predators of cabbage root fly (Coaker and Williams, 1963). ELISA's have been promoted as a technique to quantify prey proteins in the gut of predators (Sunderland, 1987; Greenstone, 1989) and have been developed to identify invertebrate predators of *Nezara viridula* (L.) and aphids in agroecosystems (Ragsdale *et al.*, 1981; Sunderland *et al.*, 1987; Sopp, 1987).

Immunological assays rely on the specificity of the antisera to the antigen and problems can arise when antisera cross react to other proteins. Slug

mucopolysaccharrides are similar in structure to earthworm mucopolysaccharrides (Symondson and Liddell, 1993a). Mucopolysaccharrides produce immune responses (J.Wheeler, personal communication) which may be non-specific.

Problems can also arise when quantifying data: Serological techniques measure the biomass of ingested prey rather than the numbers of prey eaten, killed or injured at each feed. Errors in the quantification of predation may occur when scavenging of dead prey results in positive reactions (Boreham and Ohiagu, 1978). However, meaningful interpretations from ELISA data can be made when used in conjunction with laboratory observations (Sopp *et al.*, 1992).

3.2 Raising slug antibodies

Antibodies were raised against slug proteins in two adult rabbits and two adult rats held at the University of Newcastle's Comparative Biology Centre (CBC) under licence from the Home Office.

3.2.1 Protein extraction

A number of *D.reticulatum*, *M.budapestensis*, *A.hortensis* and *Deroceras caruanae* (Pollonera) were collected from Close House and placed in plastic containers at 12°C and starved for seven days. This allowed gut evacuation of any ingested proteins which may have produced non-specific reactions.

One hundred ml of quarter strength Ringers solution was made by dissolving one tablet of Ringers in 500 ml of distilled water. This was autoclaved along with approximately 1600 ml of distilled water. Equipment used in the protein extraction was wrapped in tin foil and heat sterilised. An electric homogeniser was surface sterilised with sodium hypochlorite and then rinsed with distilled water. Homogenisation of the slugs took place in a negative pressure room on work benches which had been sprayed with a 70 percent alcohol solution.

Twenty grams of slugs were weighed out (Table 3.1) and placed in a sterile container. Individual slugs were placed on a petri dish and agitated until they

Table 3.1 Species composition of the protein extraction process described in section 3.2.1.

Species	Weight (g)
Deroceras reticulatum	5.35
Deroceras caruanae	1.60
Milax budapestensis	6.45
Arion hortensis	6.60

exuded their surface mucus which was washed off with sterile water. This cleaned the slug of any surface bacteria which could produce non-specific reactions and prove toxic to laboratory animals (Boreham and Ohiagu, 1978).

Slugs were homogenised in groups of four with 25 ml of Ringers solution. The homogeniser was activated for several short bursts until the slugs had been reduced to a uniform homogenate and no further colour change was perceived. The homogenate was poured into a sterile beaker standing in an ice bath. The remaining Ringers solution was added to the homogenate giving a w/v ratio of 1:5. An excess quantity of Ringers solution increases the concentration gradient for extraction of saline-soluble proteins (Boreham and Ohiagu, 1978). The homogenate was covered in tin foil and left to extract for 24 hours at 4°C.

After 24 hours, the extract from the homogenate was poured into two surface sterilised centrifuge tubes and centrifuged for 20 minutes at 15,000 rpm. The supernatant was then decanted into a sterile glass beaker. A length of 30/32 visking dialysis tubing was boiled in water for 10 minutes with 0.1 g of EDTA to absorb metal ions. The tube was filled with supernatant and sealed. The tube was placed in one litre of 0.9 percent NaCl saline and left to dialyse for 96 hours at 4°C. The saline was changed twice during this period.

3.2.2 Contamination of the extract

A small quantity of the extract was plated out aseptically onto two nutrient agar plates, and incubated at 37°C for 72 hours. The plates were examined for the presence of bacterial colonies which could have produced none specific reactions during immunisation, and proved toxic to the animals (Boreham and Ohiagu, 1978). The remainder of the extract was transferred to a conical flask and allowed to freeze-dry overnight.

3.2.3 Storage of extract and protein estimation

A protein assay was carried out on the extract, using a modification of the Lowry method (Hartree, 1972). The extract was found to contain 20 percent protein by

weight. A minimum of two percent protein concentration has been recommended for antiserum production (Pickavance, 1970), therefore the extract was used to raise antisera. The remainder of the freeze-dried extract was aseptically transferred to 2 ml vials and stored at -20°C.

3.2.4 Injection schedule

The procedure follows that recommended by Calver (1984). The two rabbits and two rats were immunised with slug antigens. Test bleeds were taken to monitor antibody titre. At the end of the procedure, animals were exsanguinated under terminal general anaesthetic by staff at the CBC.

Ten mg of the extract was dissolved in 1 ml of saline solution. 0.5 ml of the resulting solution and 0.5 ml of Freunds incomplete adjuvant was withdrawn into a large bore syringe. The two solutions were gently agitated. Then 0.5 ml of PBS and 0.5 ml of Tween 80 were mixed together and drawn up into the syringe and mixed with the Freunds and antigen solution to produce a creamy white consistency. The resulting 2ml of injecta was then ready for administration to a single rabbit.

This procedure was repeated, with the substitution of Freunds incomplete adjuvant with Freunds complete adjuvant, to produce a second rabbit injecta which was used on all subsequent rabbit injections. The whole procedure was repeated to produce inoculate for the two adult rats, using 1/10th of the quantities.

Pre-bleeds were taken from both rabbit and rats in order to assess background antibody levels. The first injection was given using Freunds incomplete adjuvant (injection i1). Subsequent injections were given with Freunds complete adjuvant (injections i2-i5) (Table 3.2). Injections were given subcutaneously by staff at the CBC. Trial bleeds were made on day 28 and then at monthly intervals. Blood samples were placed at 4°C for 24 hours to allow clotting. The exuded serum was decanted and frozen in 2 ml vials at -20°C.

Table 3.2 Injection schedule of rabbits and rats. The first injection was made on day zero (15th May, 1991). Pb = pre-bleed, i = injection, tb = test bleed, TB = terminal bleed.

	Day number								
	0	14	28	42	56	70	84	98	112
rabbit	Pb i1	i2	tb1	i3	tb2	i4	tb3	i5	TB
rat			Pb i1	i2	tb1	i3	tb2	i4	TB

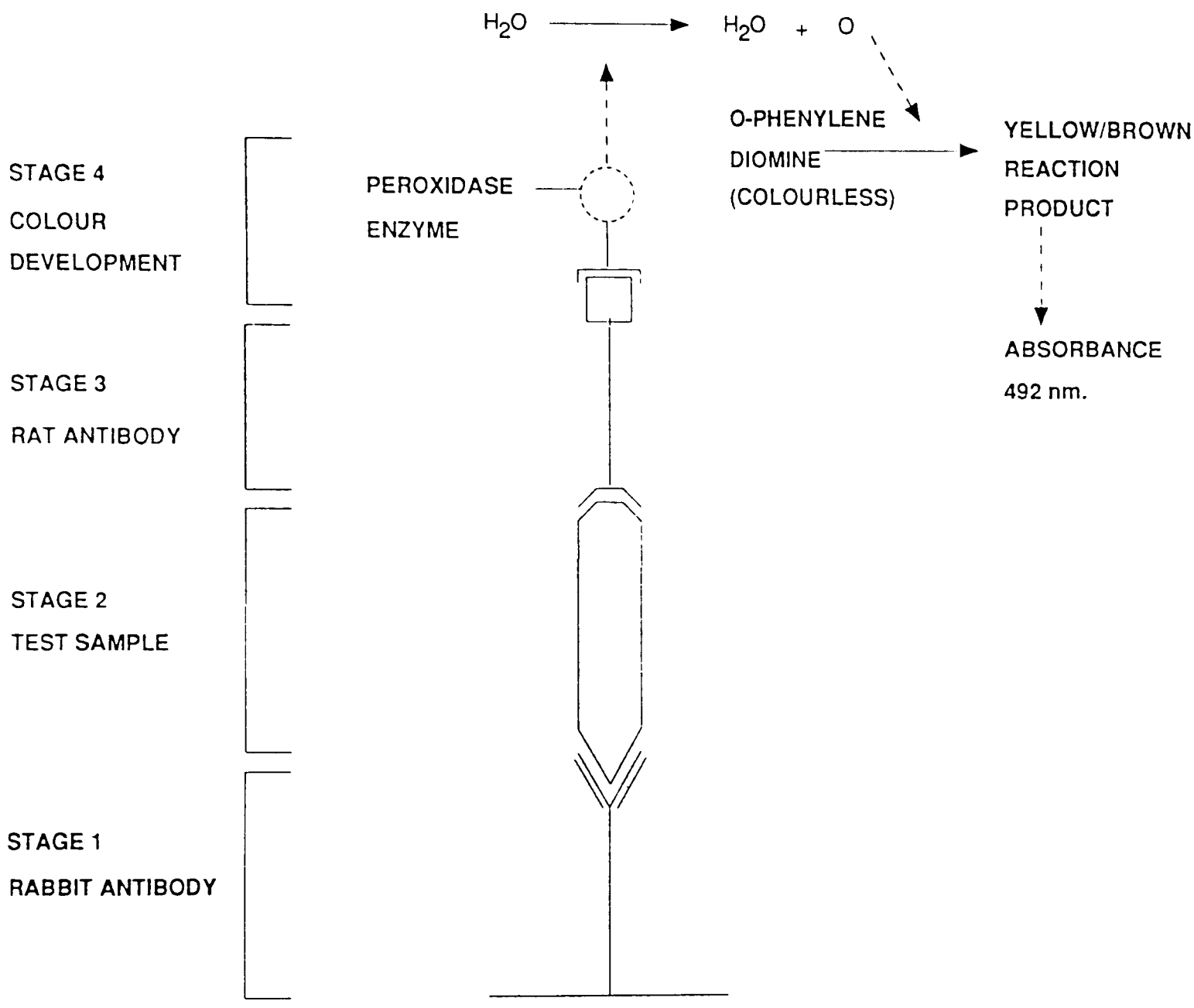
The antibodies raised against the slug antigens were monitored throughout the injection schedule using a modification of the ELISA described in section 3.3. The assay consists of a number of distinct stages. In the first stage slug antigens were coated onto the plate. In the second stage, test bleeds were used instead of test specimens. In the third stage, a conjugate was added and the plates were developed. The resulting absorbency values were used to monitor the level of slug antibodies in the rabbits and rats. Antisera are generally recovered from test animals when antibody levels begin to fall (J. Wheeler, personal communication). This criterion was used to recover the antisera by terminal bleed from the test animals. The antiserum was stored in 2 ml vials at -20°C.

3.3 Stages of the ELISA

The assay took place on disposable flexible flat-bottomed polyvinyl chloride microtitration plates (Dynatech laboratories, inc.). A microtitration plate is an arrangement of 96 miniature test tubes in a single plastic plate and is designed for use in micro-serial dilution procedures for in vitro investigational use.

The assay involved five separate stages (Fig. 3.1). Plates were inverted between each stage, shaken to dispel their contents and then washed three times in separate baths of PBS-Tween, inverting after each wash. The rabbit and rat antisera concentrations were determined using the methodology described in sections 3.4 and 3.5. Plates were first coated with rabbit antisera. In the second stage the test solutions were added. In the third stage the rat antisera were added. In the fourth stage a rabbit anti-rat conjugate labelled with an enzyme was added. The conjugate was a standard reagent sensitised to rat antigens which recognised and bound to rat antigens. In the final stage, a substrate was added which is denatured by the enzyme-conjugate. The enzyme acts by hydrolysing the substrate from a colourless to a coloured form. The degree of colour change is directly proportional to the amount of enzyme in the conjugate which is proportional to the levels of rat antibodies bound to the test samples, and is therefore a measure of the (slug) antigens in the test sample.

Fig. 3.1 The reaction stages of the ELISA. Rabbit antibodies were fixed to the surface of the wells on the microELISA plate in the first stage (see section 3.3.1).



3.3.1 Coating the plates - stage one

Coating buffer was prepared by adding 4.5ml of sodium carbonate to 8ml of sodium hydrogen carbonate and diluting to 50ml with distilled water. This produced a 0.05M carbonate buffer with a pH of 9.8. Twenty ml of rabbit antisera were added to 20ml of the buffer to give a dilution of 1:1000 and used to coat the plates. One hundred ml volumes were dispensed into each well and incubated for 3 hours at 37°C and then overnight at 4°C.

3.3.2 Preparation of test specimens - stage two

Test specimens were usually frozen beetles which were prepared by thawing and dissecting their foreguts (Calver, 1984; Giller, 1984). Guts were dissected and homogenised the day before assessment by ELISA and left to extract overnight at 4°C (Boreham and Ohiagu, 1978; Fichter and Stephen, 1981; Crook and Sunderland, 1984). The fluid portion was used in the ELISA (Fichter and Stephen, 1981 and 1984).

Each gut was placed into a predetermined well on a microELISA plate with 125ml of PBS-Tween 20 and homogenised (Crook and Sunderland, 1984; Lovei *et al.*, 1985). A multiple homogeniser was used to homogenise all 72 wells simultaneously (Ffrench-Constant and Devonshire, 1987). The homogeniser was then rinsed and stood in alcohol overnight. Particular attention was paid to quality control. Dissection instruments were washed, heat sterilised then washed again between dissections to avoid cross contamination.

3.3.3 Calibration curve and addition of test specimen - stage three

Slug standard was prepared from freeze dried slug protein by dissolving 0.01g of the protein in 2 ml of distilled water to give a stock concentration of 1000 ml/ml. The stock solution was then dispensed into 150 ml volumes and frozen at -20°C until an assessment was made. The top slug standard was made by diluting 100 ml into 1900ml of PBS-Tween 20. A dilution series was made to give slug standards at concentrations of 50, 25, 10, 1 ug/ml, 100, 10 and one ng/ml. The dilution series was made in small plastic test tubes with snap on caps, a stirrer was used to mix the

protein at each stage in the dilution.

One hundred ml volumes of the slug standard dilutions (s1-s7) and test solutions were dispensed into the appropriate wells according to the template (Fig. 3.2) and 100 ml of PBS-Tween added to the outside wells. The plates were then incubated for one hour at 37°C.

3.3.4 Rat antisera - stage four

Rat antisera was diluted to 1:1000 with PBS-Tween. One hundred ml volumes were dispensed into each well and the plates incubated for one hour at 37°C.

3.3.5 Conjugate - stage five

Rabbit antirat horseradish peroxidase (HRPO) conjugate (Dako) was diluted to 1:2000 in PBS-Tween. One hundred ml were dispensed into each well and the plates incubated for one hour at 37°C.

3.3.6 Substrate - stage six

The substrate was made up immediately before use as it is extremely light sensitive and denatures rapidly.

Five ml of chromogen was poured into 45 ml of distilled water. A one ml vial of ortho-phenylene diomine (OPD) was removed from deep freeze and allowed to thaw in the dark at room temperature. Ten ml of 30 percent hydrogen peroxide was added to the buffer to catalyse the reaction and finally the thawed OPD added. The substrate was mixed and 100 ml of the solution was dispensed into each well. The plates were incubated for 10 minutes in the dark at room temperature.

3.3.7 Colour development and the calibration curve

After 10 minutes the reaction was stopped by dispensing 50 ml of H_2SO_4 into each well. The plates were read on a Dynatech MR5000 plate reader with a 490 nm filter blanked on air. Results were recorded as absorbency units. The slug dilution series was used to generate a calibration curve. The data were best fitted by a

Fig. 3.2 MicroELISA plate design for assessing unknown specimens from the field sites. Wells B2, C2 and D2 are blank. Wells E2-G5 contain slug standard solutions at the following concentrations: s1=50, s2=25, s3=10, s4=1ug/ml, s5=100, s6=10, s7=1ng/ml. Wells B7-G11 contain homogenised gut material from unknown specimens. t1=test specimen one, etc.

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B		B	s2	s4	s6	c	t1	t7	t13	t19	t25	
C		B	s2	s4	s6	c	t2	t8	t14	t20	t26	
D		B	s2	s4	s6	c	t3	t9	t15	t21	t27	
E		s1	s3	s5	s7	c	t4	t10	t16	t22	t28	
F		s1	s3	s5	s7	c	t5	t11	t17	t23	t29	
G		s1	s3	s5	s7	c	t6	t12	t18	t24	t30	
H												

semi-log sigmoid curve. Absorbency values from test samples were converted into mg/ml of slug antigen using the calibration curve.

3.4 Assessment of rabbit and rat antisera titres

Introduction

The immunisation schedule yielded four polyclonal antisera which would react to many slug antigens. Mattinson (1965) found 13 antigens in homogenate of adult and embryonic *Deroceras* tissue using immunoelectrophoresis. Antigens in some tissues were specific to the developmental stage of the slug and some antigens were more general.

An antiserum with a high specificity and high titre are the ideal combination. A high specificity is desirable as it eliminates cross reactions to non-target organisms. High specificities can be achieved at the expense of a strong reaction to the antigens.

Rabbits injected with the same antigen give differing antibody titres (Pickavance, 1970). Therefore, the two rabbit and two rat antisera were used in different combinations in two standard ELISA's with the slug standard solution to determine which combination of rabbit and rat antisera had the highest titres.

Methods

Antisera titres were assessed by an ELISA using a modification of the method detailed in section 2.3. This allowed all four antisera to be assessed together.

Rabbit A and B antisera were diluted separately in PBS-Tween to 1:1000. One hundred ml of diluted rabbit A antisera were dispensed into each well of plate 'A'. One hundred ml of diluted rabbit B antisera were dispensed into each well of plate 'B'. The plates were incubated for one hour at 37°C.

Two slug standard dilution series (50, 25, 10, 1 ml/ml, 100, 10 and 1 ng/ml) were dispensed into both plates from columns 3-6 and 8-11 and the plates were incubated for one hour at 37°C.

Rat 1 and 2 antisera were diluted in PBS-Tween to 1:1000. One hundred ml of diluted rat 1 antisera were dispensed into columns 3-6 of plates 'A' and 'B' and one hundred ml of diluted rat 2 antisera were dispensed into columns 8-11 of plates 'A' and 'B' (Fig. 3.3). The plates were incubated for one hour at 37°C.

The conjugate was added at a concentration of 1:2000 and the plates developed according to the methodology in section 2.3.

Results

Rabbit A and rat 1 antisera gave the highest absorbency across the slug standard dilutions (Fig. 3.4). All of the slug standard dilutions were recorded as positive absorbency. The rat antisera were similar in reactivity but rabbit B antisera was less reactive than that of rabbit A.

Discussion

Laboratory animals are known to yield different titres of antibody following immunisation (Pickavance, 1970). This investigation found that a combination of rabbit A and rat 1 antisera gave the highest absorbency and therefore these two antisera were identified as having the highest titre. Thirty ml volumes of these antisera were dispensed into vials and frozen at -20°C for further investigations. The two antisera used for the rest of the investigations will now be referred to as the 'antisera'. The other two antisera were also stored at -20°C and kept in reserve.

3.5 Assessment of rabbit A and rat 1 optimum concentrations

Introduction

Two concentrations of rabbit A and rat 1 antisera were used in four combinations to determine which yielded an absorbency range beneficial for interpreting ELISA

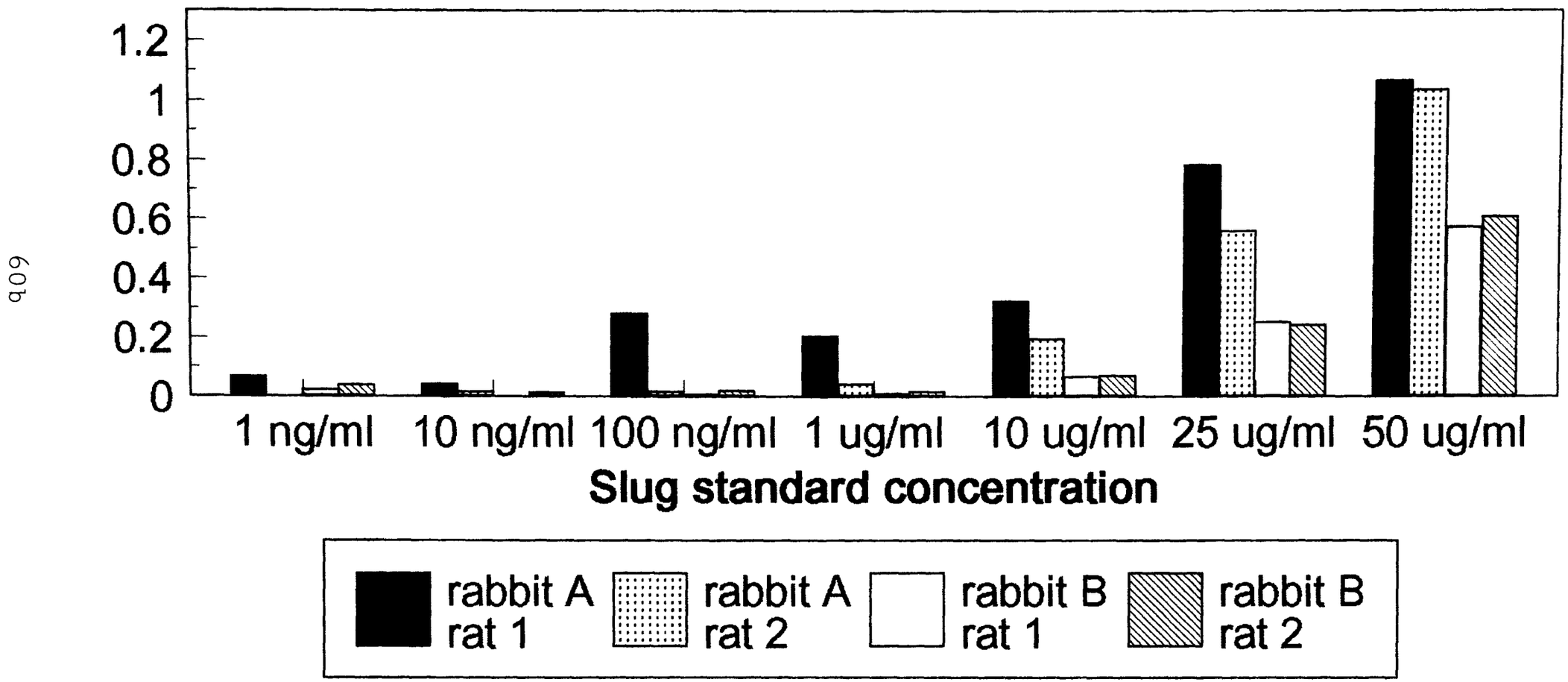
Fig. 3.3 MicroELISA plate design for assessing antisera titre. Plate A coated with rabbit A antisera and plate B coated with rabbit B antisera The strength of the slug standard solutions are as follows: s1=50, s2=25, s3=10,s4=1ug/ml, s5=100,s6=10, s7=1ng/ml.

Plate A	1	2	3	4	5	6	7	8	9	10	11	12
A												
B			s1	s3	s5	s7		s1	s3	s5	s7	
C			s1	s3	s5	s7		s1	s3	s5	s7	
D			s1	s3	s5	s7		s1	s3	s5	s7	
E			s2	s4	s6			s2	s4	s6		
F			s2	s4	s6			s2	s4	s6		
G			s2	s4	s6			s2	s4	s6		
H												
				Rat one					Rat two			

Plate B	1	2	3	4	5	6	7	8	9	10	11	12
A												
B			s1	s3	s5	s7		s1	s3	s5	s7	
C			s1	s3	s5	s7		s1	s3	s5	s7	
D			s1	s3	s5	s7		s1	s3	s5	s7	
E			s2	s4	s6			s2	s4	s6		
F			s2	s4	s6			s2	s4	s6		
G			s2	s4	s6			s2	s4	s6		
H												
				Rat one					Rat two			

Fig. 3.4 Assessment of four combinations of rabbit and rat antisera titires

The assessment was made across seven slug standard concentrations.



data from test specimens. As test data were generally in the form of small quantities of slug remains from predators guts, concentrations of antisera were required which were particularly sensitive to smaller quantities of antigen.

Methods

Two dilutions of rabbit A and rat 1 antisera were made in PBS-Tween and combined to give four treatments (Table 3.3). One hundred ml of 1:1000 rabbit antisera were dispensed into plate 'X' and 100 ml of 2:1000 rabbit antisera were dispensed into plate 'Y' (Fig. 3.5). The plates were incubated for one hour at 37°C.

One hundred ml of each slug standard dilution, from the dilution series were dispensed into wells 3-6 and 8-11 of both plates. The plates were incubated for one hour at 37°C. One hundred ml of 1:1000 rat antisera were dispensed into wells 1-6 of both plates, and 100 ml of 2:1000 rat antisera were dispensed into wells 7-12 of both plates. The plates were incubated for one hour at 37°C, developed and the absorbency compared.

Results

The highest rabbit and rat concentrations (T4) gave very high absorbencies across all seven slug standard dilutions. The highest slug standards were not within the capacity of the plate reader and the lower slug standard dilutions had poor sensitivity (Fig. 3.6). The highest slug standard absorbencies for T2 concentrations of antisera were also above the scale of the plate reader, but they were more sensitive at the lower slug standard dilutions.

T1 and T3 concentrations gave the broadest ranges of absorbencies and T1 concentrations (rabbit 1:1000, rat 1:1000) gave the most sensitive readings at the bottom end of the range at low slug standard dilutions (Fig. 3.6).

Discussion

Concentrations of antisera were required which gave a broad range of absorbency to enable accurate quantification of slug remains in the guts of predators which

Table 3.3 Assessing rabbit A and rat 1 optimum concentrations. Four treatments (T1-T4) were used which consisted of four combinations of rabbit A and rat 1 antisera.

	rabbit 1:1000	rabbit 2:1000
rat 1:1000	T1	T2
rat 2:1000	T3	T4

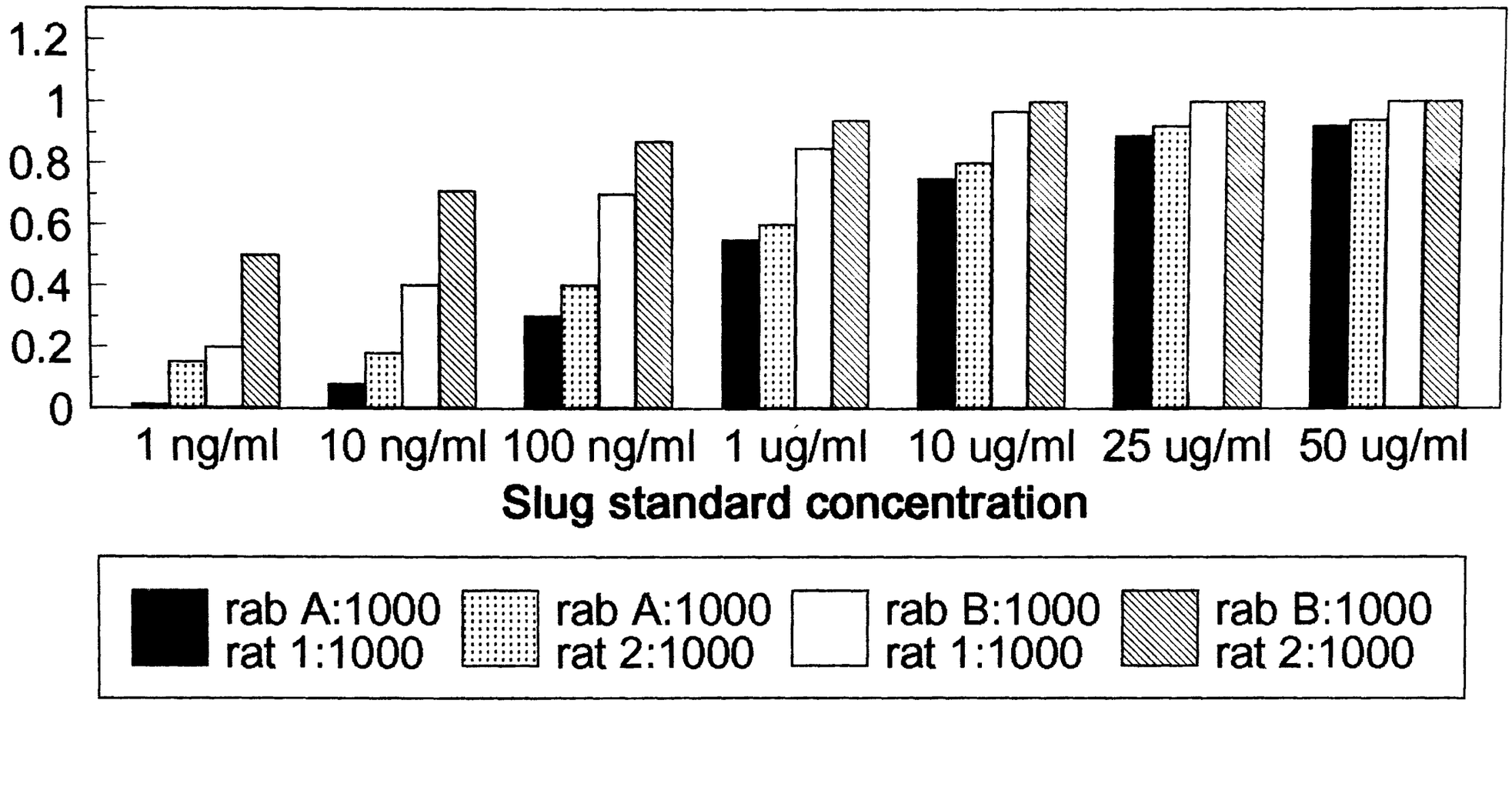
Fig. 3.5 MicroELISA plate design for assessing rabbit A and rat 1 optimum antisera concentrations. Plate x coated with rabbit A antisera at 1:1000, plate y coated with rabbit A antisera at 2:1000. The strength of the slug standard solutions are:s1=50, s2=25, s3=10, s4=1ug/ml, s5=100, s6=10, s7=1ng/ml.

Plate x	1	2	3	4	5	6	7	8	9	10	11	12
A												
B			s1	s3	s5	s7		s1	s3	s5	s7	
C			s1	s3	s5	s7		s1	s3	s5	s7	
D			s1	s3	s5	s7		s1	s3	s5	s7	
E			s2	s4	s6			s2	s4	s6		
F			s2	s4	s6			s2	s4	s6		
G			s2	s4	s6			s2	s4	s6		
H												
			Rat 1:1000					Rat 2:1000				

Plate y	1	2	3	4	5	6	7	8	9	10	11	12
A												
B			s1	s3	s5	s7		s1	s3	s5	s7	
C			s1	s3	s5	s7		s1	s3	s5	s7	
D			s1	s3	s5	s7		s1	s3	s5	s7	
E			s2	s4	s6			s2	s4	s6		
F			s2	s4	s6			s2	s4	s6		
G			s2	s4	s6			s2	s4	s6		
H												
			Rat 1:1000					Rat 2:1000				

Fig. 3.6 Assessment of rabbit A and rat 1 optimum antisera concentrations

The assessment was made across seven slug standard concentrations.



occur at unknown concentrations. Sensitivity is particularly important at lower slug dilutions. Rabbit and rat concentrations of 1:1000 were therefore chosen for future investigations.

3.6 MicroELISA plate design

Many plate designs have been used, each plate can give 96 results from test samples and some authors have used every available well including the periphery wells (e.g. Sopp, 1987). However, these wells can show atypical colour changes (Clarke and Adams, 1977; Burrows *et al.*, 1984; Herbert *et al.*, 1985).

Burrows *et al.*, (1984) analyzed a number of plates and found they expressed cluster effects, many positive wells being found in localised groups. Although this may be a feature of microtitration experiments, data control measures must be incorporated into each plate in order to gain maximum benefit from the resulting data and to help interpret test specimens (Fenlon and Sopp, 1991). Each plate should be regarded as a distinct experiment and contain all data controls (Fenlon and Sopp, 1991). This reduces the number of available wells for test specimens but increases their value as data.

A number of controls have been used to assess ELISA data. Miller (1981) used Bovine Serum Albumin (BSA). Control wells of PBS-Tween are frequently used (e.g. Crook and Sunderland, 1984). Sutula *et al.*, (1986) recommended the use of positive controls and Fichter and Stephen (1984) fed predators on alternative prey species as controls. Sunderland *et al.*, (1987) used three negative controls of PBS-Tween and two starved predators and positive controls consisting of homogenised (aphid) prey and two predators fed on aphid prey. Negative controls have consisted of starved predators (e.g. Lovei *et al.*, 1985). Fenlon and Sopp (1991) recommended that negative controls should be used which consist of predator homogenate without prey (aphid) antigen. In the plate design adopted for this project, twenty one wells were used for a seven fold dilution series of slug antigen at known concentrations. Three types of control were used (see list below) and periphery wells were not used for test samples. This left 30 wells which were used

for the analysis of test specimens (Fig. 3.2 and Plate 3.1).

1. Three negative controls of PBS-Tween.

2. Controls consisting of the gut and gut contents of starved beetles and beetles which had fed on earthworms (see section 3.8.2.2).

3. Positive controls of beetles which had fed on *D.reticulatum*.

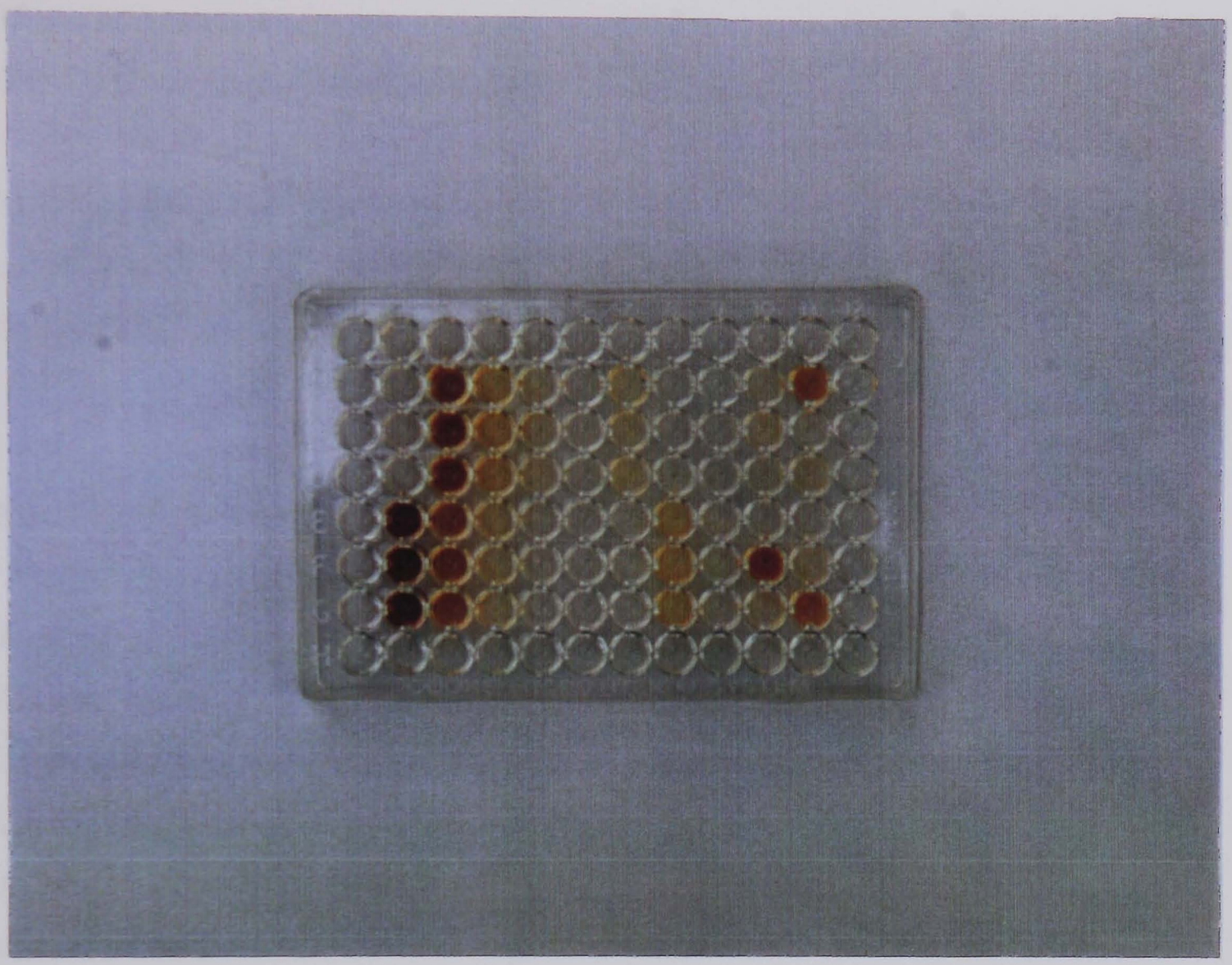
3.7 Positive - negative threshold

ELISA data gradually changes from negative to positive through gradual increments in absorbency (Heck *et al.*, 1980). The definition of a positive-negative threshold has received little attention by ecologists. In a review of 81 papers Sutula *et al.*, (1986) found 49 authors had not stated a method of quantifying ELISA data, seven had used a visual analysis (e.g. Sunderland *et al.*, 1987), 15 had used negative mean values and eight had used negative means plus a specified number of standard deviations. Frequently two standard deviations are used (e.g. Fichter and Stephen, 1981). Although widely accepted these threshold values are arbitrary and often misleading.

Histograms have been used to investigate ELISA data from healthy and infected plant material (Sutula *et al.*, 1986). Data which is distributed bimodally with two populations separated by large absorbency intervals are simple to interpret. However, if positive and negative populations are not well separated interpretation of the data is more difficult. Negative populations are not always normally distributed and often skewed to the right. This tail area has a high potential for containing false positive (Type I) and false negative (Type II) errors.

Sopp and Sunderland (1989) calculated a critical value (C) from the normal distribution of the mean and standard error of the negative controls. This value was set at the upper five percent limit of the normal distribution. Above the C

Plate 3.1 MicroELISA plate, showing the colour changes (absorbency) at the end of the assay. Slug standard solutions and blank controls were used in columns two to five. Antigens from cross-reacting earthworm species were used in column six. Test specimens from beetles caught in the field were used in columns seven to eleven (see chapter three).



limit, a positive absorbency can be said to be detected. A second, determination (D) limit was set which ensured that only five percent of the distribution whose means falls on this limit lies below the C limit. This was obtained by setting the lower five percent of this distribution to the C limit. This gives a 95 percent protection against false positive. Values which fall above the D limit are at least 95 percent certain of being detected as positive signals. Values which fall between the C and D limits are in a 'doubtful' zone (Fig. 3.7).

In this project, the C limit was calculated from the mean and standard error of the controls and the D limit calculated from the student t-distribution (Fenlon and Sopp, 1991) and set to give a 95 percent protection against false positives.

3.8 ELISA studies of slugs and predators

General methodology

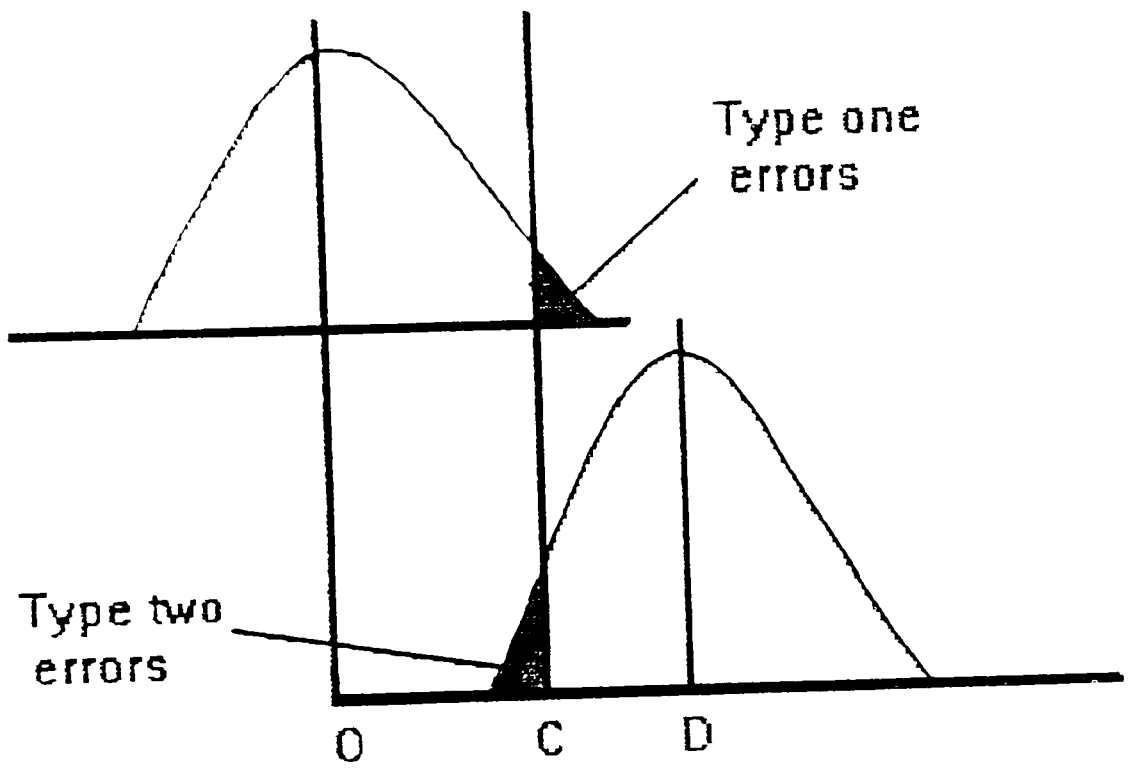
Unless otherwise stated the slugs and carabid predators used during this part of the investigation were kept in culture at 4°C and fed on a diet of lettuce and blow fly larvae respectively. Slugs which were used in specific experiments were removed from culture and separated into containers according to species, at about 6 slugs per container and starved for one week at 12°C prior to use. Carabids were removed from culture, placed in petri dishes and starved for seven days at 20°C to allow their guts to evacuate (Sunderland *et al.*, 1987).

3.8.1 Reactivity of antisera to four slug antigens

Introduction

Tissue homogenate from the four slug species were used in an ELISA to determine the reactivity of the antisera to slug antigens.

Fig. 3.7 The positive - negative threshold. The distribution of negative and positive ELISA data in terms of absorbency. Type one errors arise from false positive absorbency and type two errors arise from false negative absorbency. C = the critical limit. D = the determination limit (see section 3.7).



Methods

Several *D.reticulatum*, *D.caruanae*, *A.hortensis* and *M.budapestensis* slugs were starved, washed and one gram of each slug species weighed out and homogenised in an electric homogenator with one ml of PBS-Tween. The homogenate was left to extract overnight at 4°C. The liquid from the homogenate was then decanted and used undiluted, in the second stage of the ELISA, to determine the reactivity of soluble antigens from the four slug species to the antisera.

Results

Absorbency values were used to compare the reaction of the four slug homogenates to the antisera. Antigens from all four slug species reacted to the antisera and gave positive absorbencies (Fig. 3.8) but different levels of antigen were recovered from each species. *A.hortensis* antigens were the most reactive (mean absorbency (abs.) = 0.9, s.d.=0.05), then *D.caruanae* (mean abs.=0.69, s.d.=0.06), *D.reticulatum* (mean abs.=0.63, s.d.=0.02) and finally *M.budapestensis* (mean abs. 0.58, s.d.=0.02).

Discussion

The reactivity of the antigens to the antisera is a measure of the detectability of the slug antigens. The ELISA was able to detect target slug antigens from simple slug homogenates. *A.hortensis* tissue homogenate yielded the highest absorbencies, however antigens from *D.reticulatum*, the most important pest species also reacted strongly to the antisera.

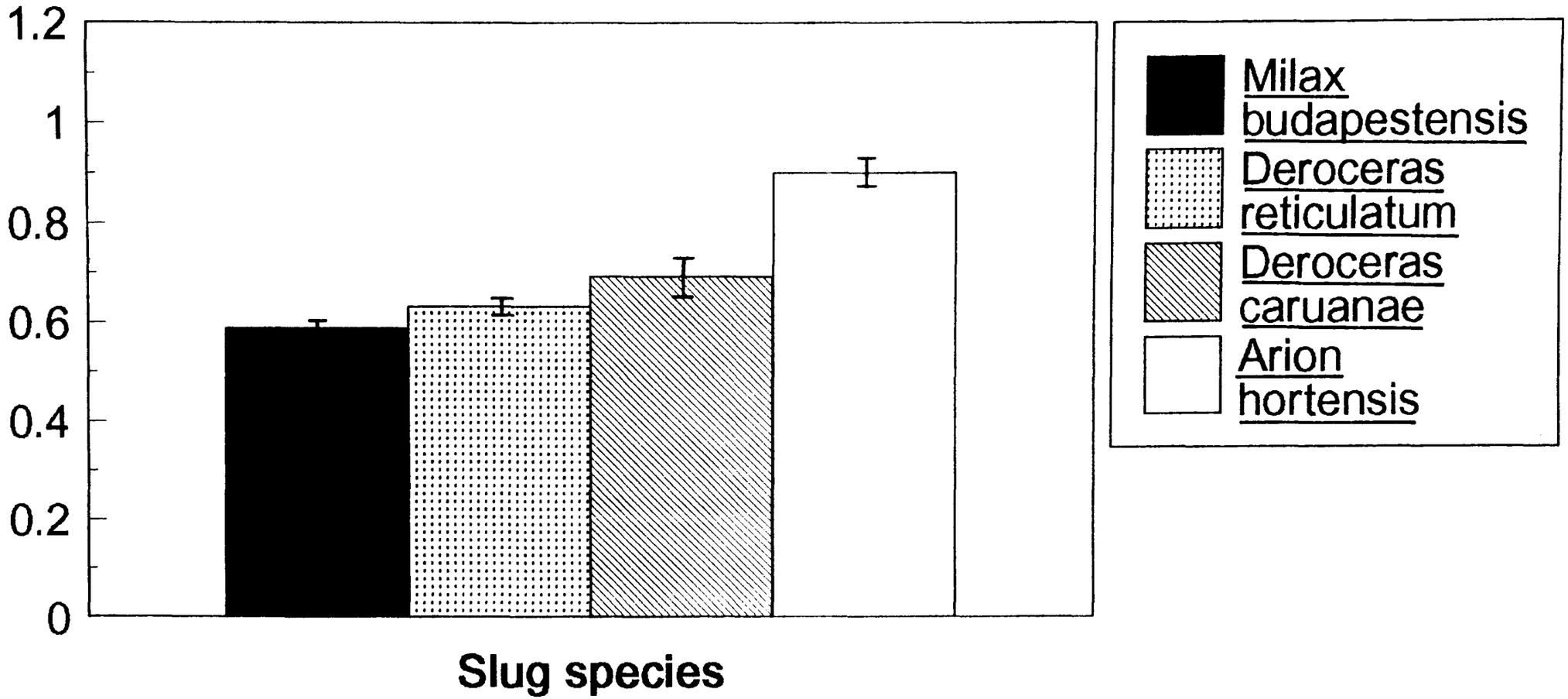
Similarities in levels of *D.reticulatum* and *D.caruanae* could be a function of their taxonomic closeness (e.g. Boreham and Ohiagu, 1978). In order to save time and utilize resources more efficiently *D.caruanae* were not included in further investigations.

3.8.2 Factors affecting detection of slug antigen in predators

Introduction

The effect of temperature, meal size, time since feeding (McIver, 1981) antigen

Fig. 3.8 Reactivity of the antisera to neat slug homogenates of the four species used to raise the antisera (section 3.2.1). I=standard error.



decay rate, predator size and sex of predator have all been cited as factors affecting the detection of target antigens in predators using serology (Sunderland, 1987; Sopp and Sunderland, 1989). These factors need to be addressed before postmortem quantification of predator's food can be made (Sopp and Sunderland, 1989).

3.8.2.1 Reactivity of antisera to ingested slug antigens

Introduction

Antigens ingested by beetle predators are subject to attack by the predator's digestive system almost immediately upon ingestion (Sunderland *et al.*, 1987). The digestive process quickly denatures the ingested food for absorption and utilisation by the predator.

The antisera were raised against undigested slug antigens. The degradation of ingested slug antigens is a critical factor in their detection. This experiment investigated the effect of slug antigen ingestion by the carabid *P.madidus* on their reactivity to the antisera.

Methods

A number of *A.hortensis*, *D.reticulatum* and *M.budapestensis* slugs and *P.madidus* adults were starved and the three slug species were homogenised separately. Starved *P.madidus* beetles were allocated a slug species and excess quantities of the appropriate slug homogenate were presented to the beetles, which were allowed to feed to satiation. The beetles were observed every 10 minutes for one hour. Beetles feeding twice or more during this period were used for the next part of the experiment, otherwise they were discarded.

Beetles which had fed were drowned separately in a weak detergent solution, and frozen at -20°C until they were assessed. When being assessed, the beetles were removed from the freezer and their foreguts were dissected and used in the second stage of the ELISA.

Results

Absorbency values were used to compare the reactivity of the three slug antigens to the antisera after ingestion by *P.madidus*. Antigens from all three test slug species reacted positively to the antisera (Fig. 3.9). *D.reticulatum* antigen were the most reactive (mean abs.=0.543, s.d.=0.49), then *A.hortensis* antigen (mean abs.=0.199, s.d.=0.107) and finally *M.budapestensis* antigen (mean abs.=0.038, s.d.=0.018).

Discussion

Antigens from all three slug species were detected by the ELISA after ingestion by the carabid *P.madidus*. However, detection of *A.hortensis* and *M.budapestensis* antigens was reduced when compared with neat homogenate (Figs. 3.8 and 3.9). Ingestion of *D.reticulatum* tissues did not greatly reduce antigen detectability.

Variations within treatments were quite large and differences between the species may simply be a result of the different amounts of slug ingested by individual predators (Crook and Sunderland, 1984; Sopp and Sunderland, 1989). It is possible that certain parts of the slug are more reactive to the antisera (Mattinson, 1965).

3.8.2.2 Specificity of the antisera

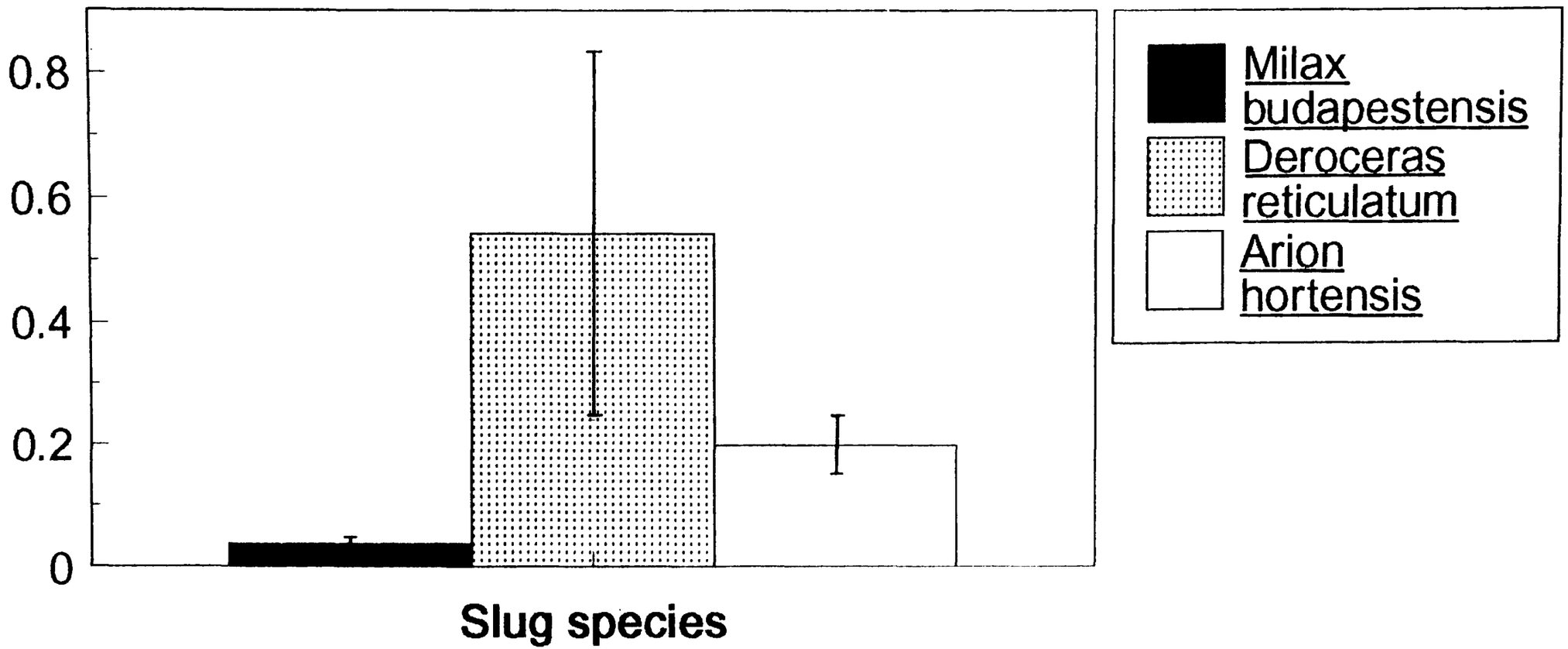
Introduction

Antisera should only detect antigens from species of prey being studied (Calver, 1984). One of the main limitations of serological studies is antisera specificity. A prerequisite of any serological study is to determine the reactivity of the antisera against other prey (Boreham and Ohiagu, 1978).

In this project, the ELISA was used as a tool to detect slug antigens in postmortem investigations of carabid predators caught in three field sites. In the field many other prey species live which provide alternative food sources for carabids predators. Many carabid species are generalists (Hengeveld, 1980a) and eat a variety of plant and animal foods (Davies, 1953). *P.madidus* feeds on plant

Fig. 3.9 Reactivity of the antisera to slug antigens after ingestion

P.madidus beetles were used to assess the reactivity of the antisera to three slug species after ingestion by the beetle. I=standard error.



material, Annelida, Arthropoda as well as Mollusca (Luff, 1974; Digweed, 1993a).

The reactivity of antigens from these alternative food sources to the antisera was assessed. This helped determine which ELISA data were true positive reactions from slug ingestion and which were false positive reactions from ingestion of cross-reacting antigens from alternative prey (Boreham and Ohiagu, 1978).

Methods

Selection of alternative prey

Prey species were collected from the ground, surface litter and growing vegetation of fields from the field sites of winter wheat and oilseed rape. Other species which were not present in the field were collected. i.e. Snail species were collected from the garden at Close House to determine the cross-reactivity of the antisera to taxonomically related species (Boreham and Ohiagu, 1978).

Carabids are known to feed on many species of aphids. Aphids have been found in the gut of *A.dorsale* (Griffiths, 1982), *P.melanarius*, *H.rufipes*, *A.dorsale*, *B.lampros* and *Tachyporus hypnorium* (Fabricius) (Sunderland, 1975). The grain aphid *Sitobion avenae* (Fabricius) is one of the most common aphid pest of winter wheat, therefore this aphid was cultured in the insectary at Close House. The cabbage aphid *Brevicoryne brassicae* (Linnaeus) was also collected.

Notiophilus spp, *Bembidion spp*, *P.madidus* and *A.parallelepipedus*, *L.pilicornis* and *N.brevicollis* feed on Collembola (Davies, 1953; Penney, 1966; Sunderland, 1975) and *A.obscurum* attacks *Podura minor* (Lubb)(Dawson, 1965). Three species of the superfamily Entomobryoidea were collected.

Diptera and their larvae are common in fields of winter wheat and oilseed rape. They have been found in the guts of *P.melanarius* and *H.rufipes* (Sunderland, 1975). *P.madidus* will feed on dipteran larvae and *A.parallelepipedus, P.madidus*, *P.niger*, *P.cupreus*, *Agonum assimile* (Paykull) all eat large blowflies (Evans, 1967). *A.plebeja* will eat diptera and *C.problematicus* and *N.brevicollis* gave positive reactions to

diptera antisera (Tipulidae) in a precipitin test (Dennison and Hodkinson, 1983). Several Calliphoridae adults, a Tipulidae larvae and several other species of Diptera larvae were collected.

Earthworms have been shown to be a source of possible cross reactions (Symondson and Liddell, 1993a). *P.madidus*, *C.piceus*, *A.parallelepipedus*, *N.brevicollis*, *P.melanarius*, *Agonum* and *Bembidio*n species all feed on earthworms (Davies, 1953; Penney, 1966; Sunderland, 1975). *C.problematicus* feeds on earthworms (Gradwell, 1954), as do other *Carabus* and *Cychrus* species (Gruntal and Sergeyeva, 1989). The earthworms *Allolobophora longa* (Ude), *Elisenia foetida* (Savigny), *Lumbricus terrestris* (Linnaeus) and *Octolasion lacteum* (Oerley) have been used in cross-reaction tests with slug antibodies (Symondson and Liddell, 1993b). These four worm species were collected.

Several carabid species have been shown to feed on spiders, including *N.brevicollis* (Davies, 1953; Penney, 1966; Sunderland, 1975), *P.melanarius* (Sunderland, 1975) and *P.madidus* (Evans, 1967). *C.violaceus* will feed on harvestspiders (Shankey, 1949). *Carabus* and *Cychrus* species have been shown to feed on spiders using serology (Gruntal and Sergeyeva, 1989). *P.niger* and *A.assimile* have been shown to feed on spiders using a precipitin test (Dennison and Hodkinson, 1983). A Linyphiidae and Gnaphosidae spider species were collected.

Many carabid species will feed on woodlice. *P.madidus* feeds on the small woodlice *Trichoniscus pusillus* (Brandt) (Evans, 1967) and *P.madidus*, *A.parallelepipedus*, *P.niger*, *P.cupreus*, *A.assimile* all eat the larger *Oniscus asellus* Linnaeus. Precipitin tests have been used to identify carabid predators of *Philoscia muscorum* (Scopoli) and *Armadillidium vulgare* (Latreille) (Sunderland and Sutton, 1980). A precipitin test identified woodlice consumption by *N.brevicollis*, *A.assimile* and *C.piceus* (Dennison and Hodkinson, 1983) and by *Carabus* and *Cychrus* species (Gruntal and Sergeyeva, 1989). *A.vulgare* has been used in cross-reaction tests against slug antisera (Symondson and Liddell, 1993b). All of these woodlice and *Porcellio scaber* Latreille and *Porcellio spinicornis* Say were collected.

A.parallelepipedus, *C.piceus*, *C.problematicus* and *Oxypoda vittata* Markel all feed on millipedes in woodland ecosystems (Dennison and Hodkinson, 1983). Symondson and Liddell (1993b) assessed *Polymicrodon polydesmoides* (Leach), *Glomeris marginata* (Villers) and *Tachypodoiulus niger* (Leach) to determine the cross-reaction of millipede antigens to slug antisera. Several millipede species were collected including *Polydesmus angustus* Latzel the common flat backed millipede, *Oxidus gracilis* Koch and the centipede *Lithobius forficatus* (Linnaeus).

P.melanarius, *H.rufipes*, *N.brevicollis, A.dorsale* and *B.lampros* feed on Coleoptera adults. These five species and *L.pilicornis* feed on Coleoptera larvae (Sunderland, 1975). *P.madidus* feeds on *P.cupreus* and *A.assimile* adults (Evans, 1967). *P.madidus* and *A.parallelepipedus* feed on Chrysomelidae pupae (Dempster *et al.*, 1959). Two coccinellid species, *Coccinella 7-punctata* Linnaeus and *Psyllobora 22-punctata* (Linnaeus), two carabid species, *Amara aenea* Degeer and *Bembidion tetracolum* Say and a *Cantharis* species were collected.

Carabids feed on Lepidoptera (Allen and Hagley, 1982). *P.madidus*, *A.parallelepipedus*, *P.cupreus* and *P.melanarius* are all important predators of winter moth pupae (Frank, 1967). Ashby (1974) used a precipitin test to identify predators of *Pieris rapae* Linnaeus. *H.rufipes* feeds on the larval stages of *P.rapae* (Dempster, 1967). The larvae of three Lepidoptera species were collected from oilseed rape including *Pieris brassicae* Linnaeus and two Noctuidae species.

P.madidus will kill the earwig *Forficula auricularia* Linnaeus (Gradwell, 1954), therefore this species was collected.

Fragments of the snail *C.minima* (=*lubricella*) have been recovered from *P.madidus* (Luff, 1974). *A.viduum, A.obscurum*, *A.thoreyi*, *A.fuliginosum*, *P.minor*, *P.diligens*, *P.strenuus* and *P.vernalis* all feed on molluscs (Dawson, 1965). Three species of Zonitoidae and a smaller Cochlicopidae species were collected.

Thirty eight alternative prey taxa were collected in total (Table 3.4). The different species were placed into separate petri dishes containing moist filter paper and starved for 48 hours to allow their guts to evacuate any foreign proteins. They were frozen separately according to species in glass tubes at -20°C, prior to assessment by the ELISA.

Alternative prey were thawed, weighed and homogenised in 125 ml of PBS-Tween until no further colour change was perceived and left to extract overnight at 4°C (Fichter and Stephen, 1981). Particular attention was paid to quality control and contamination of the homogeniser. The following day the specimens were used in the second stage of the ELISA with similarly prepared *D.reticulatum* controls.

Results

The absorbency of *D.reticulatum* antigens fell beyond the upper limit of the calibration curve. Therefore absorbency values were used to compare the cross-reactions of alternative prey antigens with the reactivity of *D.reticulatum* antigens (Figs 3.10a-c). Antigens from other mollusc species cross-reacted to the greatest extent. *Aegopinella spp.* were the most cross-reactive (mean abs.=0.484, s.d.=0.005). However, *D.reticulatum* antigen were clearly separated from cross-reacting antigens of alternative prey species.

Related groups of alternative prey species tended to give the same levels of cross-reactions. Collembolan antigens did not cross-react at all (mean abs.=0, s.d.=0) and non of the aphid antigens cross-reacted (mean abs.=0, s.d=0). Of the non-molluscan alternative prey, earthworm antigens cross-reacted to the greatest extent (*L.terrestris*; mean abs.= 0.172, s.d=0.03).

Discussion

Zonitoidae snail antigens cross-reacted to the greatest extent and the resulting absorbencies were closest to *D.reticulatum* absorbencies. This is probably a function of their taxonomic closeness (Boreham and Ohiagu, 1978). However, the absorbency values of slug and snail antigens were clearly separated from each other

Table 3.4 Alternative prey species used to assess cross-reactions with slug antisera. + + + indicates absorbency value above the scale of the plate reader. The 'N' column indicates the number of wells used to assess the species/group.

Species	Wt. (mg)	N	Slug eqv. (mg)	Absorbency	
				Mean	S.d
Collembola (Entomobryoidae)					
species 1	3.3	1	0.0	0	-
species 2	3.3	1	0.0	0	-
species 3	0.2	1	0.0	0	-
species 4	2.3	1	0.0	0	-
Diplopoda					
T.niger (Lulidae)	7.2	3	0.01	0.015	0.014
O.gracilis (Strongylosom.)	14.6	4	0.008	0.011	0.008
G.marginata(Glomeridae)	22.4	3	0.006	0.004	0.008
P.angustus (Polydesmidae)	10.9	2	0.015	0.019	0.026
Chilopoda					
L.forficatus (Lithobiidae)	5.0	2	0.003	0.034	0.016
Isopoda: Oniscoidae					
T.pusillus(Trichoniscidae)	7.8	2	0.005	0.005	0.007
P.muscorum (Oniscidae)	7.4	2	0.014	0.022	0.019
O.asellus (Oniscidae)	11.7	2	0.011	0.107	0.080
A.vulgare (Armadrillidid.)	12.7	1	0.025	0.054	-
P.scaber (Porcellionidae)	6.8	2	0.0	0.023	0.033
P.spinicornis (Porcell.)	0.4	1	0.0	0.0	-
Dermaptera *F.auricularia* (Forficulidae)	8.1	2	0.02	0.038	0.014
Lepidoptera (larvae)					
P.brassicae (Pieridae)	15.0	5	0.013	0.031	0.043
Noctuida spp.1 (Noctuidae)	15.0	3	0.019	0.097	0.018
Noctuida spp.2 (Noctuidae)	15.0	3	0.016	0.104	0.018
Arachnia					
Linyphiidae spp. (Linyphiidae)	0.35	1	0.0	0.0	-
Gnaphosidae spp. (Gnaphosidae)	0.99	1	0.007	0.017	-
Diptera					
Calliphora spp. (Calliphoridae)	5.2	4	0.001	0.034	0.049
Tipula spp. (Tipulidae)	7.0	3	0.018	0.048	0.042
Diptera larvae	3.7	1	0.001	0.0	-

Table 3.4 (continued)

Species	Wt. (mg)	N	Slug eqv. (mg)	Absorbency	
				Mean	S.d
Hemiptera					
S.avenae (Aphidiae)	2.1	2	0.0	0.0	0.0
S.avenae (alate)(Aphidiae)	1.6	1	0.0	0.0	-
B.brassicae (Aphidiae)	3.0	1	0.0	0.0	-
Coleoptera					
A.aenea (Carabidae)	15.0	1	0.003	0.031	-
B.tetracolum (Carabidae)	8.0	1	0.0	0.0	-
Cantharis spp. (Cantharoidae)	10.0	1	0.033	0.108	-
C.7-punctata (Coccinellidae)	15.0	1	0.007	0.048	-
P.22-punctata (Coccinellidae)	15.0	2	0.006	0.125	0.115
Annelidae					
L.terrestris (Lumbricidae)	15.01	3	0.083	0.172	0.030
A.longa (Lumbricidae)	5.0	3	0.045	0.114	0.054
O.lacteum (Lumbricidae)	15.0	3	0.065	0.148	0.031
E.foetida (Lumbricidae)	15.0	3	0.000	0.0	0.0
Mollusca					
Oxycilus spp. (Zonitoidae)	15.0	2	1.877	0.443	0.049
Aegopinella spp. (Zonitoidae)	15.0	2	4.775	0.484	0.005
Zonitoides spp. (Zonitoidae)	14.0	2	8.159	0.442	0.104
Cochlicopa spp. (Cochlicopidae)	6.0	2	0.131	0.169	0.028
D.reticulatum (Limacidae)	12.0	2	+ + +	1.441	0.395

Fig. 3.10(a) Cross-reactivity of alternative prey antigens to the antisera

Cross-reactivity is measured by absorbance.

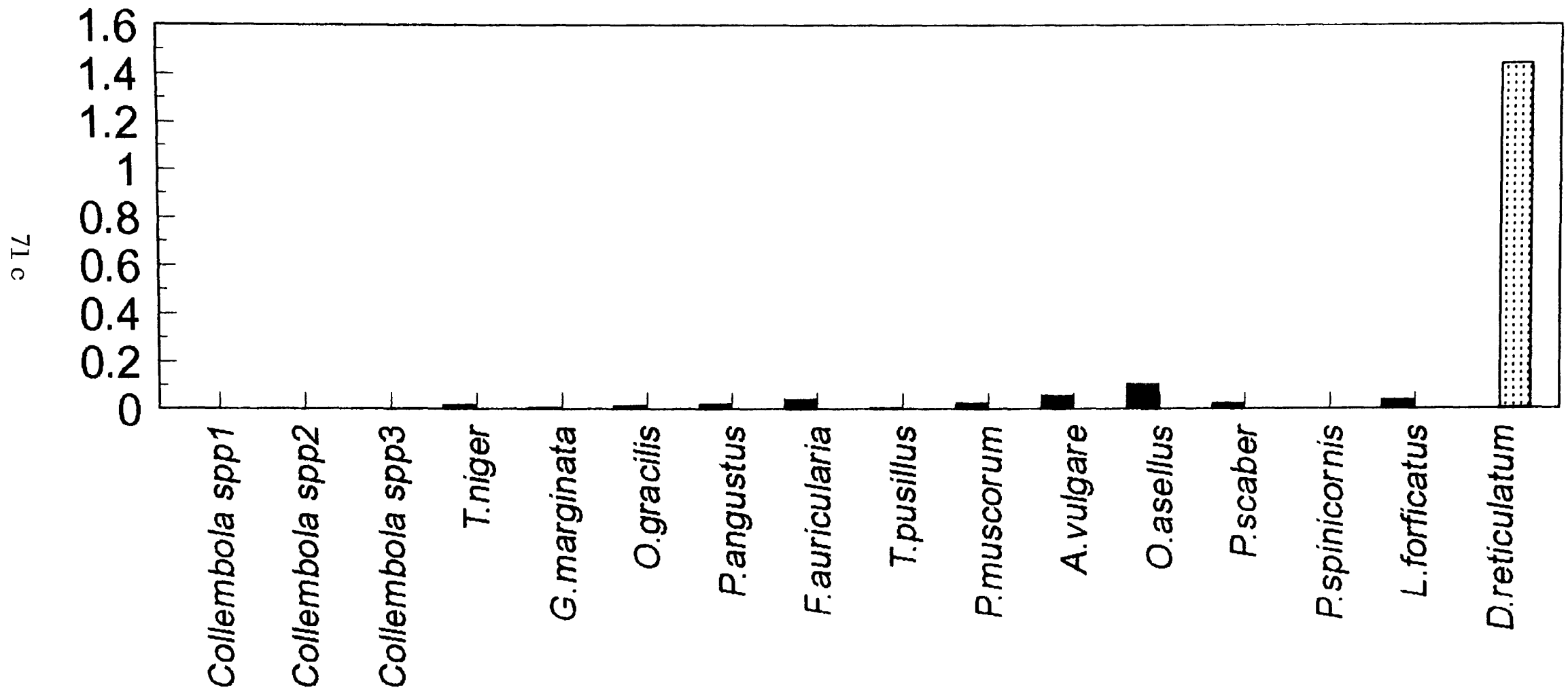


Fig. 3.10(b) Cross-reactivity of alternative prey antigens to the antisera

Cross-reactivity is measured by absorbance.

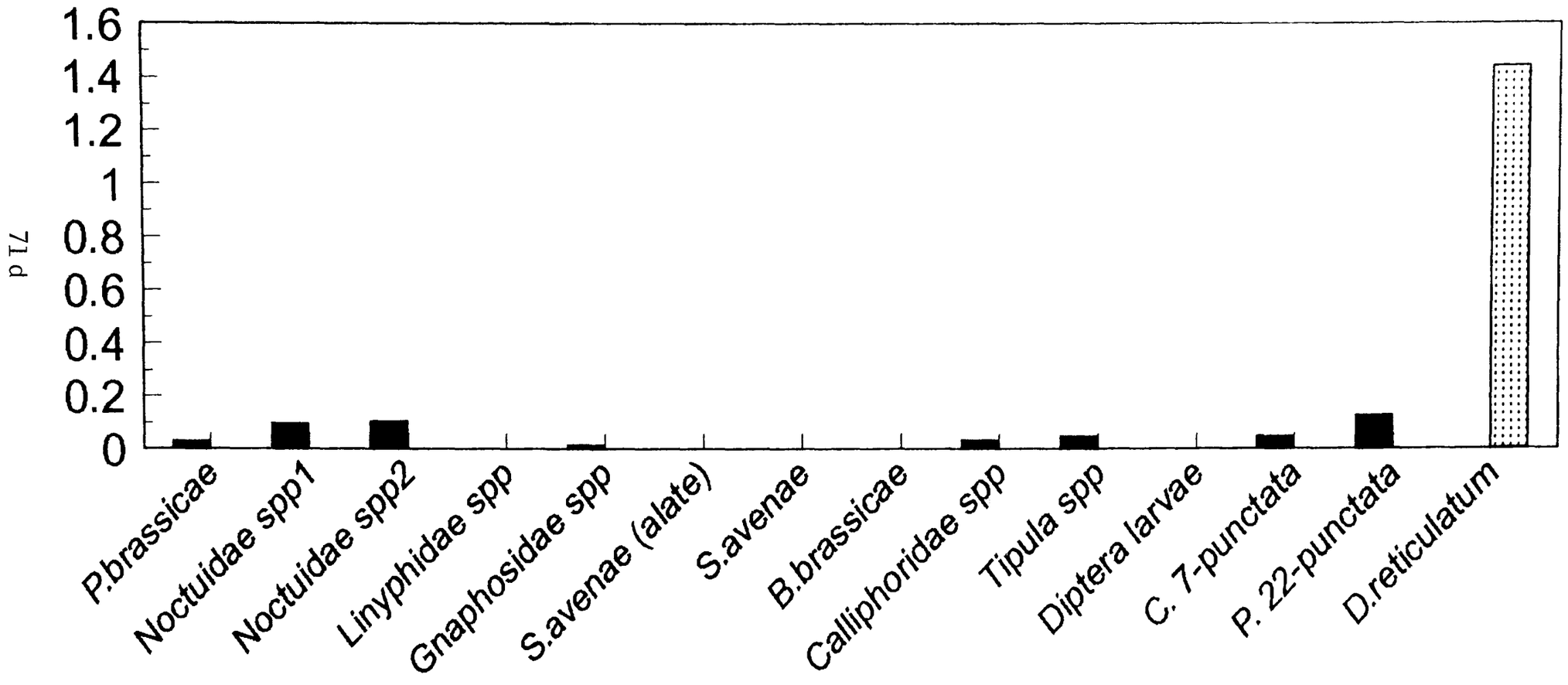
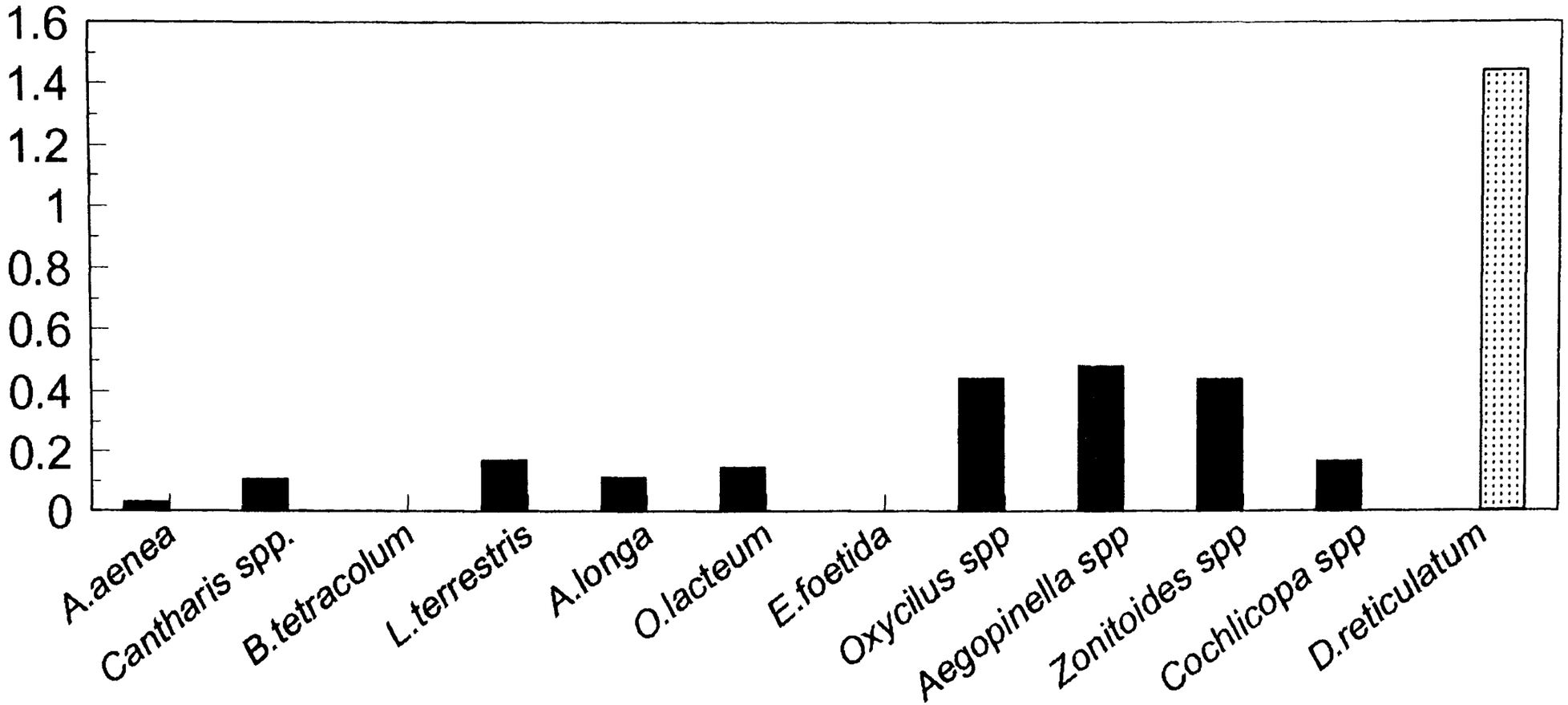


Fig. 3.10(c) Cross-reactivity of alternative prey antigens to the antisera

Cross reactivity is measured by absorbance.



and the ELISA can be used to distinguish between antigens from the two groups.

Although snail antigens cross-reacted strongly to the antisera, they were not included as controls on the ELISA plates as snails were not found in any of the study sites. However, the collection of data from the study sites concerning the occurrence of other molluscs was considered useful when interpreting ELISA data. Earthworm antigens were identified as the strongest cross-reacting non-molluscan antigens to slug antisera, probably as a result of the similarity of slug and earthworm mucus (Symondson and Liddell 1993a). Therefore, earthworm antigens were used as controls.

3.8.2.3 Reaction of antisera to ingested alternative prey antigens

Introduction

The objective of this project was to identify ingested slug antigens in the guts of carabid beetles. However, ingested slug homogenate is less reactive to the antisera than neat homogenate (3.8.2.1). The reduced reactivity to the antisera may cause problems when trying to distinguish slug and alternative prey antigens from unknown samples. This study investigated the reactivity of the antisera to slug and alternative prey antigens after ingestion by carabid beetles. This determined if the resulting absorbency could be separated.

Methods

One *Aegopinella* and one *Zonitoide* snail species were investigated as neat homogenate of these alternative prey reacted strongly to the antisera (3.8.2.2). Earthworms have cross-reacting antigens (Symondson and Liddell, 1993a), therefore *L.terrestris* was investigated. Other species included in the investigation were the aphid *S.avenae*, molluscan *Oxycilus* spp and lepidopteran *P.brassicae* larva.

Alternative prey were placed in petri dishes containing moist filter paper and incubated at 12°C, until their guts evacuated. An excess of prey were introduced into petri dishes containing starved *P.madidus* adults, one prey species to a petri

dish. Beetles were allowed to attack and feed on their prey for one hour and were observed every ten minutes. Beetles which fed twice or more during this period were individually drowned in a weak detergent solution, and frozen at -20°C until assessed by the ELISA. Beetles which did not feed, or fed only once were discarded. Controls were made by repeating the above method and using *D.reticulatum* homogenate. The beetles guts were dissected and used in an ELISA.

Results

Absorbency values were used to compare the reaction of the antisera to ingested alternative prey and slug antigens. Ingested alternative prey absorbencies were reduced when compared with corresponding 'neat' homogenate from the previous study (3.8.2.2). Lepidoptera antigens did not cross-react to the antisera after ingestion (mean abs.=0, s.d.=0) and aphid antigen cross-reacted at a low level (mean abs.=0.0008, s.d.=0.002). *L.terrestris* antigens cross-reacted more than the mollusc *Aegopinella* spp (mean abs.=0.003, s.d.=0.005), but less than the two *Zonitoides* spp. (mean abs.=0.012, s.d.=0.011). Ingested *D.reticulatum* and alternative prey antigens were clearly separated (Fig. 3.11).

Discussion

Earthworm tissues cross-react to the antisera due to the similarity of slug and worm mucopolysaccharides (Symondson and Liddell, 1993a). Earthworm antigen were chosen as controls to be incorporated into the plate design along with slug antigen. With these controls, the antisera can be used to distinguish ingested slug antigens from cross-reacting alternative prey antigens. Interpretation problems may still arise when small amounts of slug antigens are ingested or when the antigen has gone through a period of decay in the predator.

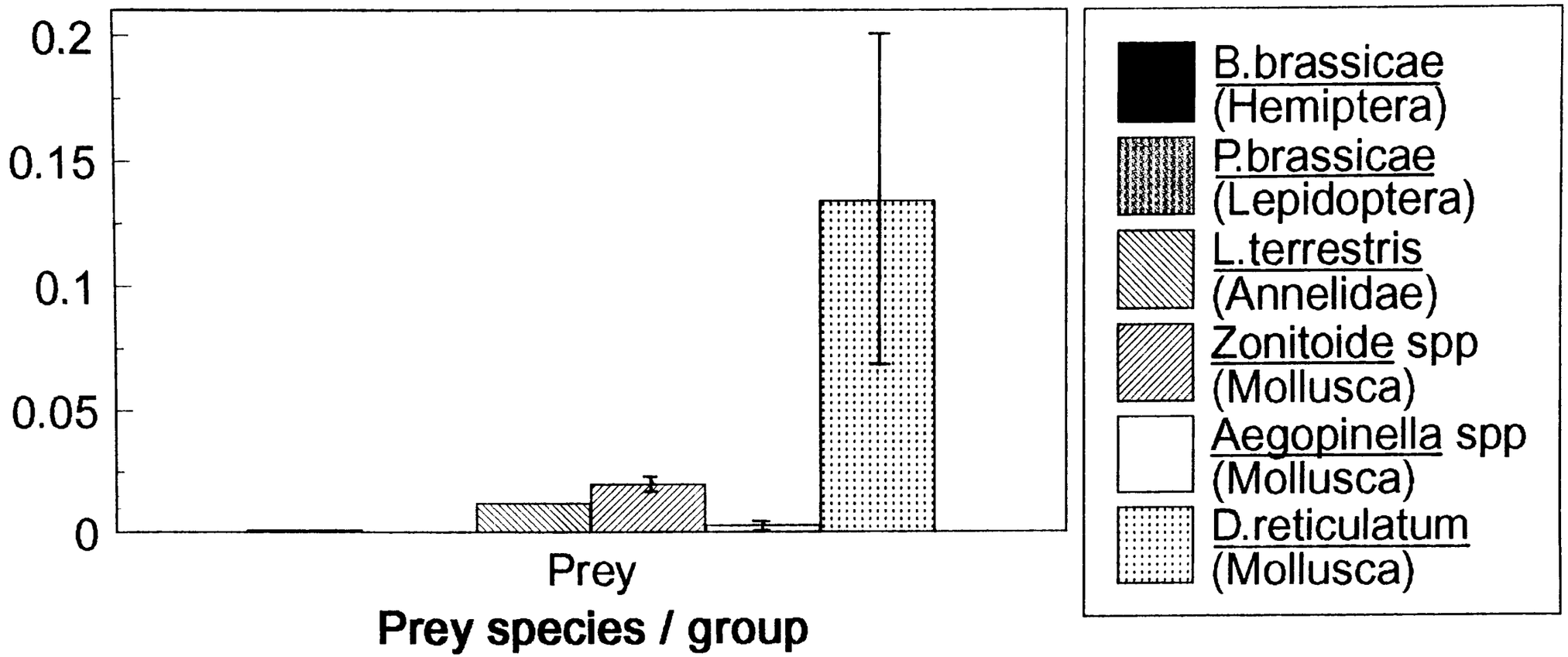
3.8.2.4 Antigen decay rate

Introduction

Digestion can start before ingestion in fluid feeding species and in the pre-oral digesting Carabini group. For most species, digestion takes place in the midgut and

Fig. 3.11 Reactivity of the antisera to ingested alternative prey antigens

Target (slug) antigens and alternative prey antigens were assessed after ingestion by Pterostichus madidus. I=standard error.



the foregut acts as a food store. Foregut volume, rate of filling and emptying are important for detecting prey in postmortem investigations (Giller, 1984).

Ingested prey antigens can denature almost immediately (Sunderland *et al.*, 1987). Predators caught in pitfall traps will have been active for an unknown period and it is not possible to determine when they last ate a slug meal. It is reasonable to assume that slug remains have gone through a period of decay in the predators digestive system before the predator is recovered from the study site and processed for analysis by ELISA.

The detection period, or the length of time the slug meal remains detectable in the gut of a predator after feeding, is critical in determining how useful the ELISA is at detecting partially digested slug meals. If antigens are digested quickly there will only be a limited period of time when the ELISA can detect them. This study investigated the length of time *D.reticulatum* antigens remained detectable in the guts of several predator species and investigated the effect of temperature on decay rate.

Methods

One hundred and eighty *H.rufipes* beetles were placed into separate petri dishes containing moist filter paper. The petri dishes were placed into incubators at 20°C and the beetles starved for seven days (Sunderland *et al.*, 1987). The beetles were then divided into batches of 36 and each batch was placed in an incubator at either 4, 8, 12, 16 or 20°C. The beetles were left to acclimatise for 24 hours.

After 24 hours, the beetles were arranged on a bench in subdued light and presented with an excess of starved, macerated *D.reticulatum* tissues and allowed to feed to satiation. The beetles were observed every ten minutes for one hour. Beetles which fed twice or more on the slug macerate during this period were returned to their allocated temperature, otherwise they were discarded.

After one hour, six beetles from each experimental temperature were removed and drowned in a weak detergent solution then stored at -20°C for future assessment by ELISA. This process was repeated after one, three, five, ten and fifteen days. The dissected guts were arranged on microELISA plates and tested for the presence of slug antigens.

This experiment was repeated using *D.reticulatum* antigens on four other predator species and one genus (see Table 3.5), which reflected the carabid fauna of the study sites (chapter five). Wherever possible, six replicates of each species were made at each temperature and time interval (see Appendix 3.1). However, predator availability and enticing the predators to feed was a major constraint in this study. In addition, predator mortality during the experiment was very high. The experiment was repeated using *P.madidus* and *A.hortensis* tissues.

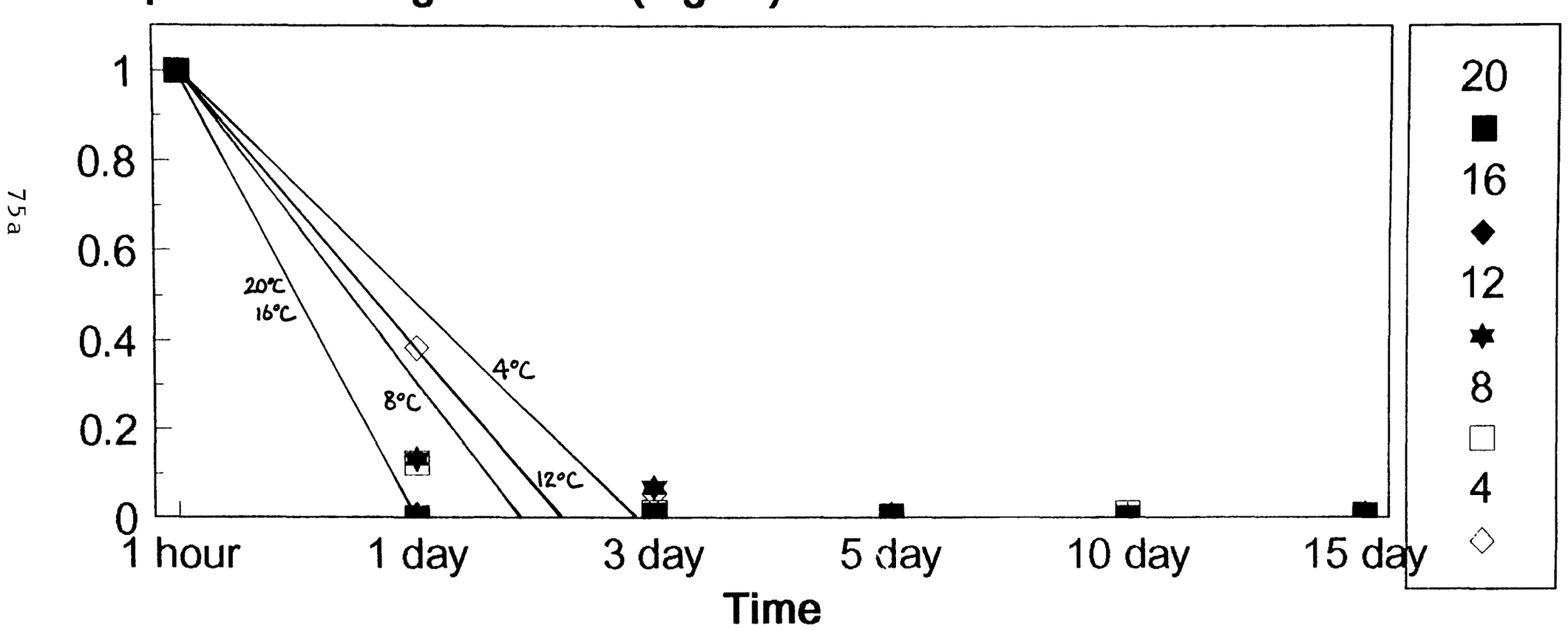
Results

Antigen decay rate

The determination limit (section 3.7) was used to identify positive reactions and absorbency values were converted to equivalent mass of slug using the calibration curve (section 3.3). The initial mean mass of slug found immediately after feeding (one hour time interval) was calculated and used to determine the proportion of slug mass left at subsequent time intervals. Mean slug mass were calculated for each predator species at each time interval at each temperature. This enabled comparisons to be made between temperatures and predator species (Sopp and Sunderland, 1989). Data were very variable, often only one beetle produced a positive absorbency for a particular treatment. This was particularly true for beetles assessed at the longer time intervals.

D.reticulatum antigen decayed exponentially in *P.madidus* and most decay took place in the first day (Fig. 3.12). Eighty percent of the antigen decayed in the first day at 8, 12, 16 and 20°C. A temperature effect was evident and decay rates decreased as temperature decreased. Decay at 4°C was slower, but by day five levels of slug remains were similar at all temperatures. *A.hortensis* antigens decayed

Fig. 3.12 Deroceras antigen decay rate in Pterostichus madidus
Antigen measured at five temperatures over 15 days, expressed as a proportion of antigen present immediately after feeding.



more slowly in *P.madidus*. In the first day, fifty percent of slug antigens remained at 20°C and over thirty percent of slug antigens were detected at 15 days (Fig. 3.13). However data were very variable.

D.reticulatum antigen decay was very rapid in *H.rufipes* at 16 and 20°C. Less than 10 percent of initial antigen levels were recovered after one day (Fig. 3.14). The rate of decay at 8 and 12°C was slower and more than 50 percent of initial antigen levels were recovered after 3 days at 12°C. However by day five antigen levels were very low.

Three antigen decay curves were evident in *A.parallelepipedus* (Fig. 3.15). Ninety percent of the decay took place in the first day at 20°C, but only 50 percent at 12°C. However, antigen levels at both temperatures were similar by day three. At 4°C decay was very slow and by day three, over 75 percent of initial slug antigen remained.

Full data sets were only available at 12°C for *N.brevicollis*. Over 70 percent of initial antigen levels were recovered on day one and over ten percent by day five at 12°C and 16°C (Fig. 3.16). Antigen decay in *H.aeneus* was complete by day three at 12 and day five at 20°C (Fig. 3.17). However at 20°C, over 80 percent of antigen were recovered on day one. Data for *Carabus* species was very erratic. Over 20 percent of antigens were recovered on day five at 20 and 8°C and also on day 15 at 12°C (Fig. 3.18).

Maximum detection limit

The data were used to determine a maximum detection period. Individual data were transformed using an angular transformation (sin-1). Means were calculated for each time interval, logged and used in a regression analysis (Lovei *et al.*, 1985). The point of intersection of the x-axis indicated the maximum detection limit (Sopp and Sunderland, 1989).

Data were very variable for most treatments. The r^2 value is a measure of the

Fig. 3.13 Arion antigen decay rate in Pterostichus madidus
Antigen measured at two temperatures over 15 days, expressed as a proportion of antigen present immediately after feeding.

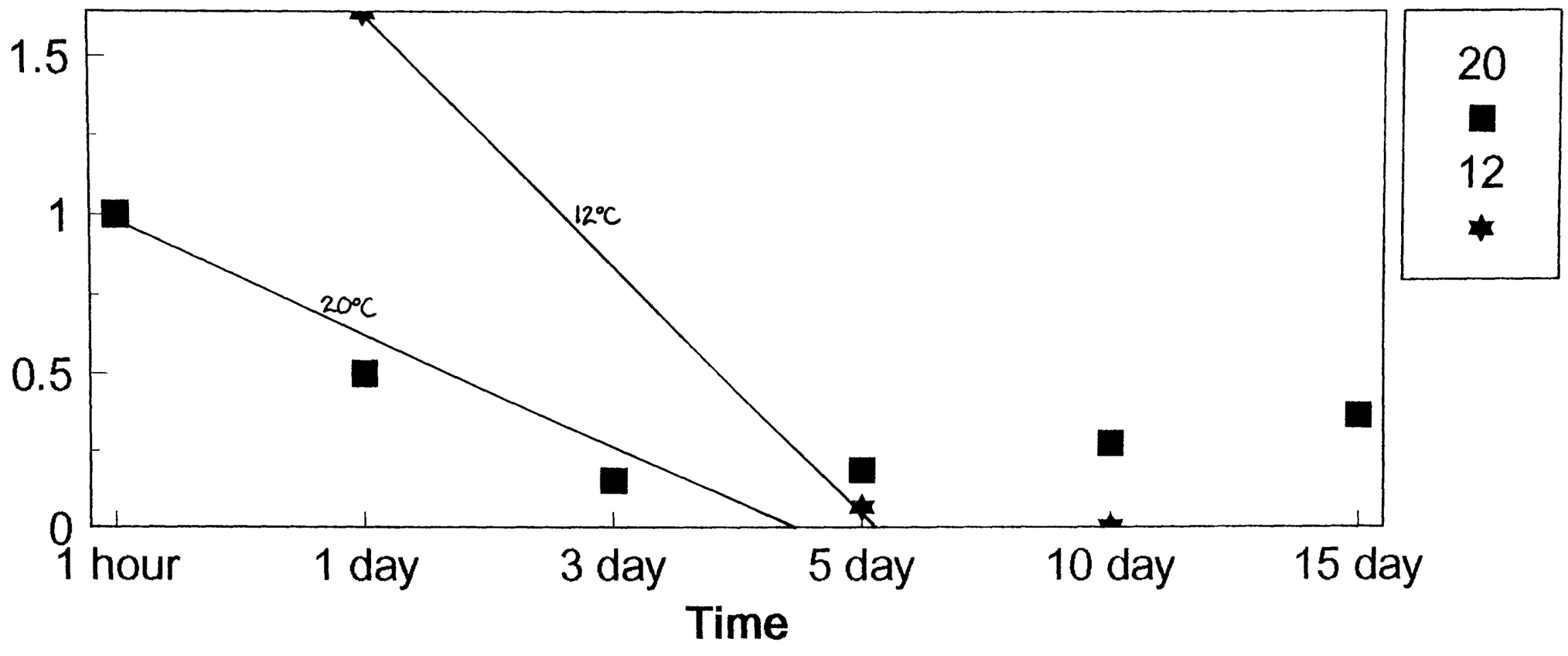


Fig. 3.14 Deroceras antigen decay rate in Harpalus rufipes
Antigen measured at five temperatures over 15 days, expressed as a proportion of antigen present immediately after feeding.

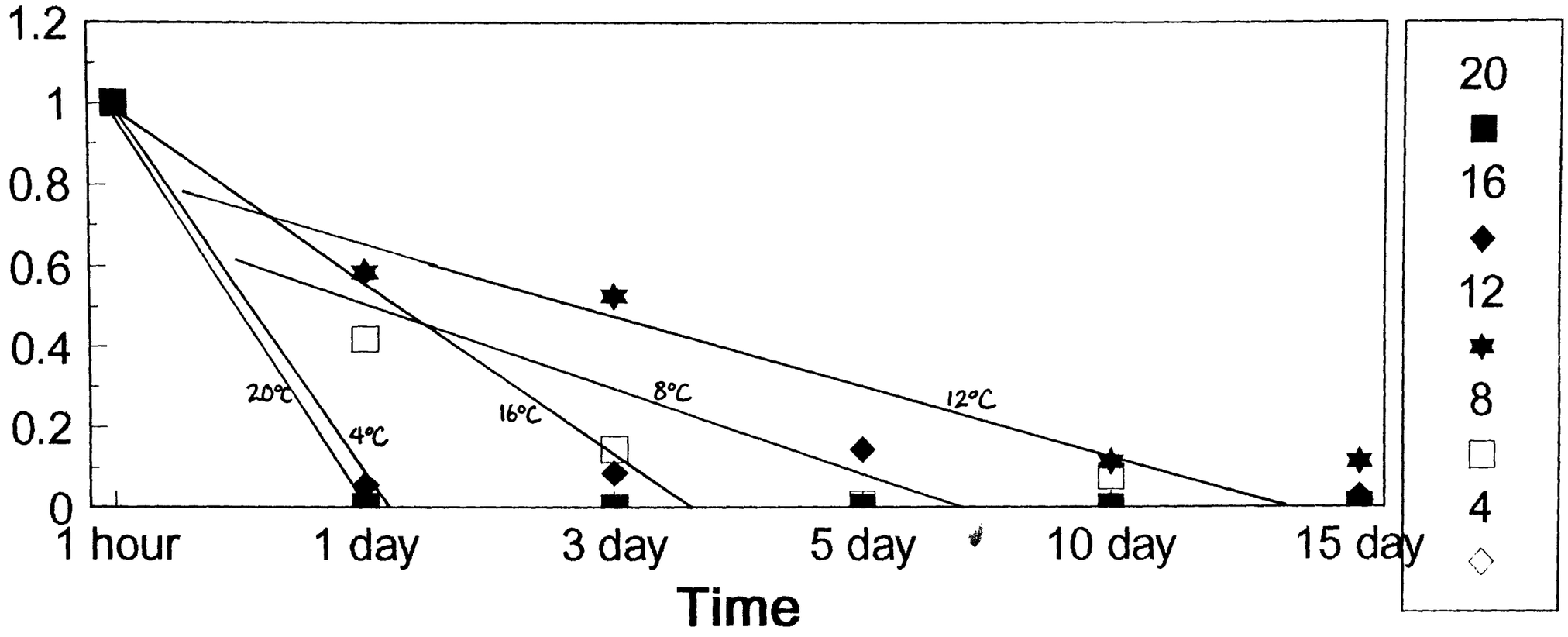


Fig. 3.15 Deroceras antigen decay rate in Abax parallelepipedus
Antigen measured at three temperatures over 15 days, expressed as a proportion of antigen present immediately after feeding.

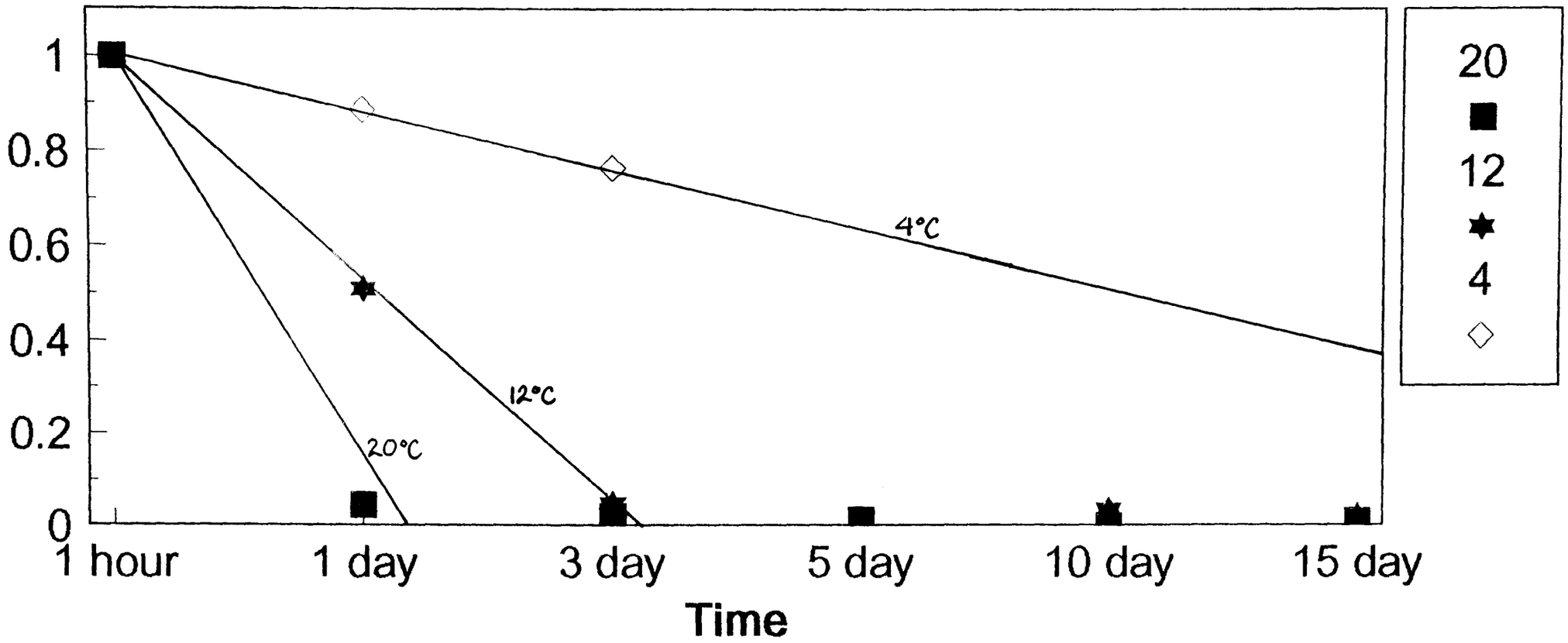


Fig. 3.16 Deroceras antigen decay rate in Nebria brevicollis
Antigen measured at three temperatures over 15 days, expressed as a proportion of antigen present immediately after feeding.

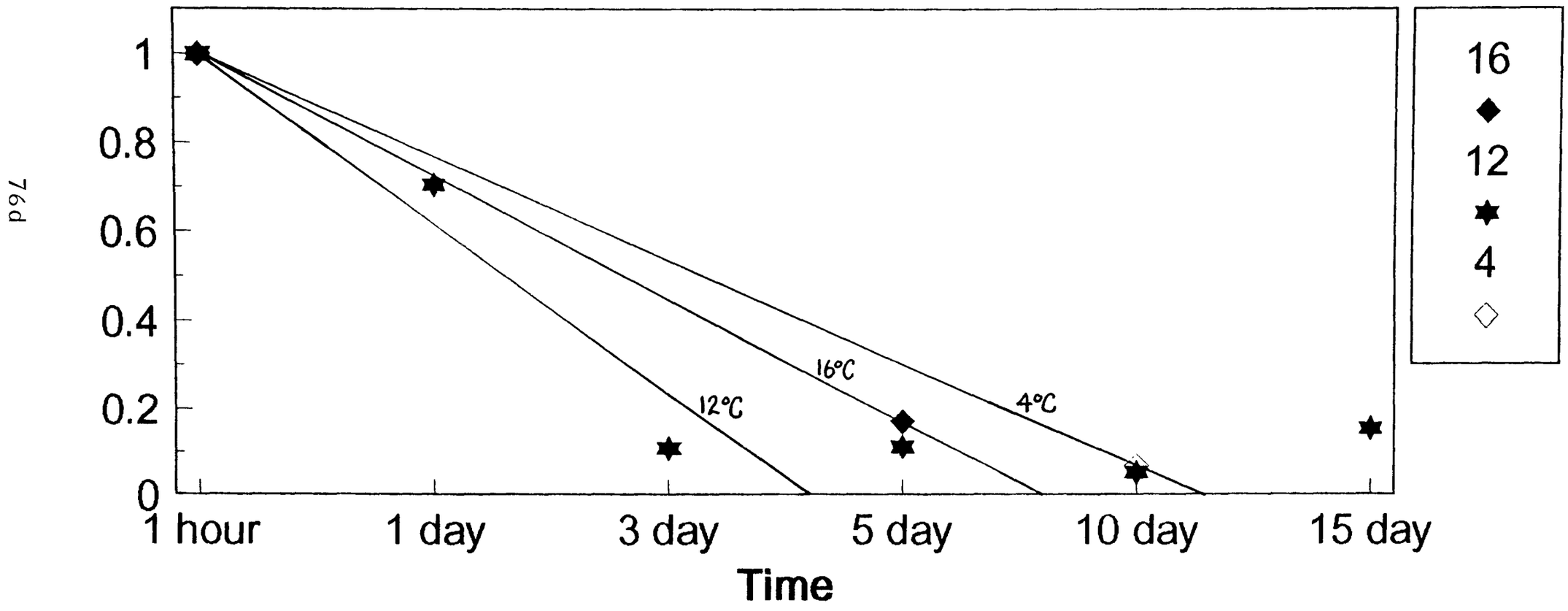


Fig. 3.17 Deroceras antigen decay rate in Harpalus aeneus
Antigen measured at two temperatures over 15 days, expressed as a proportion of antigen present immediately after feeding.

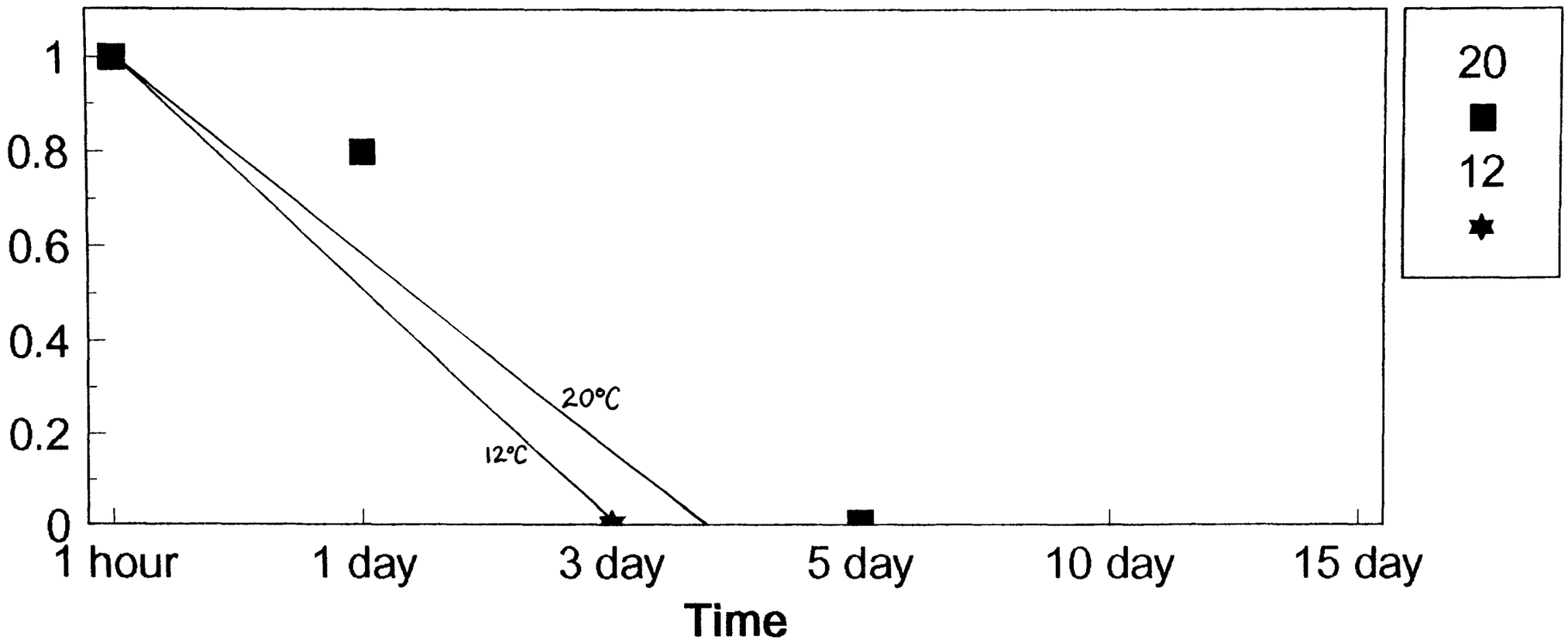
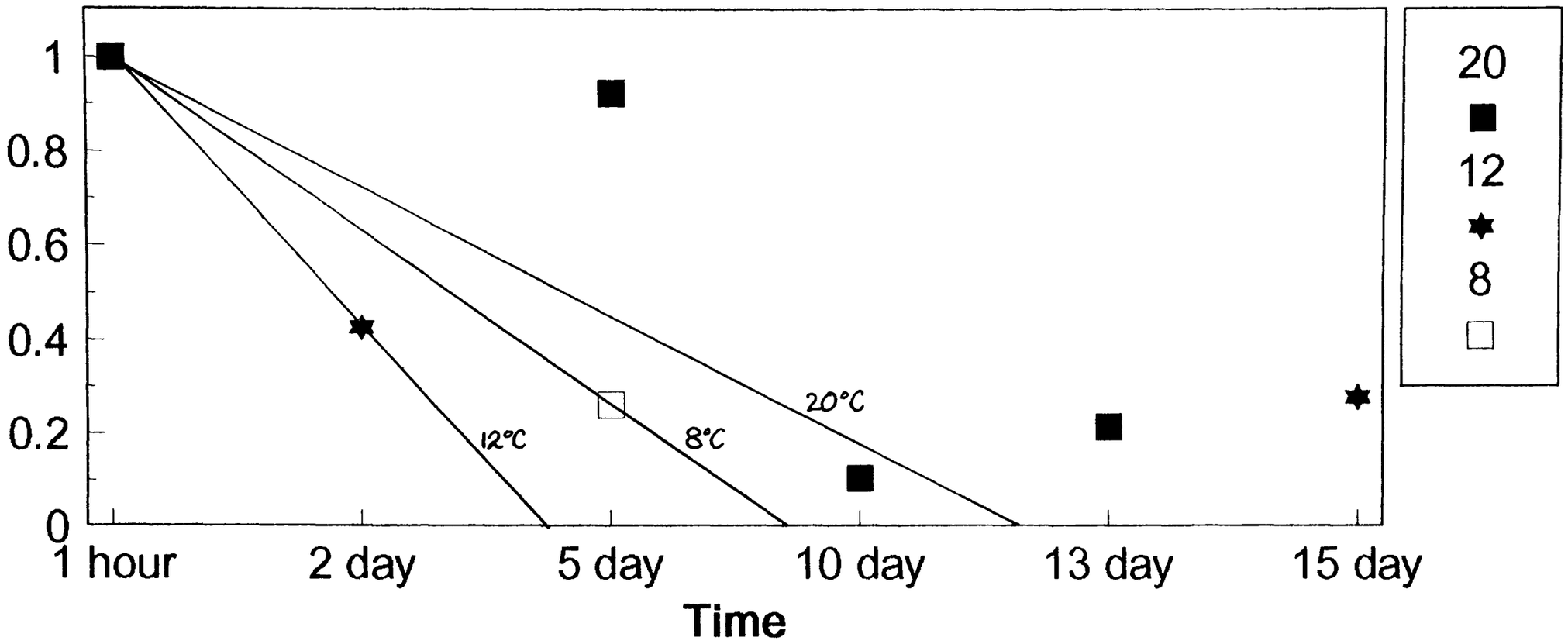


Fig. 3.18 Deroceras antigen decay rate in Carabus species
Antigen measured at three temperatures over 15 days, expressed as a proportion of antigen present immediately after feeding.



strength of the correlation. A value of 1.0 represents a strong correlation between the variables and in a weak correlation r^2 approaches zero. Borderline significant correlations were found between antigen decay and time in *A.parallelepipedus* at 4°C ($r^2=0.998$, d.f.=1, $P<0.1$) and *Carabus* spp. at 20°C ($r^2=0.91$, d.f.=2, $P<0.1$). A significant correlation was found between antigen decay and time in *H.rufipes* at 16°C ($r^2=0.907$, d.f.=3, $P<0.05$). None of the other correlations were significant. Detection periods were not always longest at low temperatures, but detection periods were shortest at 20°C in *P.madidus*, *A.parallelepipedus*, *H.rufipes* and *N.brevicollis*. Due to the overall variation in the regressions, lines were fitted by sight and detection periods estimated (Table 3.5).

There was no overall trend of increasing detection period with increasing predator size. Where most data were available (12°C), *H.rufipes* had the longest detection period. At 20°C, *H.rufipes* and *P.madidus* had the shortest detection periods and large *Carabus* predators had the longest detection periods.

Arionid antigen decay was very variable at 20°C. At 12°C the detection period for Arionid antigen was 5.0 days in *P.madidus*. At the same temperature *Deroceras* antigen was detectable for 2.2 days in *P.madidus*. Therefore, Arionid tissues remain detectable for longer than *Deroceras* tissues in *P.madidus*.

Discussion

The large variations in the amount of recovered antigen from predators in the same treatment is a function of the amount of food ingested at the beginning of the experiment (Fichter and Stephen, 1981). The variability in the amount of antigens ingested by individual predators will affect the degree of antigen detected (Fichter and Stephen, 1984).

Differences in individual beetles metabolic state before and during the experiment may affect the rate of digestion. Age, sex and development of predators all affect metabolic variation (Sopp, 1987). Different 'digestive phenotypes' may exist in a population which accounts for large variations in decay rates (Lovei *et al.*, 1985).

Table 3.5 Maximum detection period of *Deroceras* and *Arion* antigens in five species and one genus of carabid beetles. The maximum detection period is indicated in the third column. The first six treatments were made with *Deroceras* antigens. The final treatment (*P.madidus*) was made with *Arion* antigens.

species	Temperature °C	Maximum (D.p.)
P.madidus	4	2.9
	8	2.0
	12	2.2
	16	1.0
	20	1.0
A.parallelepipedus	4	30
	12	3.1
	20	1.3
H.rufipes	4	1.2
	8	7.0
	12	13.2
	16	3.8
	20	1.0
N.brevicollis	4	11.5
	12	4.5
	16	8.0
H.aeneus	12	3.0
	20	3.8
Carabus spp.	8	9.0
	12	4.5
	20	12.0
P.madidus	12	5.0
***Arion* Ags.**	20	4.5

The decay rate can also increase with increased periods of starvation (Lovei *et al.*, 1985). The long pre-experimental starvation period in this experiment may have accelerated decay and this factors indicate that maximum detection periods were underestimated.

Linear rates of antigen decay indicating constant rates of digestion, have been found in the hemipteran predator *Podisus maculiventris* Say at 20°C (Fichter and Stephen, 1981) and in Arachnid predators over seven days (Fichter and Stephen, 1984). However, decay of fruit fly antigen in the carabid *P.cupreus* is best described by a logarithmic regression (Lovei *et al.*, 1985). The same authors suggested that data from Fichter and Stephen (1981) were best fitted by a logarithmic rather than linear regression.

The data in this study were quite variable. However slug antigen generally decayed in a negative exponential curve found by other authors (Sopp and Sunderland, 1989; Symondson and Liddell, 1993c). Most digestion took place in the first three days after feeding irrespective of the amounts eaten. This is consistent with other work (e.g. Lovei *et al.*, 1985) and the rapid decline of antigen detection in *P.madidus* and *A.parallelepipedus* at 20°C agrees with work done by Symondson and Liddell (1993c).

Larger predators tend to have longer detection periods as they eat larger meals (Sopp and Sunderland, 1989). However, Symondson and Liddell (1993c) compared two generalist Pterostichini and found *P.madidus* had longer detection periods than the larger *A.parallelepipedus*. In this study *P.madidus* had similar detection periods to *A.parallelepipedus*. Therefore, the digestion rates of individual species may be of greater importance than the size of the meal (e.g. Sunderland, 1987).

A temperature effect occurred in several of the beetle species, with the shortest detection periods occurring at the highest temperatures. Sopp (1987) also found a decrease in temperature was correlated to a decrease in antigen decay rate. Decay of target antigens can be very rapid. Antigens were only detectable in

H.rufipes for one day at 20°C and inactivation (and detection) of prey antigens can be instantaneous at this temperature (Sunderland, 1987). The digestion of prey antigens in carabids is slower at low temperatures (Dawson, 1965). Antigens may persist in carabids at low temperatures due to the beetles reduced metabolism and activity. Sopp and Sunderland (1989) suggested that temperatures of 7°C halted digestion of antigens in *B.lampros*.

Pre-oral digestion by *Carabus* species does not denature slug antigens to an extent where they can not be detected. Tod (1973) suggested the oral secretions of the silphid *Phosphuga (=Silpha) atrata* (Linnaeus) denatured slug tissue to an extent where antibody-antigen reactions did not take place in a precipitin test. The differences between this and Tod's study may be the higher sensitivity of the ELISA in this study.

Antigen decay data are necessary when interpreting ELISA data (Sopp and Sunderland, 1989). These studies have shown that the ELISA can detect slug antigens in predators at high field temperatures. However, monitoring field temperatures may help interpret ELISA results (Sunderland, 1988).

3.8.2.5 Size of slug meal

Introduction

This study investigated the reactivity of small amounts of slug antigen to the antisera after ingested by a number of predators. Positive results from ELISA tests will only be obtained if the smallest meal eaten by a predator contains sufficient antigen to yield a positive signal. Small meals may be undetectable shortly after ingestion but large meals may remain detectable for much longer (Giller, 1984). Although smaller carabids such as *Agonum spp* and *P.strenuus* eat molluscs (Dawson, 1965), it is reasonable to assume that they can only tackle small slugs (chapter two) and therefore eat small meals after every kill. If these small meals are not detectable, slug feeding will be underestimated in these predators.

Predator species were chosen to reflect the sizes of carabids found at the three field sites (chapter five). Adult *P.strenuus, A.parallelepipedus, H.rufipes, H.aeneus, P.nigrita, A.fuliginosum, N.brevicollis* and larvae of *N.brevicollis* were used in the assessment.

Methods

Predators were taken from culture and individually placed into petri dishes containing moist filter paper and starved for seven days at 20°C. They were then transferred to an incubator at either 4, 12 or 20°C and allowed to acclimatise for 24 hours.

Ten, one day old *D.reticulatum* slugs were added to each petri dish and the petri dishes were returned to their incubators and left overnight. The following morning the number of slugs eaten by each predator was noted. The predators were drowned separately in a weak detergent solution and frozen before being assessed in an ELISA. The number of replicates was restricted by the availability of predators, one day old slugs and the number of predators which chose to feed on the slugs (see Appendix 3.2).

Results

Positive wells were identified using the Determination limit. The calibration curve was used to convert absorbency values to equivalent slug antigen (mg/ml) in each positive well.

Slug antigens were recovered in *A.parallelepipedus* and *P.madidus* at 20°C even when single slugs were eaten (Table 3.6). Slug antigens were only recovered in *H.rufipes* at the lowest experimental temperature (4°C) when three slugs had been eaten. Slug antigens were not recovered at all in *A.fuliginosum, H.aeneus* and *P.strenuus*. The recovery of slug antigens were very good in *N.brevicollis* larvae at all temperatures, even when single slugs were eaten. Adult *N.brevicollis* beetles needed to eat more slugs before antigens were recovered. Generally, the amount of slug antigen recovered increased with the number of slugs eaten.

Table 3.6 Slug antigen recovered (mg/ml) from predators after eating slug meals of various sizes (one to ten, one day old slugs). Predators were cultured at one of five temperatures. Temperature is abbreviated to Tm and *A.parallelepipedus* is abbreviated to *A.parallelep*.

Species	Tm °C	Number of one day old slugs eaten					
		1	2	3	4	5	10
A.parallelep.	12 20	 0.039				0.54 1.13	1.071 0.43
H.rufipes	04 12 20	 0.0 0.0	 0.0 0.0	0.16 0.0 0.0	 0.0		
P.madidus	08 12 20	0.009 0.007	0.0 0.02	0.07 0.0		 0.04 0.0	
H.aeneus	08 20	0.0	 0.0				
N.brevicollis	12	0.0	0.003				
P.strenuus	20	0.0					
P.nigrita	20			0.037			
A.fuliginosum	20	0.0					
N.brevicollis larvae	04 08 12 16 20	0.055 0.020 0.011 0.008 0.001			0.486		

Discussion

The consumption of a single hatched slug is enough to ensure recovery of slug antigens in *A.parallelepipedus* and *P.madidus* adults and *N.brevicollis* larvae. At 20°C, some predators need to consume more than a single slug before slug antigens are recovered (e.g. *H.rufipes*, *H.aeneus*, *N.brevicollis*, *P.strenuus* and *A.fuliginosum*). Slug antigens were detected in *N.brevicollis* and *P.nigrita* but not in *H.aeneus,* suggesting that *H.aeneus* has a faster digestion rate than similar sized predators. As digestion rates vary between species (e.g. Sunderland, 1987), slug predation is likely to be underestimated in those carabid species with quick digestion rates.

Prevailing climatic conditions may influence antigen detection. In *N.brevicollis* larvae, a temperature effect is operating and antigen detection decreases with increasing temperature. *A.parallelepipedus* and *P.madidus* were the only adult predators to have a single slug meal detected at 20°C, all of the smaller species needed to eat more than one slug. This indicates that some beetles may need to eat large numbers of slugs before slug antigens are detected.

3.9 Mode of predator death

Introduction

The ELISA was used to assess the gut contents of beetles caught in pitfall traps in the field (chapter four). The pitfall traps were filled with water and a dilute detergent solution which quickly drowned predators which fell into the traps. This prevented predators escaping from the traps and prevented smaller predators scavenging slugs killed in the traps by larger predators.

Predators were recovered from the traps and were frozen at -20°C for several months before being assessed in an ELISA. This study investigated the effects of drowning predators on the detection of ingested slug antigens.

Methods

H.rufipes and *P.madidus* adults were removed from culture and placed into

individual petri dishes containing moist filter paper. The beetles were placed in an incubator at 20°C and starved for seven days. Several starved *D.reticulatum* slugs were macerated and presented to the beetles, which were allowed to feed to satiation. The beetles were observed every 10 minutes for one hour. If the beetles fed twice or more during this period they were used in the next part of the experiment, otherwise they were discarded.

Predators which had fed on the slug macerate were split into two treatments. In the first treatment predators were frozen and in the second treatment predators were drowned individually in a weak detergent solution and then frozen. Controls consisted of beetles which had been starved and frozen and beetles which had been starved and drowned.

Results

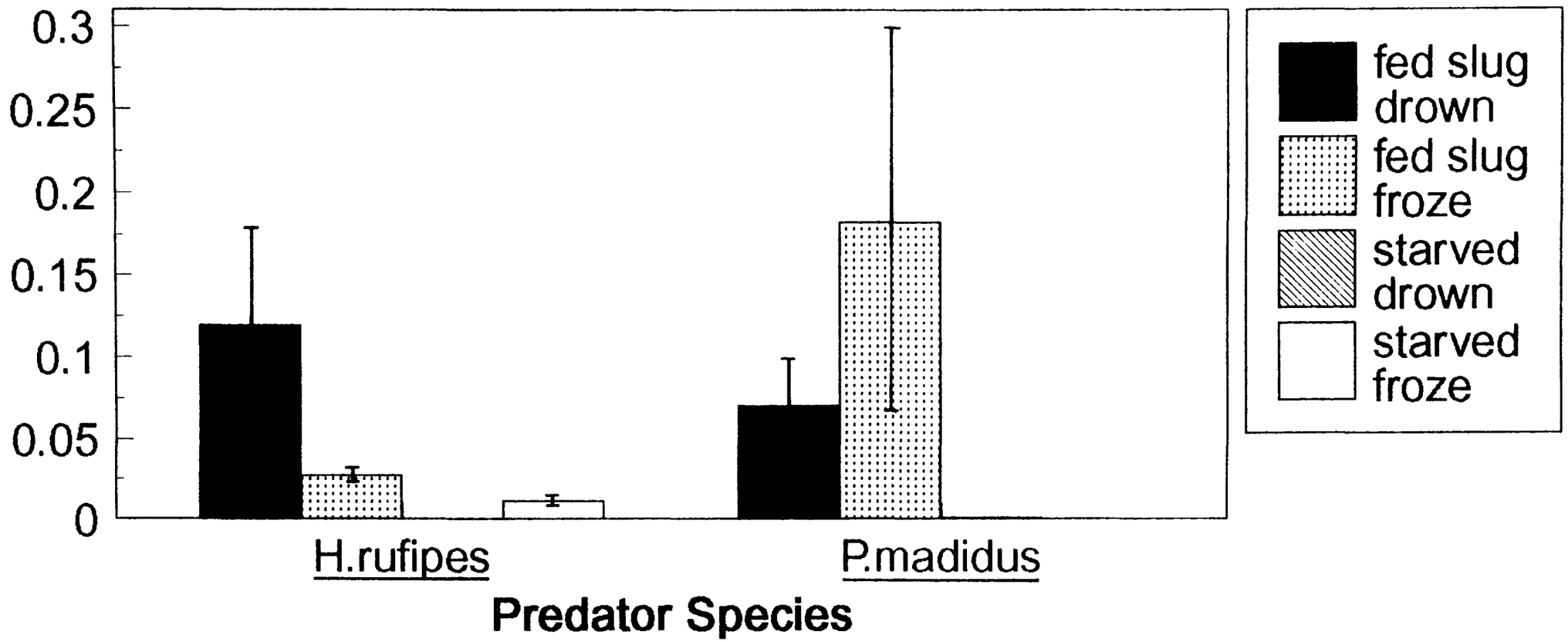
The numbers of beetles used in each treatment are presented in Appendix 3.3. Absorbency values were used to assess the reaction of the antisera to slug antigen in the guts of the predators. The antisera were most reactive to *H.rufipes* beetles which had fed on slugs and were drowned (mean abs.=0.119, s.d.=0.19). The antisera were most reactive to *P.madidus* beetles which had fed on slugs and were frozen (mean abs.=0.182, s.d=0.233). The lowest absorbencies values were from starved and drowned *H.rufipes* beetles (mean abs.=0.011, s.d.=0.011) and starved and frozen *P.madidus* beetles (mean abs.=0.005, s.d.=0.0007). Beetles which had fed on slugs were clearly separated from beetles which had not fed on slugs (Fig. 3.19).

Discussion

Drowning the beetles has no detrimental effects on the detection of slug antigens. Preliminary investigations indicated that diluting the meal increased antigen recovery by reducing the viscosity of the test solution. This allowed freer movement of the slug antigens to binding sites during the second stage of the ELISA (H. Robertson personal communication). Therefore, during the field studies (chapter four), predators guts (and contents) were diluted in a standard 125 ml volume of

Fig. 3.19 Mode of predator death on antigen detection

Deroceras reticulatum antigen detected in two carabid species by ELISA after drowning and freezing starved/fed beetles. I=standard error.



PBS-Tween.

3.10 Conclusions

The studies in this chapter detailed the development of a double sandwich ELISA and microELISA plate design which would detect slug remains in the guts of carabid beetles caught in the field.

Some cross-reactions to the antisera occurred with non-molluscan antigens. However, controls built into the design of the microELISA plate allow distinctions to be made between cross-reacting alternative prey antigens and slug antigens.

The decay of slug antigens in different predator species occurs at different rates and is temperature dependent. The calculated maximum detection periods indicate that at higher temperatures antigen decay is very rapid in some species. The size of the slug meal is also important in the recovery of slug antigens. For smaller predators which kill only small slugs, many slugs may need to be eaten before the slug meal is detected. It is likely that underestimation of predators will occur because of this.

Wet pitfall traps do not interfere with antigen detection in drowned predators and can be used to capture predators in the field.

Chapter four

ELISA Field Studies

4.1 Introduction

This chapter is arranged into three parts. In the first part (section 4.2), slug remains were identified in carabid beetles collected from three field sites using the Enzyme-Linked Immunosorbent Assay (ELISA), developed in chapter three. These beetles' role as either slug predators or scavengers is considered.

In the second part (section 4.3), ELISA data were combined with beetle activity densities and population density estimates to calculate predation rates on slug populations. In the final part (section 4.4), ELISA data were combined with antigen decay rates and activity density to rank each slug predator.

4.2 ELISA results of field caught carabids

4.2.1 Introduction

Many carabid species occur on arable land (e.g. Sunderland, 1975; Jones, 1976 and 1979; Attah, 1986). Some species are known to feed on molluscs (e.g. Davies, 1953; Tod, 1973; Luff, 1974; Symondson, 1992) and therefore have a potential to exert an impact on populations of slugs. In this study, carabid beetles were collected from three commercial fields on two farms in the Tyne valley, Northumberland. The proportions of each beetle species containing slug tissues were assessed using the quantified ELISA.

4.2.2 Description of the sites

The three field sites under investigation are located on the North side of the Tyne valley. Square field (OS reference 54.574N, 1.566W) and Bog field (54.579N, 1.57W) are located on Peepy farm. Clayton field (54.592N, 1.476W) is located on Heddon Bank farm.

Square field covers 10 ha and is bordered by a tree lined stream on one side and hedgerow on the other three sides. One side of the field is adjacent to pasture and the other three sides are adjacent to other arable fields, apart from a forty metre section which is adjacent to a wooded area. Square field is in close proximity to a large wooded area, it has a west facing aspect and is well drained.

Bog field covers 14 ha and is bordered on one side by a tree lined stream. A hedgerow and drainage ditch borders another side and hedgerows run along the other two sides. Three sides are adjacent to other arable fields and the fourth side is adjacent to a road. The field has a flat aspect and tended to become waterlogged during periods of heavy rain in 1993. When this occurred, pitfall traps were often flooded.

Clayton field covers 12 ha and is bordered on one side by a small stream and Clayton's wood. A second side is adjacent to a meadow, which until 1993 had remained unploughed for 15 years. A third side is adjacent to an arable field and the fourth side is adjacent to a narrow strip of trees which back onto a dismantled railway line. Clayton field has a flat aspect and is well drained.

In 1992 all three fields were under oilseed rape. The rape was harvested at the end of July and winter wheat was sown in September and harvested in August of 1993.

4.2.3 Methods

4.2.3.1 Predator samples

Plastic beakers were used as pitfall traps to catch ground active predators. The mouth of the traps measured 72mm in diameter. The traps were 105mm deep and the diameter of the base measured 55mm.

Twenty pitfall traps were arranged in each field in a five by four block. Traps were spaced at five metre intervals along the edges of tram lines approximately 40 metres

into the crop (Fig. 4.2.1). Traps were sunk into the ground so the lip of the trap was level with the surface of the ground and filled to 75 percent capacity with water and a weak detergent (e.g. Sunderland, 1975). The detergent lowered the surface tension of the water and quickly drowned any predators. The area around the traps was cleared of litter and was maintained in this way throughout sampling. The pitfall traps were left 'open' throughout sampling.

In 1992, pitfall traps were set on June 15th and next visited on June 22nd. They were then visited on June 24th, 25th and 29th and thereafter every working day until July 27th when the crops were harvested. The fields were sown with winter wheat in September of 1992. The traps were reset in the same positions on October 26th after the wheat plants had emerged. They were first sampled on November 2nd, again on November 16th and thereafter every week until December 21st. The traps were visited regularly during the winter months and the first carabid of 1993 was recovered on April 12th. The traps were next visited on April 26th and thereafter every week until June 1st. The traps were then visited on June 14th and then every week until the end of sampling on August 11th.

On each visit, the beetles were removed from the trap and transferred to a freezer box for transportation to the laboratory. The beetles were washed, sorted, identified following the nomenclature of Kloet and Hincks (1945) and frozen at -20°C. The catches were split into four seasons. A summer and autumn season in 1992 and a spring and summer season in 1993 (Table 4.2.1).

In 1993, a second set of twenty pitfall traps were set in Clayton and Square field to make population density estimates of carabid beetles in a mark-release-recapture (MRR) programme (Fig. 4.2.1). These density estimates were used in predation rate estimates in this chapter and are discussed thoroughly in chapter five.

4.2.3.2 Slug densities

Twenty ceramic tiles measuring 15x15 cm were placed at five metre intervals within the sampling area and used to assess the activity density of *D.reticulatum* over the

Fig. 4.2.1 The position of the pitfall traps in the three field sites. o = Pitfall traps used to collect beetles for ELISA analysis (see section 4.2) and in comparative studies of carabid abundance (see section 5.4). x = Pitfall traps used for population density estimates in a mark-release-recapture study (see section 5.3).

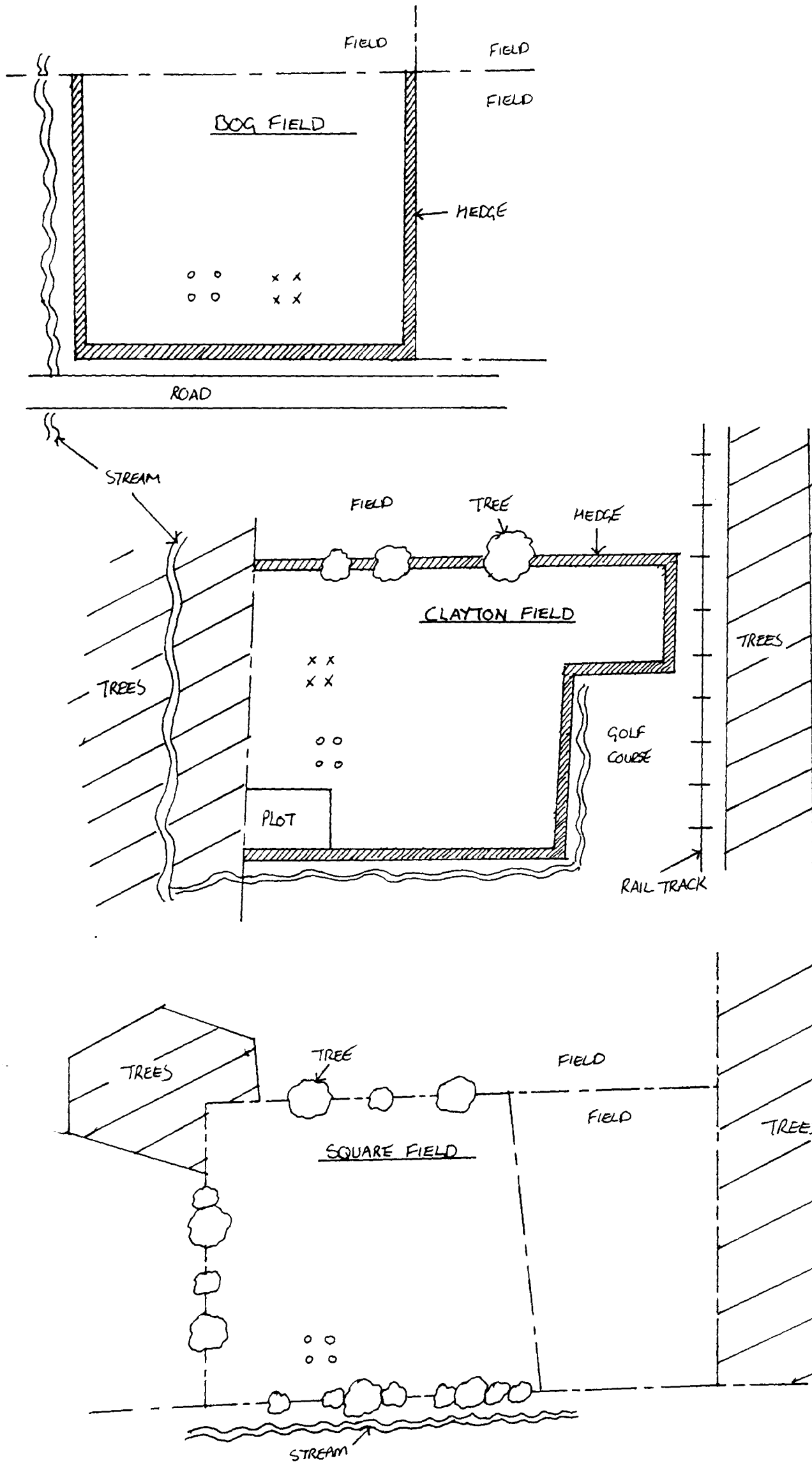


Table 4.2.1 Definition of four seasons used to make comparative studies of the carabid fauna.

Date	Season
June 16th - July 27th , 1992	Summer 1992
Nov. 10th - Dec. 21st, 1992	Autumn 1992
April 20th - June 01st, 1993	Spring 1993
June 15th - July 26th, 1993	Summer 1993

summer periods in 1992 and 1993. Other molluscs were noted by their presence. On June 30th and July 8th 1992 and July 7th 1993, slugs were collected from the study areas and taken back to the laboratory to be weighed. The following day, the slugs were returned to the study areas in the appropriate fields.

4.2.3.3 Field temperature

The ground air temperature was measured at each site throughout June and July of 1992 and 1993 using maximum-minimum thermometers. The thermometers were placed at ground level in the crop out of direct sunlight and the maximum-minimum temperatures were recorded at the end of each week.

4.2.3.4 Analysis of the predators by ELISA

Rabbit and rat antisera were used in an ELISA analysis (described in chapter three) to identify slug tissues (antigens) in the guts of field caught carabids. Carabids were prepared for the assay the day before the ELISA was run; they were removed from the deep freeze, thawed and their foreguts dissected into a well of a microtitration plate containing 125ml of PBS-Tween20. The predators' foreguts and contents were homogenised with a multiple homogeniser and allowed to extract overnight at 4°C.

The design of the microELISA plate is discussed in chapter three. The plates consisted of a seven fold serial dilution of a slug standard solution and a number of controls. Starved predators and predators fed on alternative prey have been used as controls (Fichter and Stephen, 1984; Lovei *et al.*, 1985). In this study, two sets of control were used. The first consisted of predators fed on earthworm tissues as they were found to contain the most cross-reactive non-molluscan antigens to the antisera. The second controls consisted of predators fed on *D.reticulatum* tissues (see chapter three).

The outside wells were used as heat buffers, therefore each microELISA plate consisted of the gut contents of 30 predators collected from the study sites and (where possible) six controls. Generally, only one carabid species was tested on

each microELISA plate. Beetles used to generate the control gut contents were of the same species as those being assessed.

For each plate, the predator species was noted, along with the date and time of collection, and site of origin. Results were recorded as absorbency units. Absorbency values of the test wells were identified as positive or negative using the determination limit (D) described in chapter three.

4.2.4 Results

A total of 2184 carabid specimens representing forty species were collected from the three study sites. Not all specimens could be assessed by the ELISA due to the time constraints of the project.

Three criteria were used to choose predator species for ELISA analysis. The first was predator size, larger species are more likely to eat slugs than smaller species (Tod, 1973). Therefore, the majority of the analysis in this project was aimed at larger predator species.

Secondly, predator abundance in the field was considered. Species which were rare or occurred infrequently were considered to be unlikely to exert an effect on slug populations. Therefore, only frequently occurring beetles were assessed. Exceptions to this were predator species which were very large and/or known slug specialist species, three of which occurred at low densities.

Finally, the results from the laboratory feeding trials in chapter two were considered. Some species, such as *L.pilicornis* did not feed on slugs in laboratory trials. *Loricera* are specialised collembolan feeders (Hengeveld, 1980a). The sensory equipment and predatory behaviour of *L.pilicornis* are adapted to overcome the high speed escape mechanism of their collembolan prey (Bauer, 1982). Therefore this beetle was unlikely to be feeding on slugs in the field. Information from laboratory feeding trials should be interpreted with caution: Luff (1974) found *P.madidus* did not feed on molluscs in the laboratory, but did in the field. Tod

(1973) collected 56 *L.pilicornis* specimens from the field and found none contained mollusc remains. Although *L.pilicornis* was abundant at Square and Bog field in 1992, only one specimen was assessed as it was not considered to be a slug predator.

Each species was considered carefully before being included or excluded in the ELISA assessment. Nineteen of the forty species, including 1312 specimens were assessed by the ELISA. Details of the number of beetles which had fed on slugs are presented in Appendix 4.1-4.3.

4.2.4.1 Carabid species containing slug tissues

The beetle species assessed by ELISA are shown in Table 4.2.2. Thirteen of the nineteen beetle species assessed were found to contain molluscan tissues. No snails were found in the study sites (Appendix 4.4), therefore the beetles were considered to have fed on slugs. The beetle species were compared with each other by calculating the proportion of each species containing slug tissues (Table 4.2.3).

N.brevicollis fed most frequently on slugs (Fig. 4.2.2). Thirty seven percent of *N.brevicollis* beetles contained slug tissues. Twenty five percent of *H.rufipes* beetles fed on slugs (Fig. 4.2.3) and twenty seven percent of *A.similata* beetles fed on slugs (Fig. 4.2.4).

Two *Pterostichus* species fed on slugs in similar proportions. Ten percent of all *P.madidus* beetles fed on slugs (Fig. 4.2.5) and nine percent of all *P.melanarius* beetles fed on slugs (Fig. 4.2.6).

Several large beetles and known slug predators were assessed. All four *C.violaceus* beetles fed on slugs. One of two *C.caraboides* beetles fed on slugs. A single *A.parallelepipedus* beetle was recovered which had eaten a slug meal and a single *C.problematicus* beetle was recovered which had not fed on slugs. Only one of thirteen *P.niger* beetles fed on slugs.

Table 4.2.2 The number of each beetle species tested for slug remains by ELISA in 1992 and 1993 from the three field sites. The number of each beetle species found to contain slug tissues is given in the 'positive' column. The data from all three sites are combined.

	1992		1993	
	tested	positive	tested	positive
P.melanarius	76	5	43	6
P.madidus	58	3	42	7
P.niger	10	1	3	0
P.nigrita			2	1
P.cristatus	6	0	1	0
A.parallelepipedus	1	1		
H.rufipes	243	71	86	12
H.latus	2	1	1	0
H.aeneus	15	0	8	0
A.similata	571	158		
A.aenea	5	0		
A.lunicollis	10	4		
A.aulica	5	1		
A.plebeja	1	0		
C.violaceus	4	4		
C.problematicus			1	0
C.caraboides	2	1		
L.pilicornis	1	0		
N.brevicollis	115	43		

Table 4.2.3 The number of each beetle species tested for slug remains by ELISA in 1992 and 1993 at each of the three field sites. The proportion of beetles of each species found to contain slug tissues is given in the 'P+' column. *C.problematicus* is abbreviated to *C.problemat.* and *A.parallelepipedus* is abbreviated to *A.parallelep.*

	Clayton		Square		Bog	
	test.	P+	test.	P+	test.	P+
1992						
P.melanarius	25	0	47	10	4	0
P.madidus	47	6	4	0	5	0
P.niger	5	0	5	20		
P.cristatus	3	0	3	0		
A.parallelep.	1	100				
H.rufipes	238	29	3	33	2	0
H.latus	2	50				
H.aeneus	15	0				
A.similata	465	30	80	18	26	0
A.aenea	4	0			1	0
A.aulica	5	20				
A.lunicollis	4	75	6	16		
A.plebeja	1	0				
C.violaceus	4	100				
C.caraboides	1	0			1	100
L.pilicornis					1	0
N.brevicollis	9	44	95	40	11	9
1993						
P.melanarius	14	14	29	13		
P.madidus	34	14	7	28	1	0
P.niger	1	0	2	0		
P.cristatus			1	0		
P.nigrita	2	50				
H.rufipes	85	14	1	0		
H.latus	1	0				
H.aeneus	8	0				
C.problemat.			1	0		

Fig. 4.2.2 The proportion of N.brevicollis beetles feeding on slugs
The hatched section indicates the overall proportion of beetles feeding on slugs. Data from both years and all three sites are presented

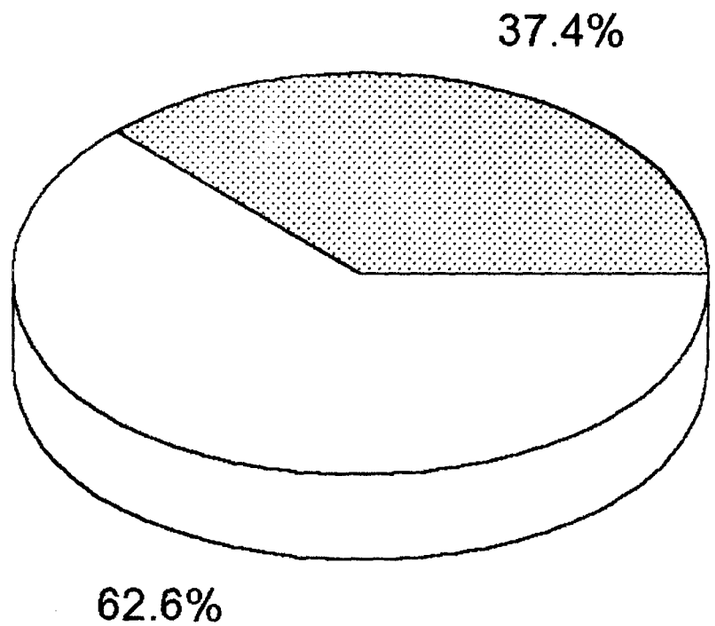


Fig. 4.2.3 The proportion of H.rufipes beetles feeding on slugs
The hatched section indicates the overall proportion of beetles feeding on slugs. Data from both years and all three sites are presented

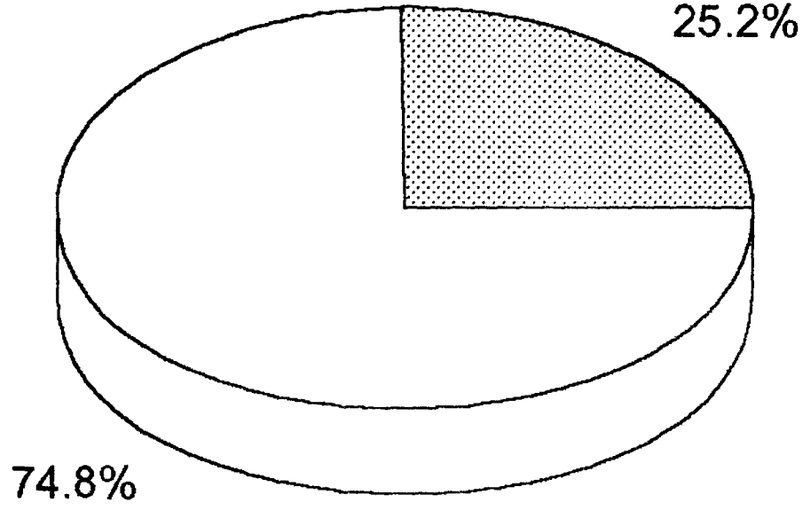


Fig. 4.2.4 The proportion of A.similata beetles feeding on slugs
The hatched section indicates the overall proportion of beetles feeding on slugs. Data from both years and all three sites are presented

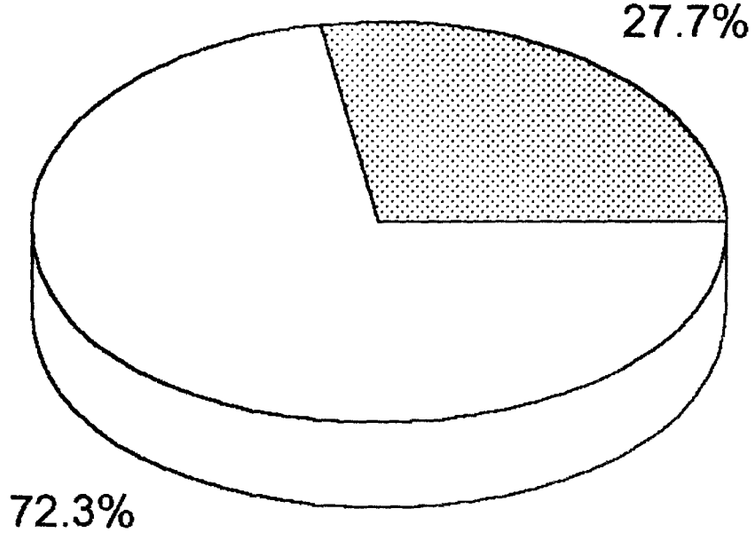


Fig. 4.2.5 The proportion of P.madidus beetles feeding on slugs
The hatched section indicates the overall proportion of beetles feeding on slugs. Data from both years and all three sites are presented

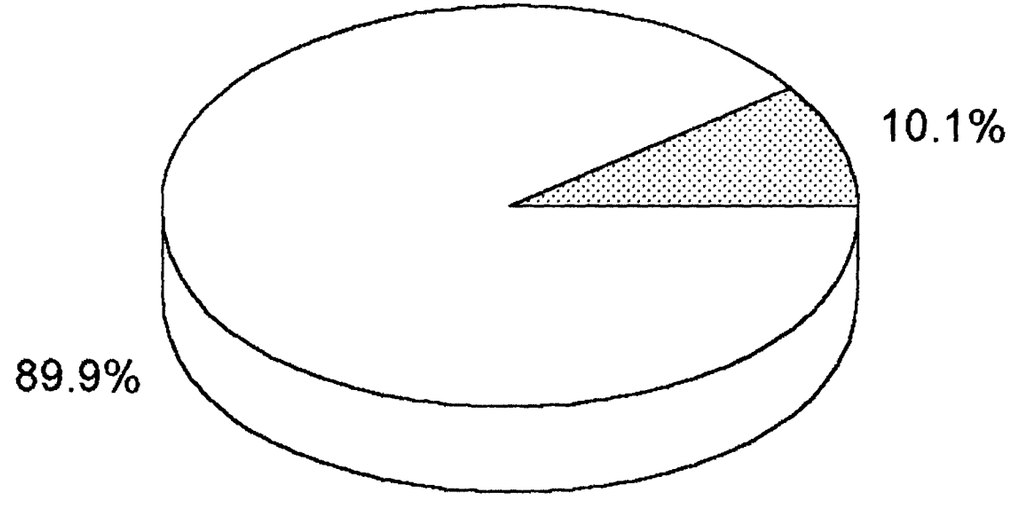
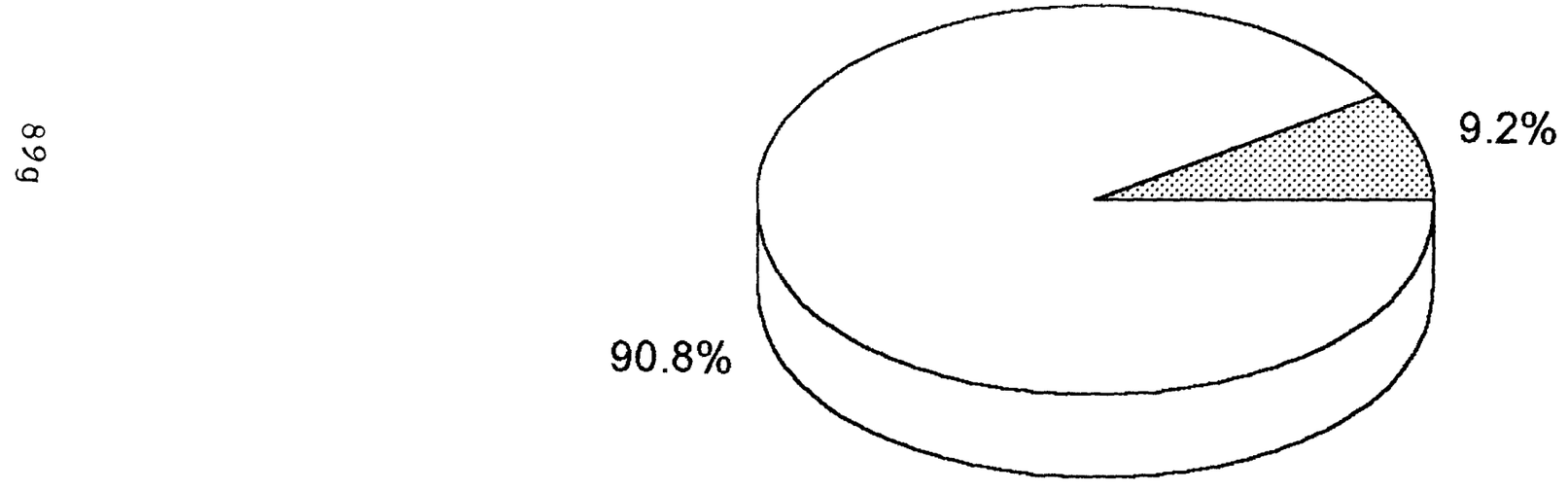


Fig. 4.2.6 The proportion of P.melanarius beetles feeding on slugs
The hatched section indicates the overall proportion of beetles feeding on slugs. Data from both years and all three sites are presented



In general, a proportion of most beetle species assessed contained slug tissues. However some species tested consistently negative and never fed on slugs. None of the twenty three *H.aeneus* beetles fed on slugs and none of the *Pterostichus cristatus* (Dufour) and *A.aenea* beetles fed on slugs. However the sample size for these latter two species were small.

4.2.4.2 Overall differences in slug feeding between the sites

The proportion of predators containing slug remains was used to make comparisons of predation at the three sites (e.g. Cherrill and Begon, 1989). Similar proportions of beetles fed on slugs at Clayton and Square field, but lower proportions of beetles fed on slugs at Bog field (Fig. 4.2.7). Contingency tables of the actual numbers of beetles feeding on slugs were used to determine if these differences were significant. Fewer beetles fed on slugs at Bog field than at Clayton field ($X^2=539.3$, d.f. = 1, $P<0.001$) and Square field ($X^2=20.6$, d.f. = 1, $P<0.001$). No other significant differences were found.

The proportion of each common and abundant beetle species containing slug remains was calculated at each site and used to compare slug feeding at each site. Similar proportions of *N.brevicollis* and *H.rufipes* beetles fed on slugs at Clayton and Square field (Figs. 4.2.8 and 4.2.9). The largest proportions of *P.madidus* and *P.melanarius* beetles containing slug tissues were from Square field (Figs. 4.2.10 and 4.2.11). The largest proportion of *A.similata* beetles containing slug tissues were from Clayton field (Fig. 4.2.12). The actual numbers of beetles feeding on slugs were used to determine if these differences were significant. Significantly more *A.similata* beetles fed on slugs at Clayton field than at Square field ($X^2=4.7$, d.f. = 1, $P<0.05$) and Bog field ($X^2=11.2$, d.f. = 1, $P<0.001$). Significantly more *A.similata* beetles fed on slugs at Square field than at Bog field ($X^2=5.6$, d.f. = 1, $P<0.05$).

P.madidus, *P.melanarius*, *H.rufipes* and *A.similata* beetles did not feed on slugs at Bog field. Only a single *N.brevicollis* and *C.caraboides* beetle fed on slugs at Bog field. Significantly more *N.brevicollis* beetles fed on slugs at Square field than at Bog field ($X^2=4.05$, d.f. = 1, $P<0.05$).

Fig. 4.2.7 The proportions of beetles feeding on slugs at each site
The hatched section indicates the proportion of beetles feeding on slugs at each site. Data from both years are presented

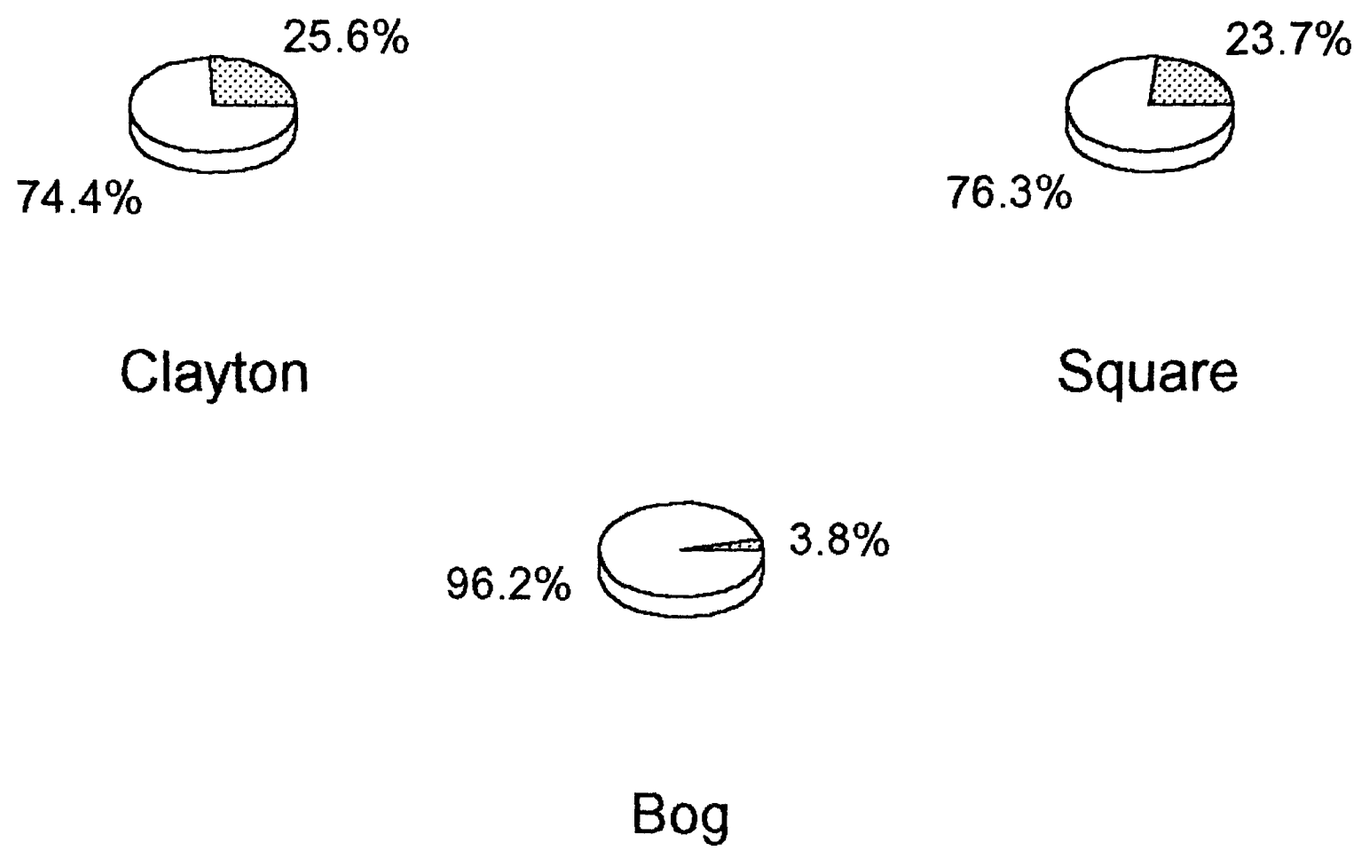


Fig. 4.2.8 The proportion of N.brevicollis feeding on slugs at each site
The hatched section indicates the proportion of beetles feeding on slugs at each site. Data from both years are presented

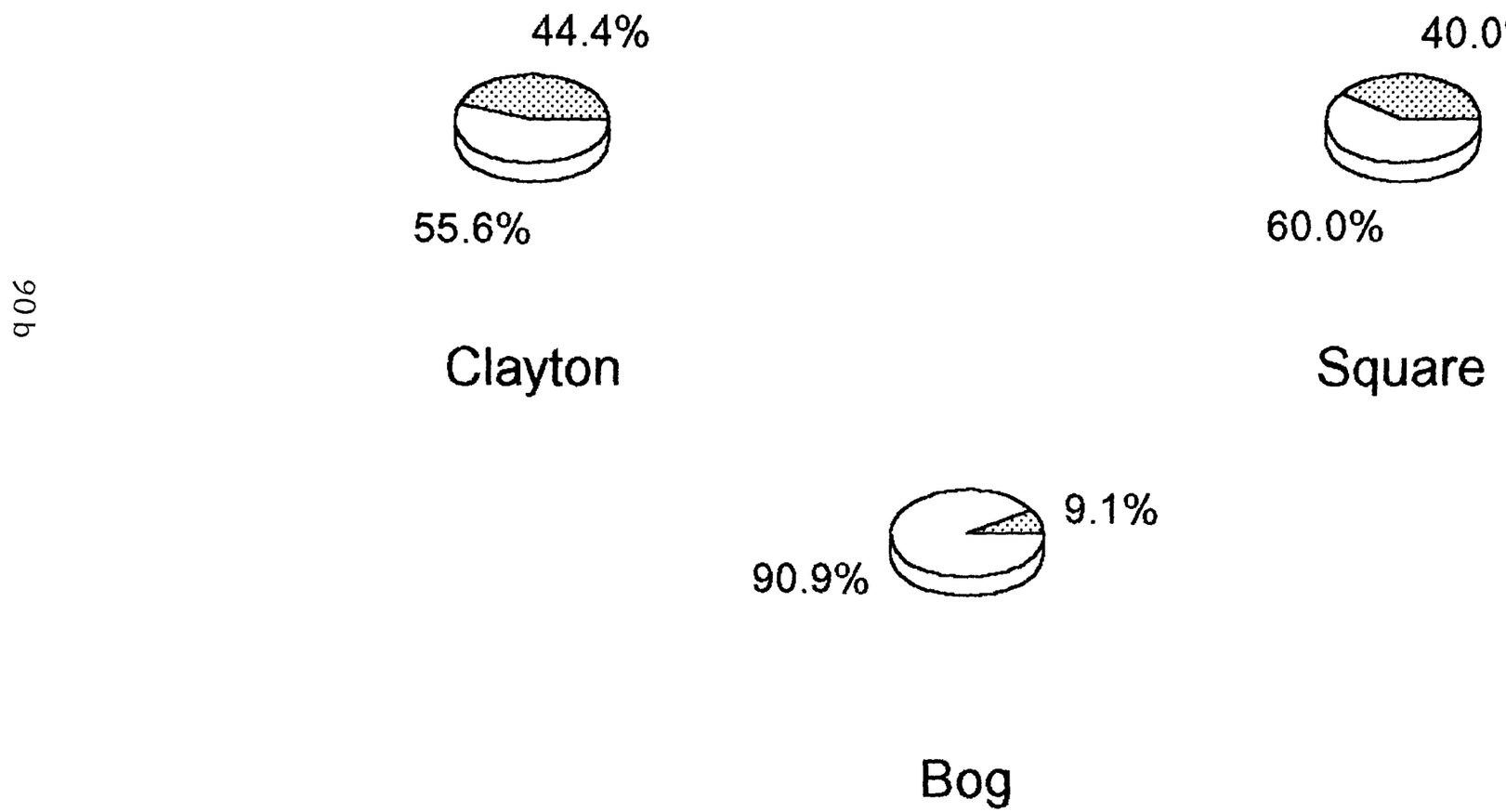


Fig. 4.2.9 The proportion of H.rufipes beetles feeding on slugs at each site
The hatched section indicates the proportion of beetles feeding on slugs at each site. Data from both years are presented

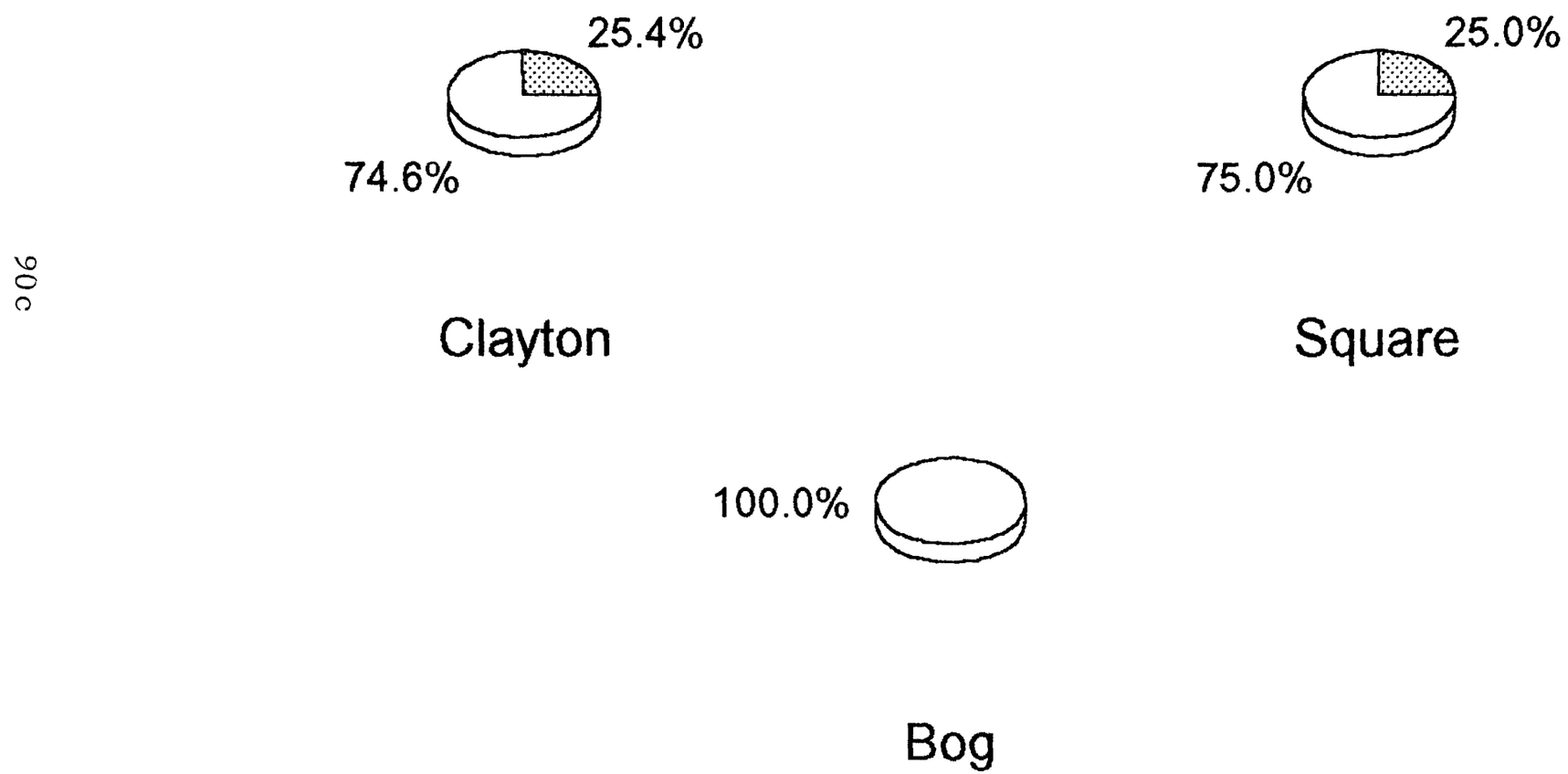


Fig. 4.2.10 The proportion of P.madidus beetles feeding on slugs at each site
The hatched section indicates the proportion of beetles feeding on slugs at each site. Data from both years are presented

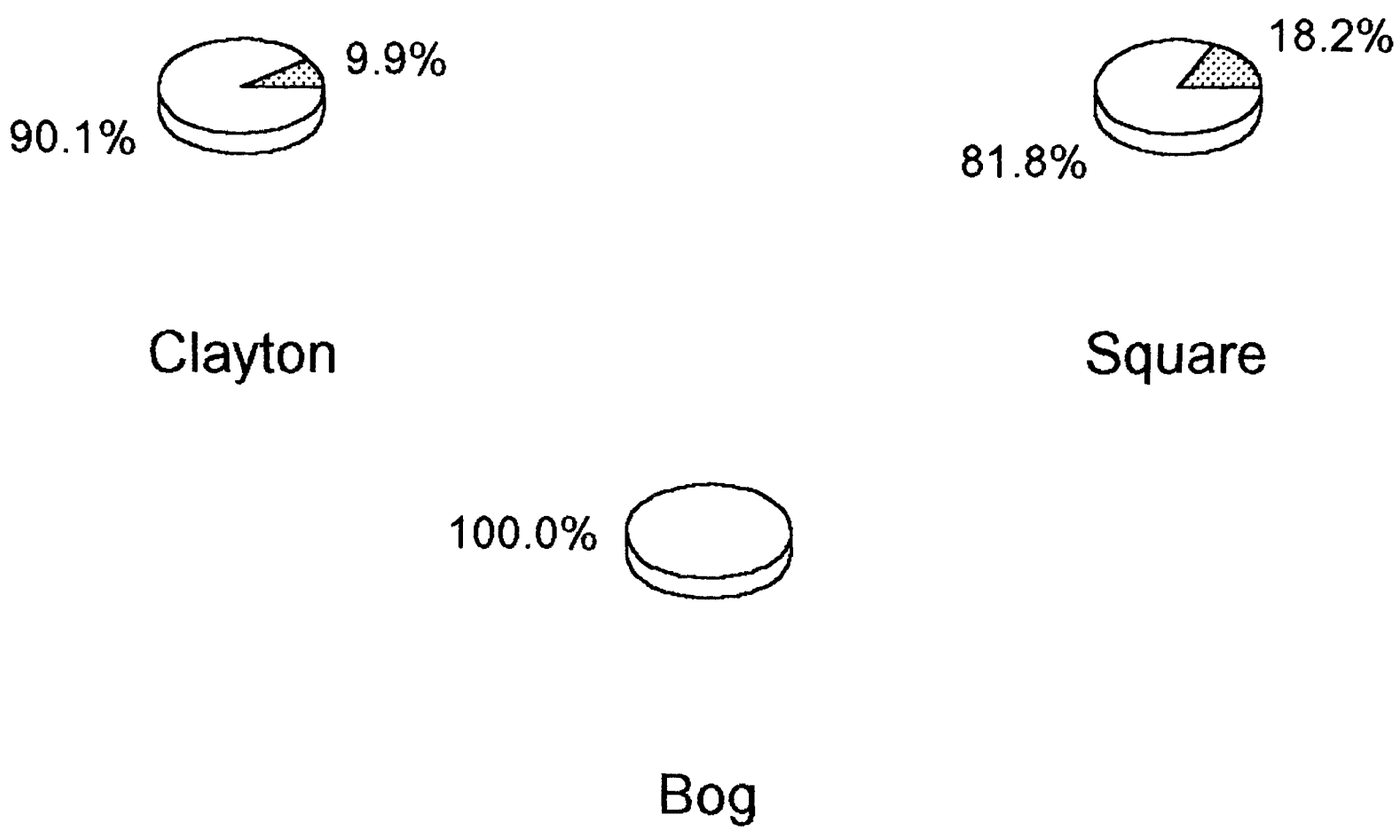
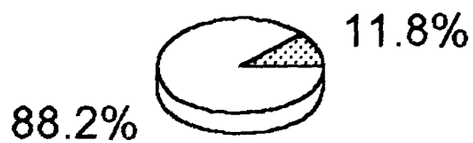
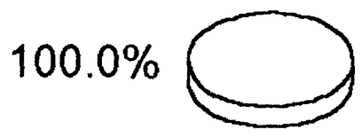


Fig. 4.2.11 The proportion of P.melanarius feeding on slugs at each site
The hatched section indicates the proportion of beetles feeding on slugs at each site. Data from both years are presented

Clayton

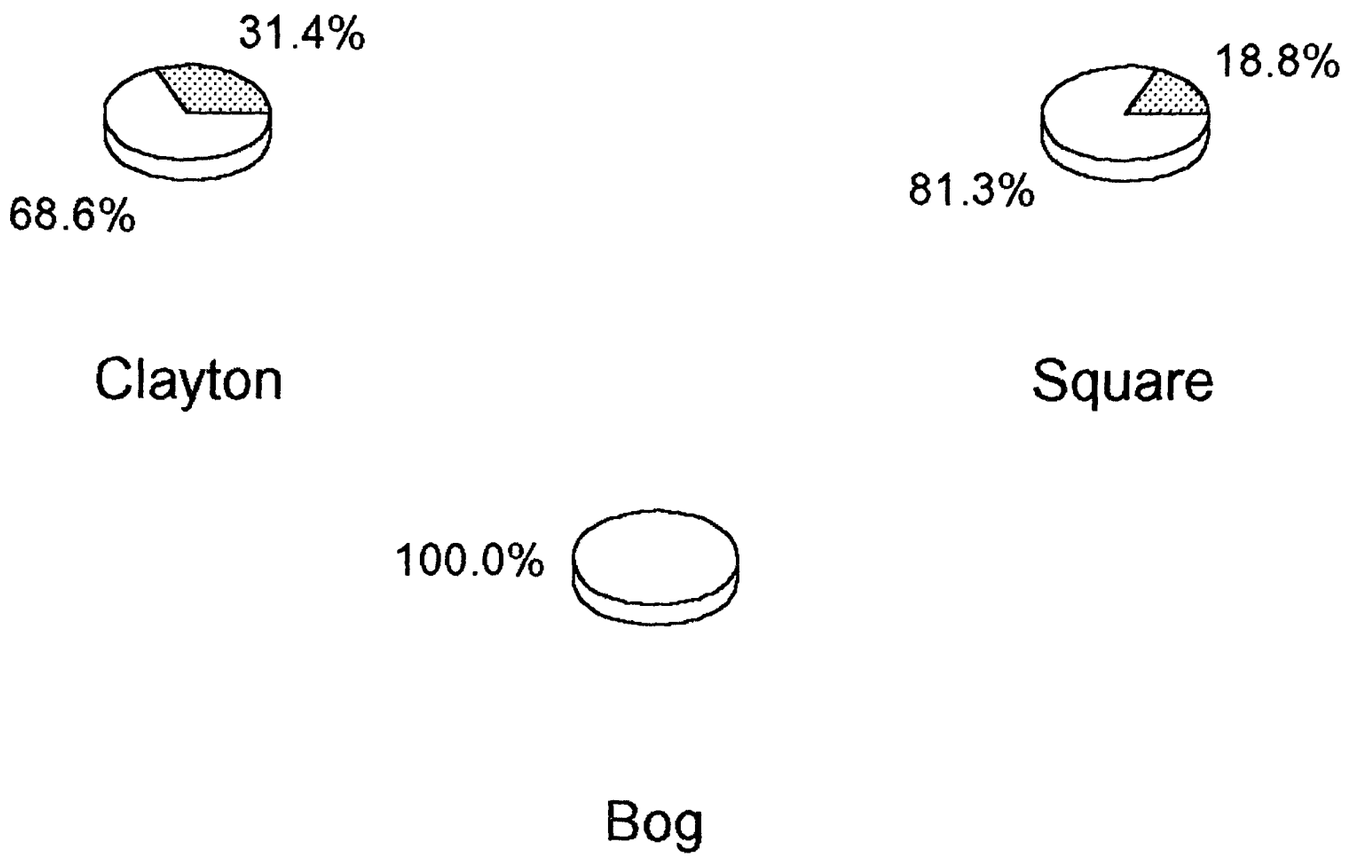


Square



Bog

Fig. 4.2.12 The proportion of A.similata beetles feeding on slugs at each site
The hatched section indicates the proportion of beetles feeding on slugs at each site. Data from both years are presented



4.2.4.3 Changes in slug feeding at the sites in 1992 and 1993

The relative numbers of beetles feeding on slugs between the years were investigated. Overall, fewer beetles fed on slugs in 1993 compared to 1992 (26 percent in 1992 and 14 percent in 1993). This decline was significant at Clayton field ($X^2 = 12.7$, d.f. = 1, $P < 0.001$) (Fig. 4.2.13), but not at Square field (Fig. 4.2.14). Similar tests were not made at Bog field as only one carabid was assessed in 1993.

The proportion of each beetle species feeding on slugs was calculated for each site in 1992 and 1993 (Table 4.2.3). At Clayton field, the decline in slug feeding in 1993 was due largely to fewer *H.rufipes* beetles eating slugs. The number of *H.rufipes* beetles feeding on slugs decreased in 1993 ($X^2 = 7.8$, d.f. = 1, $P < 0.01$). However, more *P.melanarius* and *P.madidus* beetles fed on slugs in 1993 (Table 4.2.3).

The carabid catch at Bog field was particularly impoverished in 1993 and only one large carabid (*P.madidus*) was caught throughout the sampling period. This was the only beetle assessed in 1993 from this site and it did not contain slug tissues.

Summer periods

The proportion of beetles eating slugs in the summer periods of 1992 and 1993 were calculated. The decline in slug feeding were similar to the yearly data (26 percent in 1992 to 14.5 percent in 1993). This decline in slug feeding was significant at Clayton field ($X^2 = 5.6$, d.f. = 1, $P < 0.05$) but not at Square field.

However, some species fed more frequently on slugs in the summer of 1993. Contingency tables were used to compare the proportion of *P.madidus* and *P.melanarius* beetles feeding on slugs in both summers. Slug feeding significantly increased in *P.madidus* in the summer of 1993 ($X^2 = 4.1$, d.f. = 1, $P < 0.05$). Although more *P.melanarius* fed on slugs in the summer of 1993, the increase was not significant. The numbers of both *Pterostichus* species found with slug tissues in their gut were small (Table 4.2.2).

The sampling regime used in 1992 increased the likelihood of identifying beetles

Fig. 4.2.13 The proportion of beetles feeding on slugs at Clayton field
The hatched section indicates the proportion of all beetles feeding on slugs at Clayton field in 1992 and 1993

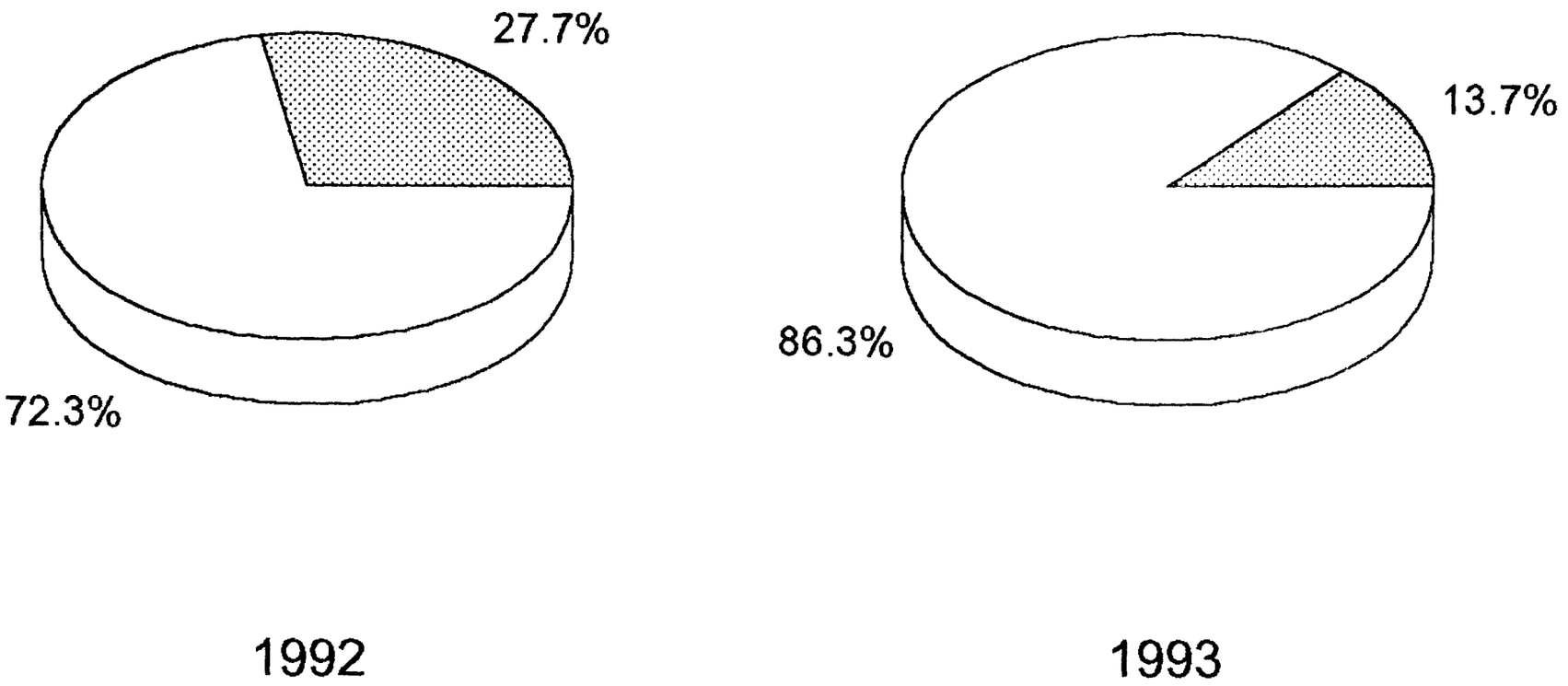
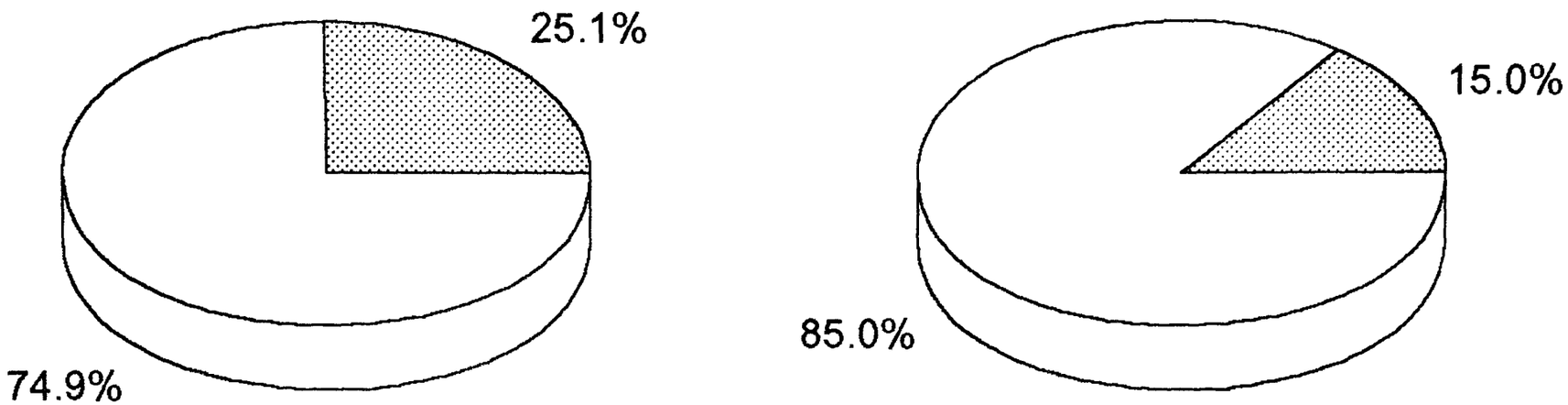


Fig. 4.2.14 The proportion of beetles feeding on slugs at Square field
The hatched section indicates the proportion of all beetles feeding on slugs at Square field in 1992 and 1993



91 b

which had fed on slugs. Beetles were collected more frequently in 1992 and slug antigens had less time in which to decay before the predators were frozen. The sampling regime used in 1993 may have resulted in underestimating the number of beetles of some species which fed on slugs. Despite the sampling regime, more *P.melanarius* and *P.madidus* beetles fed on slugs in 1993 compared to 1992. This increase may have been underestimated due to the sampling regime adopted in 1993.

4.2.4.4 Effect of slug activity density on beetle feeding

Site differences

The effect of slug activity density on the proportion of beetles containing slug tissues was investigated. The mean slug activity density at Bog field was similar to those at Clayton field in 1992 (Table 4.2.4). Despite the similar slug activity density, the proportion of beetles feeding on slugs at Bog field was lower than at Clayton field (section 4.2.4.2). *P.madidus* and *A.similata* fed on slugs at Clayton field but not at Bog field and *N.brevicollis* beetles ate slugs in lower proportions at Bog field compared to Clayton field. At Bog field, insufficient *P.madidus* were recovered to detect any real differences in slug feeding between the sites. However, *A.similata* was numerous at all sites and the differences in slug feeding at the three sites are probably real differences.

Paired sample t-tests were used to compare the weekly summer activity densities of *D.reticulatum* between the three sites (see Appendix 4.5). In 1992, slug activity densities at Square field were significantly higher than those at Clayton ($t=2.997$, $d.f.=5$, $P<0.05$) and Bog field ($t=3.405$, $d.f.=5$, $P<0.05$). Despite the higher slug activity densities at Square field, the proportion of beetles feeding on slugs was similar at Clayton and Square field.

Availability of slugs in the summer periods

Each site was investigated separately by regression analysis to determine the relationship between the weekly slug density and the proportion of all beetles

Table 4.2.4 The mean density of *D.reticulatum* slugs at each site in 1992 and 1993. The mean is calculated from the weekly densities of slugs found under tile traps over the two summer seasons at each site.

	1992			1993		
	Mean	n	S.E.	Mean	n	S.E.
Clayton field	0.31	6	0.08	0.84	5	0.54
Square field	1.38	6	0.38	0.22	5	0.09
Bog field	0.30	6	0.12	1.00	5	0.31

feeding on slugs. The analysis were made in both summer periods, except at Bog field in 1993. Therefore five analysis were made. The r^2 value was used to measure the significance of the analysis. None of the correlations were significant at the five percent level (1992: Clayton $r^2=0.66$, d.f.=4; Square $r^2=0.12$, d.f.=4; Bog $r^2=0.02$, d.f.=4: 1993: Clayton $r^2=0.40$, d.f.=3; Square $r^2=0.15$, d.f.=3).

Each beetle species was analyzed separately by regression analysis at each site and year. The only positive correlation between slug activity density and slug feeding was found in *N.brevicollis* at Square field in 1992 ($r^2=0.75$, d.f.=4, $P<0.1$)(Fig. 4.2.15). In the summer period of 1992, two peaks in slug activity densities occurred at Square field on July 6th and July 27th. *P.melanarius* was caught throughout the summer period at this site, but only fed on slugs on the two occasions when slug activity density peaked (Fig. 4.2.16).

Yearly differences

Two tailed t-tests were used to compare the weekly summer activity density (number of slugs per trap) of *D.reticulatum* between 1992 and 1993 at each site. Slug activity density increased significantly at Square field ($t=2.704$, d.f.=9, $P<0.05$), but decreased at Bog field ($t=2.175$, d.f.=9, $P<0.1$) in 1993. More slugs were trapped at Clayton field in 1993 compared to 1992, but this was not significant.

4.2.4.5 Effect of slug weight on beetle feeding

The weight of individual slugs was considered as a factor affecting their consumption by beetles in the summer period of 1992.

The weights of *D.reticulatum* slugs trapped between June 30th and July 8th 1992, were used to compare the population structure between the sites. Slugs were of a similar size at Square and Clayton field (Square field, mean = 0.468g, s.d = 0.292; Clayton field, mean = 0.465, s.d = 0.241), but larger at Bog field (mean = 0.646g, s.d = 0.395). Most of the very large slugs (>1.0g) were found at Bog field (see Appendix 4.6), this coincided with the lowest proportion of beetles feeding on slugs.

Fig. 4.2.15 Slug density and slug feeding by N.brevicollis

The relationship between slug activity density and the proportion of N.brevicollis beetles feeding on slugs at Square field in 1992

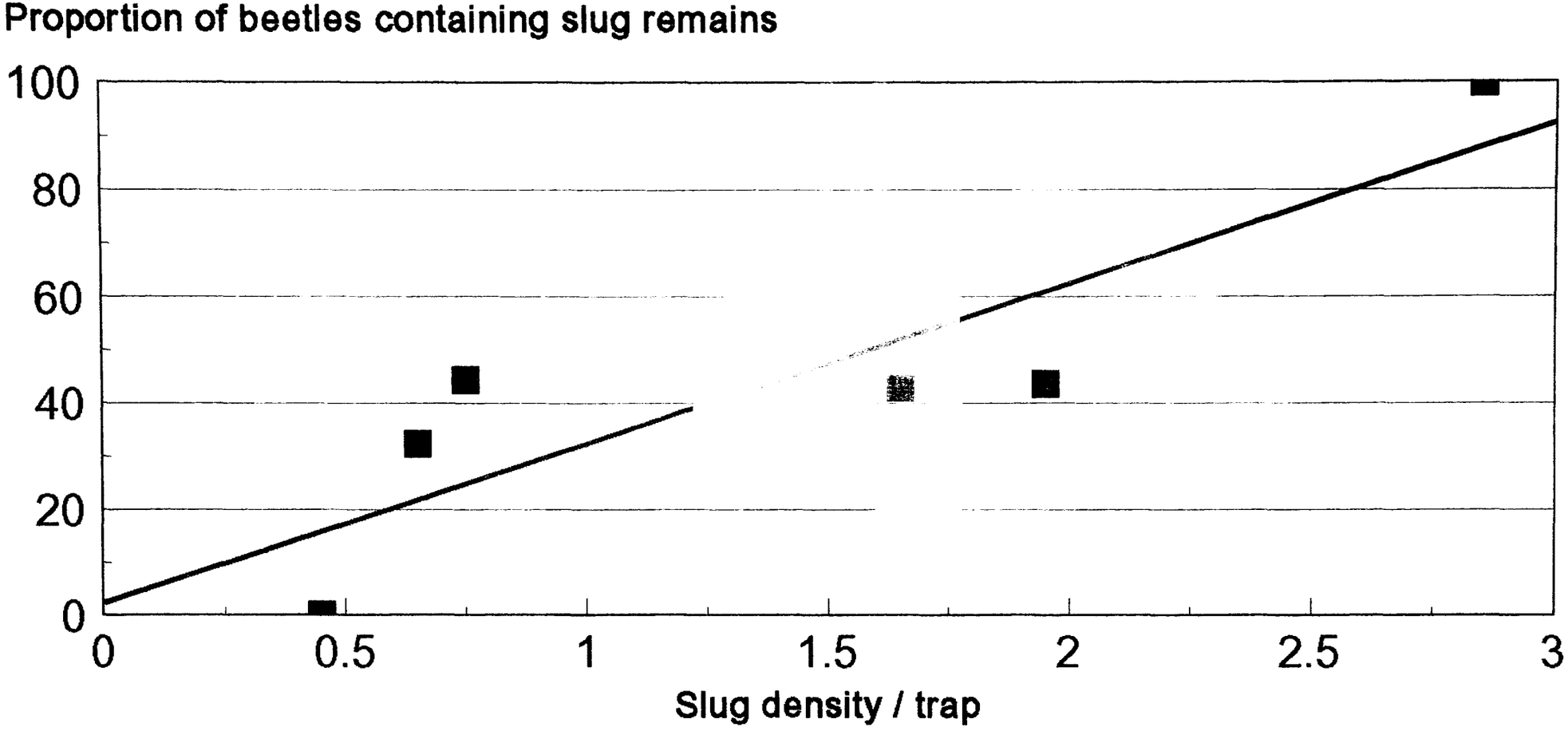
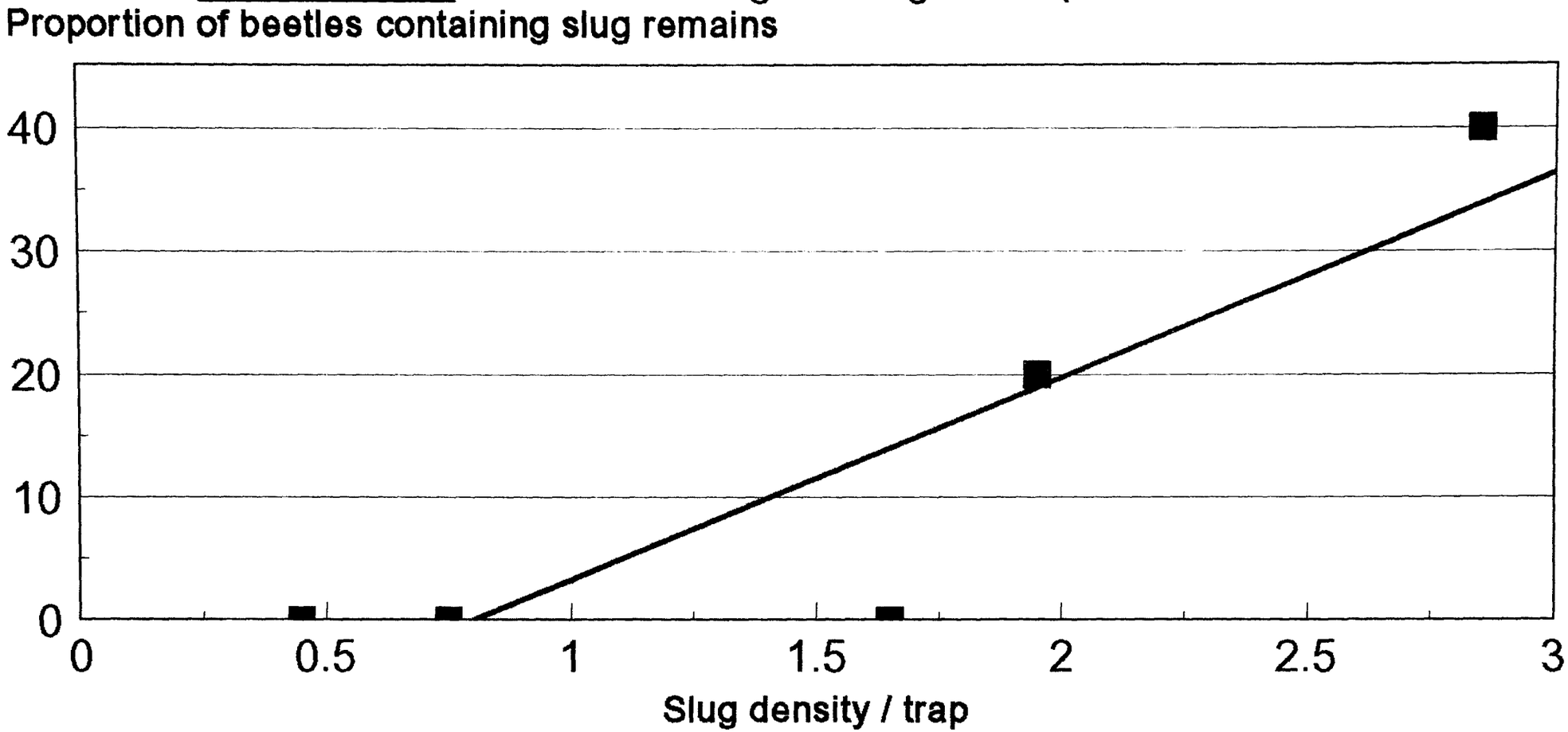


Fig. 4.2.16 Slug density and slug feeding by P.melanarius
The relationship between slug activity density and the proportion of P.melanarius beetles feeding on slugs at Square field in 1992



Slugs were significantly heavier in weight at Bog field compared with slugs at Square field ($t=2.036$, d.f.$=70$, $P<0.05$). No other significant differences were found in slug weight between the sites.

4.2.5 Discussion

The thirteen carabid species which fed on slugs in this study are compared with four other studies in Table 4.2.5. The proportion of beetles feeding on molluscs differed between the four studies. The relatively low proportion of beetles containing mollusc remains in Davies (1953) investigation may be a reflection of the technique used to identify mollusc remains. Microscopic gut analysis can only identify molluscs when the radula is ingested. However, similar numbers of some species (*P.madidus*) have been found with slug remains in their guts, using dissection and serological techniques (Tod, 1973; Luff, 1974).

Fourteen of twenty six species fed on slugs in Tod's (1973) study and nine of these species were tested in this study. A higher proportion of each species fed on slugs in Tod's (1973) study compared with this study, with the exception of *C.violaceus* and *N.brevicollis* beetles.

The relative proportion of beetles feeding on slugs in the four studies will reflect the ecosystem from which the beetles were trapped. In the other studies, beetles were collected from a walled garden (Luff, 1974), a hillside, spruce wood, hardwood and grass pasture on the Pentland hills (Tod, 1973), a conifer and deciduous wood (Symondson, 1989) and unspecified inland sites (Davies, 1953). In this study, the beetles were collected from arable sites. Differences in the availability of slugs and alternative prey in these ecosystems may account for the variation in slug feeding between the studies.

4.2.5.1 Carabid species containing slug tissues

As many beetle species ate dead macerated *D.reticulatum* tissue in laboratory studies (chapter three), it is likely that all of the carabid predators investigated in this study scavenged dead slugs to some degree. However, the extent of scavenging

Table 4.2.5 Comparison of the proportion of each beetle species containing mollusc tissues in this study with four other studies. * indicates ten or less beetles were assessed. Symondson (1992) is abbreviated to Symond.

	This study	Tod (1973)	Davies (1953)	Symnd. (1992)	Luff (1974)
P.melanarius	9	35	0*		
P.madidus	10	20	5	44	23
P.niger	7	43	0*		
P.nigrita	50*	0*	0*		
P.cristatus	0*				
A.parallelepipedus	100*		16	92	
H.rufipes	25		0		
H.latus	33*	0*	0*		
H.aeneus	0		0*		
A.similata	27		0*		
A.aenea	0*		0*		
A.lunicollis	40*				
A.aulica	20*		0*		
A.plebeja	0*		0*		
C.violaceus	100*	100*	0*		
C.problematicus	0*				
C.caraboides	50*	73	0*		
L.pilicornis	0*	0	0*		
N.brevicollis	37	2	0		

may vary from species to species.

Most large and medium sized carabids kill slugs (chapter two). *P.melanarius* eats slugs in the field (Cornic, 1973; Tod, 1973) and was considered to be killing slugs in this study. *P.madidus* kills slugs (chapter two) and eats mollusc material in the field (Davies, 1953; Luff, 1974; Symondson, 1992) and was considered to be killing slugs in this study. *A.parallelepipedus* kills large slugs (chapter two) and has been used to control *D.reticulatum* in experimental plots (Symondson, 1992). *A.parallelepipedus* feeds on slugs in other studies (Davies, 1953; Symondson, 1992) and was considered to be predating slugs in this study.

Slugs are part of the diet of *Carabus* and *Cychrus* species (Gruntal and Sergeyeva, 1989) and some *Cychrus* species are mollusc specialist (e.g. Larochelle, 1972; Loreau, 1984; Evans and Forsythe, 1985). A high proportion of these two genera fed on slugs in this study and in Tod's (1973) study. In this study, all *C.violaceus* beetles and one of two *C.caraboides* beetles assessed had fed on slugs. The *Carabus* and *Cychrus* beetles in this project were considered to have predated their slug meals.

H.rufipes has often been regarded as a seed feeder. Reports of it feeding on strawberry seeds (see Briggs, 1965) date back to the last century (McLachlan, 1897). Davies (1953) found no animal remains in any Harpalini specimens, including *H.rufipes*. He concluded that although the liquid food found in two of the specimens were of animal origin, this was a predominantly vegetarian group. The liquid food found in Davies' study may represent the remains of snails (molluscs) (Hengeveld, 1980a). A high proportion of *H.rufipes* beetles can contain this liquid food (Sunderland, 1975), indicating that it may feed on slugs extensively. *H.rufipes* is certainly known to feed on slugs in the field (Cornic, 1973) and this species killed slugs in laboratory investigations (chapter two). Therefore, *H.rufipes* was considered to be a slug predator.

Other beetle species were more limited in their ability to kill slug prey by virtue of

their size and the size of individual slugs. *N.brevicollis* eats small prey including Diptera, Collembola, mites, spiders and earthworms under 4mm in length (Penney, 1966). This beetle's size and feeding preferences indicate that it is unable or unlikely to kill large slugs. *N.brevicollis* has not fed on slugs to a great extent in other studies (Tod, 1973). However, *N.brevicollis* killed and ate hatchling slugs in laboratory studies (chapter two) and 37 percent of beetles fed on slugs in the field. Therefore, *N.brevicollis* specimens containing slug tissues in this study had either predated small slugs or scavenged dead slugs.

The Amarini are generally considered to be phytophagous (Aubrook, 1949; Davies, 1953). However, *A.convexior* will kill other Coleoptera (Allen, 1953) and *A.plebeja* will kill aphids (Sunderland and Vickerman, 1980). A number of *Amara* species contained slug remains in this project, including a large proportion of *A.similata* beetles. Three *Amara* species killed hatchling slugs in laboratory studies (chapter two) including *A.similata*. However, the relatively small size of *A.similata* indicates it would be unable to kill all but the smallest slugs. Therefore *A.similata* specimens containing slug remains in this project had probably either predated small slugs or scavenged dead slugs.

D.reticulatum breeds throughout the year. Slugs which hatch in the spring die in the autumn and winter and slugs which hatch in the autumn die in the spring and summer the following year (Hunter, 1968a). Therefore, dead slugs are available for carabids to scavenge throughout the year. Additionally, partially consumed dead slugs are exposed to scavenging by species which can not kill them and beetles which have no impact on slug mortality may have given positive signals in the ELISA analysis (see Frank, 1971). Few large slug killing predators were found at Bog field, consequently fewer dead slugs may have been available from which *A.similata* could scavenge. This may explain why no *A.similata* beetles fed on slugs at Bog field.

Other carabid species were found in the field sites which were not assessed by ELISA. *C.melanocephalus* was found at all three sites in both years of this project

but were not assessed by ELISA. This species also feeds on molluscs in the field (Tod, 1973). However, laboratory studies (chapter two) indicated that *C.melanocephalus* did not kill hatchling slugs. *A.dorsale* was one of the most abundant species at Clayton field in 1992 and *Agonum* species do feed on molluscs (Dawson, 1965). Therefore, this is another potentially useful slug predator.

Other mollusc species occurred at very low densities in the field sites (Appendix 4.4). Therefore, those beetles which contained mollusc material were considered to have fed on *D.reticulatum*.

4.2.5.2 Site differences in beetles feeding on slugs

Differences in the carabid fauna of the three fields partly explains the differences in the proportion of beetles feeding on slugs. *H.rufipes* was much more abundant at Clayton than Bog field, and this species frequently contained slug tissues. Similarly, *N.brevicollis* beetles were much more abundant at Square than Bog field and again, this species frequently contained slug tissues.

Some species occurred at each site, but ate slugs at different proportions at each site. *A.similata* fed on slugs at Square and Clayton field, but not at Bog field, despite similar slug densities at Clayton and Bog field. Similarly, *P.madidus* and *P.melanarius* did not feed on slugs at Bog field but did at the other two sites. The availability of alternative prey influences the impact that *P.melanarius* exerts on other pests, such as codling moth larvae (Hagley *et al.*, 1982) and may have contributed to the differences in slug consumption at the three sites.

4.2.5.3 Proportion of beetles feeding on slugs in 1992 and 1993

The yearly change in the overall proportion of beetles containing slug tissues reflects the abundance of those species which feed on slugs. The majority of beetles containing slug tissues in Clayton and Square field in 1992 were *A.similata* beetles, but this species was found only at extremely low densities in 1993.

Fewer carabids were caught in pitfall traps in 1993 compared to 1992 (chapter five).

The reduced abundance of carabids may reflect the reduced abundance of other Coleoptera at the field sites in 1993. *P.madidus* and *P.melanarius* beetles both eat other Coleoptera (Sunderland, 1975). The increase in slug consumption by these two species in 1993 may reflect the reduced availability of Coleoptera prey. *H.rufipes* also feeds on other Coleoptera (Sunderland, 1975), but it can change its dietary habit throughout a season. It is carnivorous in summer and carnivorous and phytophagous in the autumn (Cornic, 1973). As this species exploits different food sources, it may have switched feeding from slugs to an alternative food source in 1993.

Summer periods

Fewer beetles fed on slugs in the summer period of 1993 compared with the same period in 1992. The increase in slug feeding by *P.melanarius* and *P.madidus* and decreased slug feeding by *H.rufipes* in the summer of 1993, may be a response to changes in the availability of alternative prey (discussed above).

4.2.5.4 Effect of slug activity density on beetle feeding

Site differences

There was no evidence to suggest that differences in slug activity density affected the proportion of beetles feeding on slugs in 1992. Slug activity density was significantly higher at Square field than at Clayton field in 1992, but the overall proportion of beetles feeding on slugs at the two sites was similar. Slug activity density at Clayton and Bog field were similar but the proportion of *P.melanarius*, *P.madidus*, *N.brevicollis* and *A.similata* feeding on slugs was always lowest at Bog field. This indicates that slug activity density at the field sites is not affecting slug feeding by the predators.

Yearly differences / availability of slugs during the summer

Individual species expressed a feeding response to slug activity density over the course of a season. More *N.brevicollis* beetles fed on slugs at Clayton and Square field as slug density increased. *N.brevicollis* also feeds more on other pests such as

aphids when aphid densities are high (Sunderland and Vickerman, 1980).

P.melanarius fed on slugs more as the slugs activity density increased (although this was not significant). At Square field in 1992, slugs were only consumed on two occasions when the two highest slug densities were recorded. *P.melanarius* feeds on other prey which are most abundant at a particular time (Pollet and Desender, 1985). Cornic (1973) found *P.melanarius* ate slugs on rainy days (presumably because slugs were moving around on the ground surface). This indicates that *P.melanarius* changes its diet according to the availability of particular prey and may feed on slugs if they are abundant.

Slugs are difficult animals for carabids to handle and other prey occur in arable land which *P.melanarius* may find easier to kill. *P.melanarius* will feed on the aphid *Rhopalosiphum padi* (Linnaeus) when aphid populations are both high and low (Chiverton, 1984). If easier to handle, alternative prey are available, *P.melanarius* may chose to ignore slugs altogether and may only feed on slugs when slug density are high or other alternative prey are unavailable.

4.2.5.5 Effect of slug weight on beetle feeding

The size of a prey species can affect its vulnerability to predation by carabids (Penney, 1966; Ernsting and van der Werf, 1988). *H.rufipes* can kill early instars of large Lepidoptera prey such as *P.rapae* and cut worm larvae. However, as these two Lepidoptera grow, predation by *H.rufipes* decreases (Dempster, 1967; Frank, 1971). Similarly, slugs may grow to sizes which make them impossible for many carabid species to attack and kill. This is probably more critical for small carabids, as larger beetles are more capable of killing slugs than smaller ones (Tod, 1973).

The acceptance of prey by invertebrate predators has been defined by prey weight (Nentwig and Wissel, 1986), as prey weight increases, prey acceptance decreases. In this study, slug size is defined by slug weight. Two features of the prey and predator data at Bog field in 1992 were the heavy weight of individual slugs and the small proportion of beetles feeding on slugs. The low numbers of beetles feeding

on slugs may be due to the large size of the slugs at this site which protected them from predation.

P.melanarius and *P.madidus* beetles never ate slugs at Bog field. However, only nine *Pterostichus* specimens were assessed at this site and care should be taken when interpreting such sparse data. More conclusively, large proportions of *A.similata* fed on slugs at Clayton and Square field but not at Bog field. If *A.similata* can only kill small slugs, then a low proportion of beetles feeding on slugs could indicate that few small slugs were available at Bog field which this predator could exploit. Slug age-structures are discussed more fully in section 4.2.5.7.

4.2.5.6 Effect of sampling regime on antigen detection

In 1992, pitfall traps were visited most working days and any trapped beetles spent a maximum period of 72 hours in the pitfall traps before they were collected. In 1993, pitfall traps were visited once a week and any trapped beetles spent a maximum period of seven days in the pitfall traps before they were collected. The longer time spent by predators in the pitfall traps in 1993 meant that slug antigens ingested by the predators had longer time in which to decay. The sampling regime adopted in 1993 was almost certainly responsible for the reduction in the number of beetles found with slug meals (see section 4.2.2.1).

Differences in the proportion of predators feeding on prey may reflect differences in feeding or digestion rates (Cherrill and Begon, 1989). The digestion rate of a predator species strongly influences the proportion of that species containing detectable prey remains at any given time (Sunderland *et al.*, 1987).

Maximum field temperatures often rose above 20°C (mean=20.6, s.d=3.1, range 13-27°C)(see Appendix 4.7). As *D.reticulatum* antigens only remain detectable for one day in *H.rufipes* and *P.madidus* at 20°C (see chapter three), it is possible that the proportions of *H.rufipes* and *Pterostichus* beetles feeding on slugs were underestimated.

4.2.5.7 Slug population structure

The mean weight of surface dwelling *D.reticulatum* slugs is greater than those found in soil samples (Hunter, 1968c). Soil samples give better representative data on the age distribution of slug species than other methods, as small slugs are just as likely to be collected as large slugs. Small slugs may move underground during the day to avoid desiccation. Therefore the daytime surface sampling method used in this project was biased and the mean weight of the slug population was considered to be an overestimation. However, the error was constant at each site and comparisons between the sites are valid.

Weight frequency categories have been used to describe the age structure of *D.reticulatum* populations (Bett, 1960; Hunter, 1968a). Slugs in the smallest weight category dominate slug populations in gardens and in arable ground from May to December (Bett, 1960; Hunter, 1968a). Therefore small slugs are available throughout the summer period when carabids are active and small/medium sized beetle species which predate small slugs (see chapter two) have an opportunity to exert an impact on slug populations in the field.

D.reticulatum breeds throughout the year, but two hatching peaks occur in the spring and autumn (Hunter, 1968a). The autumn breeding peak may occur too late in the season for many carabid species to utilize the abundance of small newly hatched slugs, as many beetle species are becoming inactive at this time. Slug generation intervals are affected by prevailing weather conditions and vegetation (Bett, 1960) and the availability of small slugs may change from year to year. This will affect the extent to which some carabid species feed on slugs. The extent of carabid predation of other pests such as aphids is dependant on the degree of synchronisation between the life cycle of the predator and the phenology of the aphid species (Sunderland and Vickerman, 1980).

Slugs which hatch in the spring, mature in July and August (Bett, 1960), but slugs which hatch in the autumn take seven months to complete their life cycle (Hunter, 1968c). The rate at which the autumn generation develops partly depends on the

prevailing temperature. Mild winters may lead to an early spring generation which may result in a summer abundance of large slugs which many carabid predators cannot utilize. This may have occurred at Bog field, where the carabid fauna were unable to exploit the slug population. Only two beetles from this site contained slug tissues and one of these was a slug specialist (*C.caraboides*). Although the slug sampling technique did not yield accurate age-structure data, the above example highlights the need to understand prey ecology to elucidate predator-prey interactions in the field.

4.3 Predation equations

4.3.1 Introduction

In this project, the ELISA measured the mass of slug tissue consumed by each beetle and not the number of slugs killed or eaten. The mass of slug tissue present in the beetle, could have been part of a recent meal or the remains of a previous meal which has been partially digested (Sunderland, 1988). Assuming slugs were predated and not simply scavenged, the rate at which a predator is found with prey remains in its gut is a simple way of assessing that species performance as a predator.

Predation equations have been reviewed by a number of authors (e.g. Calver, 1984; Sunderland, 1988; Sopp *et al.*, 1992). These equations combine serological data with predator densities and can be used to estimate the impact of predator species on prey populations.

Serological data gathered in this study were used to estimate the predation rates by carabid beetles on slugs at the three field sites using predation equations developed by Rothschild (1966) and Dempster (1967).

4.3.2 The Dempster predation estimate

Dempster (1967) developed an equation to estimate the number of *P.rapae* eaten

by the carabid *H.rufipes* in a brussel sprout crop. The equation involved laboratory and field data. Precipitin tests were used to measure the proportion of *H.rufipes* beetles which had fed on *P.rapae* in the field. Laboratory tests were used to calculate the maximum detection period of *P.rapae* antigens in *H.rufipes*. These were used together with estimates of *H.rufipes* populations in the field and the availability of the prey. The main limitation of this equation was the assumption that each positive reaction is equal to one prey item killed and consumed. The equation does not consider the consumption of a second prey item within the detection period of the first (Calver, 1984).

Predation rates were calculated for carabids in this project using the following equation, described by Calver (1984) and used by Sunderland and Sutton (1980).

$$\frac{\text{predator density}}{\text{catching rate (No/M}^2\text{)}} \times \frac{\text{proportion positive}}{\text{(percentage)}} = \text{Predation rate}$$

$$\text{detection period (days)}$$

4.3.2.1 Methods

The mean field temperatures from the three sites in July of 1992 were, maximum 20.6°C and minimum 11.2°C (see Appendix 4.7). Detection period data concerning the decay of *D.reticulatum* antigens were determined for a number of carabid species at several temperatures (chapter three). Detection period data at 20°C were used to calculate the predation rate as this was comparable with the average daytime maximum temperature.

Short detection periods occur at higher temperatures. When these data are used in the equation they return high predation rates in comparison to long detection periods which occur at lower temperatures. Therefore, the detection periods used in this investigation returned the highest predation rates possible. Where no detection period data existed for a particular species, the data from a related species or similar sized beetle were used.

Dempster used Bailey's (1952) triple catch method to estimate the total predator population size. Abundance of predators in pitfall traps have been used in other investigations to measure the activity density of ground active predators (e.g. Sunderland *et al.*, 1987). In this project, simple activity density data were available from pitfall traps which were used to collect specimens for ELISA analysis. These data were available from all three sites in both 1992 and 1993.

The total number of each beetle species caught over both summer periods (in ELISA pitfall traps) at each site was calculated and divided by the number of pitfall traps used to make the catch (20) and the number of weeks in the sampling programme (six). This gave a 'catching rate' (Luff, 1973 and 1982) which describes the activity density of each species.

Population density estimates from the (MRR) programme (chapter five) were available for *P.melanarius* in Clayton field in June and July of 1993, which gave data on the number of beetles/m^2. Population density estimates for *P.madidus* were calculated indirectly at Clayton field in 1993 (using MRR data) by comparing the population density estimate of *P.melanarius* with the number of *P.melanarius* beetles caught in ELISA pitfall traps. Population density estimates of *P.madidus* were then made by multiplying the number of *P.madidus* beetles found in MRR traps by this factor (Frank, 1967). The MRR data were used to calculate the mean *P.melanarius* and *P.madidus* densities in June and July, these were compared with activity density data collected over the same period.

4.3.2.2 Results

In 1992, the combined impact of all the beetle species at all three sites on slug populations was 1.12 slugs killed per day. This declined to 0.17 slugs killed per day in 1993. Predation rates for each beetle species varied between the three sites. *A.similata* had one of the highest predation rates at Clayton and Square field in 1992, but had a predation rate of zero at Bog field (Table 4.3.1). *N.brevicollis* had a higher predation rate at Square field compared to Clayton field, due to a high activity density at Square field and a low activity density at Clayton field.

Table 4.3.1 Predation rates for the carabid fauna in the summer season of 1992 and 1993 calculated using Dempster's equation. Density calculated as the number of beetles caught per trap/week (or number of beetles/m^2 in the MRR section). The detection period is presented as days. The predation rates are calculated as the number of slugs killed/day.

	Density	Proportion Positive	Detection period	Predation rate
Clayton 1992				
A.similata	3.966	0.307	3.8	0.3204
A.lunicollis	0.033	0.750	3.8	0.0065
A.aulica	0.058	0.200	3.8	0.0030
H.rufipes	2.058	0.294	1.0	0.6050
H.latus	0.016	0.500	1.0	0.0080
N.brevicollis	0.091	0.444	8.0	0.0050
P.madidus	0.425	0.063	1.0	0.0267
A.parallel.	0.008	1.000	1.3	0.0061
C.violaceus	0.033	1.000	12.0	0.0027
Square 1992				
A.similata	0.700	0.187	3.8	0.0344
A.lunicollis	0.050	0.166	3.8	0.0021
H.rufipes	0.025	0.333	1.0	0.0083
N.brevicollis	0.891	0.400	8.0	0.0445
P.melanarius	0.400	0.106	1.0	0.0424
P.niger	0.041	0.200	1.0	0.0082
Bog 1992				
N.brevicollis	0.108	0.090	8.0	0.0012
C.caraboides	0.008	1.000	12.0	0.0006
Clayton 1993				
H.rufipes	0.541	0.166	1.0	0.0898
P.madidus	0.258	0.190	1.0	0.0490
P.melanarius	0.133	0.090	1.0	0.0119
Square 1993				
P.madidus	0.050	0.250	1.0	0.0125
P.melanarius	0.150	0.076	1.0	0.0114
MRR data Clayton 1993				
P.madidus	0.0110	0.190	1.0	0.0020
P.melanarius	0.0625	0.091	1.0	0.0056

In 1993, the predation rate for *H.rufipes* decreased at Clayton field from 1992 levels as the proportion of beetles feeding on slugs decreased and the activity density declined. This may have been compounded by the sampling regime used in 1993. In 1993, the predation rate for *P.madidus* increased at Clayton field as the proportion of beetles containing slug tissues increased. Predation rates for the slug specialists *C.violaceus* and *C.caraboides* and the large generalist predator *A.parallelepipedus* are low due to their low activity densities.

4.3.2.3 Carabid catching rate and population density estimates

Predation rates were calculated for *P.melanarius* and *P.madidus* in Clayton field in 1993 using the population density estimates (beetles/m^2) from the MRR programme. The data were used to calculate the number of slugs killed over the experimental area (500 m^2) and the experimental period (42 days). *P.madidus* killed 42 slugs over the summer season and *P.melanarius* killed 117.6 slugs over the summer season.

The population density estimates from the MRR programme are a more accurate measure of the population size than the activity densities. The predation rates calculated using *P.melanarius* and *P.madidus* population density estimates, are lower than the predation rates using the activity densities (Table 4.3.1). As the predation rates for the other carabid species are calculated from the activity densities, slug predation by the other carabid species is probably overestimated.

4.3.3 The Rothschild predation estimate

Rothschild (1966) developed a predation estimate to quantify the impact of a number of litter layer predators on the Homoptera *Conomelus anceps* (Germar). Rothschild's equation is similar to Dempster's equation in that it requires a measure of predator density and a measure of the proportion of predators containing prey antigens. However, in Rothschild's equation, the maximum detection period data can be substituted by a laboratory measured attack rate of the predators on the prey. The equation assumes that predators were feeding at their maximum rates (Calver, 1984).

Predation rates were calculated for carabids in this project using Rothschild's equation described by Calver (1984) and used by Sunderland and Sutton (1980):

$$\begin{array}{c} \text{predator density} \\ \text{(catching rate No/M}^2\text{)} \end{array} \times \begin{array}{c} \text{proportion positive} \\ \text{(percentage)} \end{array} \times \begin{array}{c} \text{feeding rate} \\ \text{(No/day)} \end{array}$$

Sopp and Wratten (1986) calculated laboratory consumption rates for nine species of carabids feeding on aphids. However, aphids are small prey items and increases in aphid sizes occur at discrete intervals (instars). Calculating a consumption rate for slugs is more problematic as they are available in a variety of sizes and consumption rates are likely to vary according to the size of slug attacked. Stephenson (1965) compared seven species of carabids and found *P.melanarius* ate five *D.reticulatum* in 14 days and *P.madidus* ate one *D.reticulatum* in 15 days. When six *P.madidus* and three *P.melanarius* were confined with twenty *D.reticulatum*, all of the slugs were eaten in 24 days. The sizes of the slugs were not specified.

Slugs collected from the field in this study ranged in weights up to a maximum of 1.4 g, and obviously hatched at a fraction of that weight. A beetle feeding on one large slug may be satiated for several days, but the same beetle may consume several dozen small slugs over the same period.

4.3.3.1 Methods

Preliminary laboratory investigations in this study indicated that *P.madidus*, *H.rufipes* and other large carabid species could predate several hatchling slugs daily. However, even large predators such as *A.parallelepipedus* consumed large slugs at a maximum rate of one per day (chapter two, section 2.3). Slugs occur in the field in a variety of sizes (see Bett, 1960 and Hunter, 1968a) and these may all available to a predator at any one time. For the purposes of this investigation, hatchling slugs were used as a standard slug size. By using this standard size, the smaller beetle species were not excluded from the calculation and a maximum slug kill rate could be calculated for these smaller carabid species.

Laboratory investigations indicated that the *Amara* could eat one slug per day,

N.brevicollis could eat two slugs per day and *H.rufipes* four slugs per day. *P.madidus* ate five slugs per day, but an upper limit for this species was not defined. Similarly *A.parallelepipedus* could eat ten slugs per day, but again an upper limit for this species was not defined. When a feeding rate was not calculated for a particular carabid species, the feeding rate of a related species were used (e.g. *P.madidus* data were used for *P.melanarius*).

The predation rates were calculated using activity density and population density estimates of *P.melanarius* and *P.madidus*. The two predation rates were compared.

4.3.3.2 Results

In 1992, the combined impact of all the predator species at all three sites was 5.8 slugs killed per day. This decreased to 1.2 slugs killed per day in 1993. However, these mortality rates may be underestimated as the upper threshold for the number of slugs killed by some beetle species was not determined in the laboratory experiments (the number of slugs killed by each beetle species are presented in Appendix 4.8).

The predation rate for each beetle species varied between the sites (Table 4.3.2). In 1992, *A.similata* had a higher predation rate at Clayton field when compared to Square field. This was due to a smaller proportion of *A.similata* beetles feeding on slugs at Square field and a lower activity density.

H.rufipes had the highest predation rate at Clayton field in 1992. This species predation rate decreased in 1993 at Clayton field due to a lower activity density and a smaller proportion of beetles feeding on slugs in 1993. Again, this may have been compounded by the different sampling regime used in 1993. The proportion of *P.madidus* beetles feeding on slugs increased at Clayton field in 1993. This resulted in a higher predation rate for *P.madidus* at this site in 1993, despite a lower activity density.

Table 4.3.2 Predation rates for the carabid fauna in the summer season of 1992 and 1993 (the Rothschild equation). ? indicates predators laboratory predation rate is unknown and presumed to be one slug per day. ( ) indicates laboratory measured predation rate based on *P.madidus*. + indicates that the maximum laboratory predation rate may be more than the value indicated. The density is calculated as the number of beetles caught/trap/week (or number of beetles/m^2 in the MRR section).

	Density	Proportion positive	Laboratory feeding (No.slugs/day)	Predation rate
Clayton 1992				
A.similata	3.966	0.307	1	1.2175
A.lunicollis	0.033	0.750	1	0.2475
A.aulica	0.058	0.200	1	0.1160
H.rufipes	2.058	0.294	4	2.4202
H.latus	0.016	0.500	?	0.0080 ?
N.brevicollis	0.091	0.444	2	0.0808
P.madidus	0.425	0.063	10 +	0.2677 +
A.parallel.	0.008	1.000	10 +	0.0800 +
C.violaceus	0.033	1.000	?	0.0330 ?
Square 1992				
A.similata	0.700	0.187	1	0.1309
A.lunicollis	0.050	0.166	1	0.0083
H.rufipes	0.025	0.333	4	0.0333
N.brevicollis	0.891	0.400	2	0.7128
P.melanarius	0.400	0.106	(10) +	0.4240 +
P.niger	0.041	0.200	(10) +	0.0820 +
Bog 1992				
N.brevicollis	0.108	0.090	2	0.0194
C.caraboides	0.008	1.000	?	0.0080 ?
Clayton 1993				
H.rufipes	0.541	0.166	4	0.3592
P.madidus	0.258	0.190	10 +	0.4902 +
P.melanarius	0.133	0.090	(10) +	0.1197 +
Square 1993				
P.madidus	0.050	0.250	10 +	0.1250 +
P.melanarius	0.150	0.076	(10) +	0.1140 +
MRR data Clayton 1993				
P.madidus	0.0110	0.190	10 +	0.0209 +
P.melanarius	0.0625	0.091	(10) +	0.0568 +

4.3.3.3 Carabid catching rate and population density estimates

Predation rates were calculated for *P.melanarius* and *P.madidus* using population density estimates from the MRR programme in Clayton field in 1993. The population density estimates were used to calculate the number of slugs killed over the experimental area (500 m^2) and the experimental period (42 days). *P.madidus* killed 438.9 slugs over the summer season and *P.melanarius* killed 1192.8 slugs over the summer season. However these may be underestimates as the maximum number of slugs killed by these two predators was not determined in laboratory experiments (see Appendix 4.8).

The population density estimates yielded lower predation rates than the activity density data. As the population density estimates are a more accurate measure of the beetle population size, the predation rates for the other carabid species are probably overestimated.

4.3.4 Discussion of predation equations

Sunderland and Sutton (1980), used both Dempster's and Rothschild's equations on the same data to estimate two predation rates of various arthropods on woodlice in a dune ecosystem. They concluded that the true predation rate lay between the two estimates. In this study, the two equations yielded extremely different predation rates for each species. This is due to the emphasis placed on different components of the predator's feeding.

4.3.4.1 Application to field

The Rothschild equation is suitable for calculating predation inside prey aggregations and the Dempster equation is more suitable when a low background density of prey is present (Sunderland and Sutton, 1980). Slugs aggregate in favourable habitat patches and oviposition occurs within these habitat patches (South, 1965). Consequently small hatchling slugs are aggregated at the beginning of the hatching season (Hunter, 1966). In the context of this investigation, the feeding rates were calculated for beetles feeding on hatchling slugs. Therefore the Rothschild equation is applicable to slug distribution at this time.

There are two main limitations in using these equations to describe predator-prey interactions between carabids and slugs in the field. The first is making an accurate estimation of the predator population density. The second is in making some meaningful estimate of the number of prey killed by a predator in a prey population of mixed size and age.

4.3.4.2 Predator density, activity and prey availability

In this study the predator density was estimated as an activity density or catching rate (Luff, 1973 and 1982). The activity density of *P.melanarius* and *P.madidus* were higher than the population density estimates made in the MRR programme and subsequently gave higher predation rates. However, the population density estimates made using MRR methods are a more reliable measurement of beetle population size. Therefore, the predation rates of the other beetle species, which were measured as a activity density, are probably overestimated.

P.melanarius and *P.madidus* were not abundant at Clayton field in 1993 (see chapter five). However, using Rothschild's equation, these two species killed at least 1631 slugs in the experimental area (500 m^2) in 42 days in June and July at Clayton field in 1993. These data were generated using small hatchling slugs. There is evidence in the literature to suggest that small slugs are available in abundance at this time of the year (Bett, 1960; Hunter, 1968a). Therefore, these two predators could kill large numbers of slugs even at relatively low beetle densities.

The temporal availability of slugs and alternative prey may reduce slug predation. *P.madidus* may eat fewer molluscs in June (Luff, 1974) as molluscs move underground during the dry summer weather. *H.rufipes* and *P.madidus* have long activity periods in Northumberland, lasting from March to November (Luff, 1973 and 1980). The long activity periods of such predators, increase the length of time that predators can inflict mortality on slug populations.

Sunderland *et al.*, (1987) and Sopp *et al.*, (1992), described a further method of assessing the predation rate involving the mass of prey detected in predators and

the mean mass of individual prey in the field. Although slug mass data was collected from the field in this project, it was considered unrepresentative of the slug population and therefore inadequate for such an analysis.

4.4 Predator indices (ranks)

4.4.1 Introduction

Serological data can be used with field density data to rank predators by means of an index (e.g. Sunderland *et al.*, 1987). The index is an indication of the relative value of each species as a predator (Sunderland *et al.*, 1987). Ranking predators helps identify candidate species for integrated control programmes (Wratten *et al.*, 1984). An abundant species, which rarely feeds on slugs could have the same impact as a less abundant species which often feeds on slugs.

The proportion of predators containing aphid remains in gut dissections and predator field density have been used in predator indices (Sunderland and Vickerman, 1980). The same two parameters have been used with, maximum detection period data (D_{max}) in the following equation (Sunderland *et al.*, 1987):

$$(\%\ \text{positive} / D_{max})\ \text{mean predator density}$$

In this equation pitfall trap data was used to estimate the predator density as 'the number of predators passing over 1 m^2 of ground during 24 hours' (calculated from the number caught per pitfall). The index took no account of meal size, feeding rate or satiation of the predator.

4.4.2 Methods

Predator indices were calculated for each beetle species at each of the three sites, for both summer seasons (after Sunderland *et al.*, 1987) . Predators were then ranked according to their index score. A combined data set was calculated using the pooled data for each species from each site and the summer seasons of 1992

and 1993. The catching rates were altered to take into account the combined number of pitfall traps involved (60) and the combined sampling weeks (12).

4.4.3 Results

The position of each species in the rank changes between the three sites and between years (Table 4.4.1). In 1992, the proportion of *N.brevicollis* containing slug remains was similar at Clayton and Square field, but the indices and ranks at the two sites were very different. This was due to the higher activity densities at Square field. At Clayton field, *H.rufipes* and *A.similata* dominated the rank in 1992 as a high proportion of both species had fed on slugs and both had high activity densities.

The rank was heavily influenced by the activity density of predators, the most abundant species dominating the rank. *C.violaceus* and *A.parallelepipedus* always fed on slugs but had low ranks because they were present at low activity densities.

4.4.4 Discussion

A.similata had a high overall rank in this project, however it is not a documented slug feeder, indeed Davies (1953) considered the Amarini tribe to be exclusively vegetarian. This tribe do kill small slugs (chapter two), but their size suggests that they will be unable to overcome larger slugs. However, the high position of *A.similata* in the rank may be justified, as small slugs are available in arable land over the summer period (Hunter, 1968a) and *A.similata* is capable of predating hatchling slugs.

The rank takes no account of the number of prey killed by each predator. Several other beetles with a lower rank than *A.similata* are more voracious slug predators (chapter two) than *A.similata* which kills only one slug per day; *N.brevicollis* kills up to two slugs per day, *P.madidus* kills several slugs per day and this species is more capable of killing large slugs than *A.similata* by virtue of its size (Tod, 1973). In addition, *A.similata* inflicts only a low slug mortality (ten percent, see chapter two) but a high proportion of slugs die (80 percent) when exposed to *N.brevicollis*

Table 4.4.1 Predator indices and ranks (after Sunderland *et al.*, 1987) for each site and year over the summer season. Combine data from all three sites over the two summer seasons. Density is calculated as the number of beetles caught/trap/week and has been altered for the combined data set (see section 4.4.2). The proportion positive is given in the second column. The detection period is presented as days.

	Density	P+	Detection Period	index
Combined data				
Harpalus rufipes	0.4444	27.1	1.0	12.0432
Amara similata	0.7930	27.6	3.8	5.7596
Pterostichus madidus	0.1347	9.7	1.0	1.3065
Pterostichus melanarius	0.1541	7.0	1.0	1.0787
Nebria brevicollis	0.2013	37.3	8.0	0.9385
Pterostichus niger	0.0208	8.3	1.0	0.1726
Amara lunicollis	0.0138	40.0	3.8	0.1452
Harpalus latus	0.0041	33.3	1.0	0.1365
Abax parallelepipedus	0.0013	100.0	1.3	0.1000
Carabus violaceus	0.0055	100.0	12.0	0.0458
Amara aulica	0.0069	20.0	3.8	0.0363
Cychrus caraboides	0.0027	50.0	12.0	0.0112
Clayton 1992				
Harpalus rufipes	2.058	29.4	1.0	60.5052
Amara similata	3.966	30.7	3.8	32.0411
Pterostichus madidus	0.425	6.3	1.0	2.6775
Harpalus latus	0.016	50.0	1.0	0.8000
Amara lunicollis	0.033	75.0	3.8	0.6513
Abax parallelepipedus	0.008	100.0	1.3	0.6153
Nebria brevicollis	0.091	44.4	8.0	0.5050
Amara aulica	0.058	20.0	3.8	0.3052
Carabus violaceus	0.033	100.0	12.0	0.2750
Square 1992				
Nebria brevicollis	0.891	40.0	8.0	4.4550
Pterostichus melanarius	0.400	10.6	1.0	4.2400
Amara similata	0.700	18.7	3.8	3.4447
Harpalus rufipes	0.025	33.3	1.0	0.8325
Pterostichus niger	0.041	20.0	1.0	0.8200
Amara lunicollis	0.050	16.6	3.8	0.2184
Bog 1992				
Nebria brevicollis	0.108	9.0	8.0	0.1215
Cychrus caraboides	0.008	100.0	12.0	0.0666
Clayton 1993				
Harpalus rufipes	0.541	16.6	1.0	8.9806
Pterostichus madidus	0.258	19.0	1.0	4.9020
Pterostichus melanarius	0.133	9.0	1.0	1.1970
Square 1993				
Pterostichus madidus	0.050	25.0	1.0	1.2500
Pterostichus melanarius	0.150	7.6	1.0	1.1400

(chapter two).

A high proportion of the large slug specialist species fed on slugs, but they all have low ranks due to their low field densities. This masks their overall impact on slug populations. In video tape analysis (chapter two) *C.violaceus* made multiple kills of large slugs in a single night (this study and Stephenson, 1965). All of the *C.violaceus* specimens assessed by ELISA contained slug remains. This species voracity indicates that it may have a far greater impact on slug populations than the abundant *A.similata*.

Parameters such as the season of activity, field penetration and the response to prey heterogeneity have been considered in ranks by other authors (Wratten *et al.*, 1984). In this investigation, laboratory studies provided additional information to assess a predators importance. This is helpful when considering predation of multi-sized prey which are difficult to overcome and exist in mixed age populations. Caution must be taken in using these ranks. Activities such as scavenging may hugely exaggerate the importance of some species, which may only infrequently kill the prey. This is discussed in section 4.4.4.1.

4.4.4.1 Effects of scavenging

Many authors have assumed that positive precipitin tests have indicated predation of prey in the field. Sunderland and Sutton (1980) found eight species of arthropod were unable to kill woodlice in the laboratory, but would scavenge dead woodlice. Serological data from the same study indicated that these species had eaten woodlice in the field. These results would have incurred considerable error in the estimated field consumption of woodlice had all the positive reactions in the precipitin test been assigned to predation. Similarly, two *Amara* species are unable to kill larval cutworm in the laboratory, but field caught beetles frequently contain cutworm antigen (Frank, 1971). Again this suggests scavenging may seriously bias postmortem studies.

The impact of scavenging needs serious consideration when assessing and applying

serological results to ranks. The indices used in this study indicated that *A.similata* is exerting a high impact on slug populations relative to larger predators which are more capable of killing slugs by virtue of their size (e.g. *A.parallelepipedus*) (Tod, 1973) and specialisation on slug prey (e.g. *C.violaceus*). The comparatively large slugs at the field sites indicates that scavenging from dead slugs may account for the large proportion of *A.similata* beetles containing slug tissues, as this species can only overcome small slugs.

4.5 Conclusions

The proportion of beetles feeding on slugs varied between the three field sites. This is partly due to the occurrence and abundance of beetle species which eat slugs at each of the sites.

Slug eating species did not feed on slugs to an equal extent at each site. The proportion of beetles which ate slugs depended on the site from which the beetle was recovered. The changing carabid fauna, availability of alternative prey and abundance of slugs may all contribute to the variable proportion of beetles feeding on slugs between years.

In the context of this study, the ELISA technique has two main limitations. It can only measure mass of ingested prey antigens and does not indicate the number of slugs attacked or killed. The ELISA cannot distinguish between predation and scavenging. Most of the species investigated in this project were probably scavenging dead slugs to some degree and beetle which merely scavenge dead slugs have no impact on the slug population.

The work has shown that slugs are active throughout the summer season under crops of winter wheat and oilseed rape. Slugs are a readily available source of food and results from this chapter have shown that many carabids exploit this food source. Unfortunately, some carabid species are limited in their ability to exploit slugs due to their size and consequently their inability to overcome all but the smallest slugs. Therefore, the age structure of the slug population may contribute

to the number of beetle species able to exploit slugs.

The use of predation rates and ranks helps to identify and assess useful predators. However, extreme caution should be taken in interpreting results for prey such as slugs which are difficult to overcome and exist in mixed age populations. Species which occur at low densities and have low ranks may have more of an impact on slug populations than small abundant species which always feed on slugs. If certain species were to be selected for control programmes, their predatory and scavenging habits would need careful consideration. Laboratory studies, including video tape techniques can yield valuable evidence to support a predator species importance.

Finally, agricultural crops are host to many invertebrate species which carabid predators may exploit at the expense of killing slugs. The numbers and variety of alternative prey may change according to crop type and time of the year. These variables will all influence the overall impact of a predator on populations of slug pests.

Chapter five

Population Density Estimates and Comparative Studies of Carabids in Arable Land

5.1 Introduction

The two studies in this chapter investigated the carabid community of three fields sown with arable crops. The first study (section 5.3) was made in June and July 1993 and estimated the carabid population density in fields of winter wheat in Clayton field and Bog field. The second study (section 5.4) was a comparative study which investigated the changes in abundance of the carabid fauna between three fields over four seasons. This study commenced in June 1992, when the fields were in oilseed rape and ended in July 1993 when the fields were in winter wheat. This study was made at Clayton, Bog field and Square field (Fig. 4.2.1). The fields are described in chapter four.

5.2 The use of pitfall traps

Pitfall traps were used in this project to collect beetles for population estimates and to compare beetle abundance between the three fields and between years.

Pitfall traps are generally used by ecologists to collect ground active invertebrates. Although almost universally used, pitfall traps have been widely criticised. They can give unrepresentative data on the fauna of a given area. The number and type of beetles caught are primarily determined by size of the population at risk and level of activity (Greenslade, 1964b). Active species are more likely to be caught than sedentary species. The term 'Activity density' has therefore been used to describe pitfall trap data (Thiele, 1977) as it measures activity and abundance (Mitchell, 1963b; Luff, 1982).

Species show varying susceptibility to trapping according to size, behaviour and the ground vegetation in which they are active (Greenslade, 1964b). Climate and substrate affect the efficiency of pitfall traps for any species (Luff, 1987).

Therefore, the physical characteristics of every study site must be considered when comparing catches between sites (Greenslade, 1964b).

Predator behaviour can alter in different habitats. *P.melanarius* attempts to hide immediately after release in corn plots. However, it is caught more readily in corn plots compared with oat plots (Rivard, 1965). This is due to increased levels of beetle movement in unfavourable habitats, as the beetles try to move to more favourable habitats (Baars, 1979a).

Ground vegetation type can affect trapping efficiency due to its resistance to horizontal movement (Frank, 1967). Sparse or compressed vegetation offers little resistance to beetle activity. This can result in higher catching rates in open areas compared with areas with dense ground vegetation. Catches of *N.brevicollis* can be negatively correlated with increasing litter depth (Greenslade, 1964b) or positively correlated to increasing litter depth (Penney, 1966). The catches of other species are not dependant on litter depth.

The prevailing weather conditions and climate are two important factors affecting beetle activity. The catches of some species are correlated with increasing humidity (Rivard, 1965). *P.madidus* is more active during wet rather than dry weather (Dempster *et al.*, 1959) but *C.melanocephalus* and *Pterostichus versicolor* (Sturm), are less active with prolonged abundant rain (Baars, 1979a).

The catches of some species, such as *P.melanarius*, *P.madidus*, *P.versicolor, C.melanocephalus* and *H.rufipes* are positively correlated with increasing temperature. Catches of *B.lampros*, *N.brevicollis* and *N.biguttatus* are negatively correlated with accumulated temperature (Jones, 1976 and 1979; Baars, 1979a; Luff, 1982). This suggests that larger species are able to withstand periods of hot dry weather. For day active species the maximum temperature is more important in regulating activity and the minimum temperature is more important for night active species (Baars, 1979a).

Beetle size and trap size can affect the catching rate. Small traps only catch small beetles efficiently (Luff, 1975). Large, fast moving predators which cover large areas of ground, increase their likelihood of encountering a trap and are trapped in higher numbers than smaller more sedentary species (e.g. Greenslade, 1964b). Other factors, such as a species ability to perceive the pitfall trap edge may be more important than beetle size, speed of movement or diurnal behaviour (Halsall and Wratten 1988). A few species such as *Demetrias atricapillus* (Linnaeus) are adept climbers and can escape from pitfall traps (Halsall and Wratten, 1988). Some species are therefore more readily caught than others and species moving at similar speeds and occurring at similar densities may differ in their rate of capture.

Greenslade (1964b) argued that pitfall trapping could not be properly used for the quantitative assessment of the carabid fauna of any habitat, or to compare the numbers of one species in different habitats. However, they can be used as a sampling method in capture-recapture work and for investigating the distribution of one species in a single vegetation type. In other studies, pitfall catches have correlated well with population density estimates (Luff, 1982) and linear relationships have been found between beetle densities in several habitats over several years and the numbers of beetles trapped (Baars, 1979b).

Pitfall traps minimise attention from unwanted visitors and enable set numbers to be placed discretely in fields. They are robust and not easily damaged by heavy farm machinery moving over them. In the following two studies, pitfall traps were used in a mark-release-recapture (MRR) programme to estimate population densities of surface active carabids and to compare predator abundance in two agricultural monocultures (Meijer, 1974). This satisfies the criteria identified by Greenslade (1964b) for the use of pitfall traps. However, the dependence of the traps on predator activity and the parameters which affect activity, demands careful interpretation of the results (Luff, 1987).

5.3 Carabid population density estimates

5.3.1 Introduction

Inverse relationships have been found between the number of aphids and the number of carabids in crops of winter wheat (e.g. Edwards *et al.*, 1979). Carabids can reduce slug numbers in arable land (Altieri *et al.*, 1982) and slug numbers are higher when beetles numbers are artificially reduced (Burn, 1992). *A.parallelepipedus* is known to reduce slug damage to lettuce in polytunnels at densities of six beetle/m^2 (Symondson, 1989). In arable land, little quantitative data concerning beetles density exists.

In this study, carabid population density estimates were made in two crops of winter wheat using a MRR method. These data were compared with beetle densities which reduced slug damage on a chinese cabbage crop in the miniplot experiment (chapter six).

5.3.2 Background to population estimates

A number of methods have been used to assess predator dispersal and densities. Ectoparasitic fungi and radioactive labels have been used as markers to study the migration and dispersal of carabids (Meijer, 1975; Baars, 1979a). However, MRR methods have been most extensively used by ecologists to study carabid populations (Dempster *et al.*, 1959; Mitchell, 1963b; Frank, 1967; Dijk, 1973; Luff, 1982). MRR methods have also been used in conjunction with serological studies of carabid predator-prey interactions (Dempster *et al.*, 1959).

MRR methods have been discussed fully by Southwood (1966) and Begon (1979). In brief, animals are collected from the study site, marked and released back into the study site and allowed to mix with the population before being recaptured at discrete intervals. The number of recaptured animals is expressed as a proportion of the total population:

$$P = N \times (M/R)$$

Where M individuals have been captured, marked and released, N individuals have been recaptured, including R marked ones. P is then an estimate of the population. A number of assumptions are made about the technique and the following criteria must be satisfied (Southwood, 1966):

1. Marking a predator does not affect its chance of survival.

2. Individuals distribute themselves evenly in the population after release.

3. Every individual in the population has an equal chance of recapture.

4. Sampling must be at discrete time intervals.

Unfortunately, these criteria are not always easily met. Male and female carabids are often trapped unequally (e.g. Gordon and Mckinlay, 1986). Rivard (1966) caught 10,000 carabids in pitfall traps in agricultural crops and found 57 percent were male and 43 percent were female. He concluded that males may be more active than females. The same disproportionate capture of males and females can be found in other insect groups (e.g. *Drosophila*, Begon *et al.*, 1975). Carabid species also differ in their susceptibility to capture in pitfall traps (see section 5.2). This must be considered when assessing population density estimates.

5.3.3 Experimental procedure

Carabid population density estimates were made at Clayton field on Heddon Bank farm and Bog field on Peepy farm between June 15th and July 29th 1993.

Pitfall traps were plastic beakers measuring 65mm in diameter and 65mm deep. The bottom of the beaker was slightly smaller (58mm), therefore the sides were angled. A one centimetre diameter hole was cut away in the base of the beaker and metal gauze was glued over the hole. This allowed rainwater to drain out of the trap and prevented the trap from flooding. Damp, decaying wheat leaves were collected from the study sites and arranged in the bottom of the pitfall traps as a

refuge for smaller carabids to prevent predation in the trap (Begon, 1979). This also helped maintain high humidity in the trap and prevented desiccation of the trapped predators.

The sets of pitfall traps used for population density estimates and pitfall traps used for the comparative study (section 5.4) were placed 75 metres apart. They were arranged in similar positions in the two fields to enable comparisons to be made between the two sites. The traps were placed away from the edge of the field to minimise the overestimation of field edge predators (Rivard, 1966; Coombes and Sotherton, 1986). Comparisons between the two fields were valid as they were the same vegetative type (Greenslade, 1964b).

Ten to twelve traps are sufficient to catch ground active Coleoptera of a similar activity type in a particular habitat (Obrtel, 1971). Pitfall traps are generally arranged into grids for population estimations (e.g. Greenslade, 1964a; Penney, 1966; Luff, 1982; Attah, 1986) and spaced at 4.5, 9.1 or ten metre intervals (Greenslade, 1964a; Penney, 1966; Attah, 1986). In this study, twenty pitfall traps were arranged in a 5 x 4 grid in both fields. Traps were laid at five metre intervals (Ericson, 1977), thirty metres from the edge of the fields and parallel with the field edge (Fig 4.2.1).

The traps therefore covered an area of 300 m^2. Pitfall traps on the periphery of the sampling area caught beetles from outside the defined area. Therefore each peripheral trap had an effective area of influence outside the 300 m^2. As the traps were spaced five metres apart, the effective area was assumed to be 2.5 metres (Dub, 1971; Ericson, 1978). For the purposes of this study the sampling area was therefore increased by adding a surrounding 2.5 metre strip around the peripheral traps (Ericson, 1978; Attah, 1986). This gave a total sampling area of 500 m^2.

The traps were placed in bare areas of soil (Greenslade, 1964b; Baars, 1979b) on the edge of the tramlines to standardise catching conditions between the fields. The bare areas of soil around the traps were maintained throughout the sampling

period in order to maintain a constant catching efficiency (Baars, 1979b) and eliminate any variation in catch due to obstacles close to the traps (Greenslade, 1963b). The traps were sunk in the soil so the lip of the trap was level with the surface of the ground. Particular attention was paid to the evenness of the soil-trap interface.

On day seven of the programme, some of the traps were disturbed by a large vertebrate. Therefore 150mm squares of chicken wire were placed over the traps and held in place with 40cm thin steel rods pushed into the ground. This allowed predators to fall into the traps but protected them from the attention of vertebrates.

The traps were used to sample the carabid population twice weekly (Greenslade, 1964a). Snap on plastic lids were used to cover the traps between samples. When a sample of the population was made, the lids were removed and the traps left uncovered overnight (approximately 15 hours), which was the sampling period (Begon, 1979). This relatively long sampling period enabled day and night active predators to be captured. Although many Coleoptera, including carabids, are trapped at night (Vickerman and Sunderland, 1975; Luff, 1978) up to 33 percent of smaller Carabidae are active by day (Luff, 1978). The sampling period allowed day and night active species (e.g. *P.madidus*) to be caught (Williams, 1959).

In the morning, the predators were removed from the traps using jeweller's forceps and the traps were covered with plastic lids. The predators were transferred to several plastic containers containing moist paper towelling and transported to the laboratory. Predators were immediately placed into individual petri dishes containing moist filter paper and placed in an incubator at 8°C for fifteen minutes. Cooling the predators improved handling and reduced excessive movement after the predators were marked. Excessive movement could result in the predators removing their marks.

Clipping the elytra has been used to mark carabids (e.g. Penney, 1966) but paint is normally used (e.g. Frank, 1967). In this study predators were marked with a glossy

dope (Greenslade, 1964a; Attah, 1986) on the elytra using a sharpened matchstick. Several different colours were used in a variety of combinations to identify the day of capture. Preliminary investigations with the paint showed it to be durable for several weeks on a number of different carabid species in moist peat and soil cultures in the laboratory.

The paint was allowed to dry and the predators were transported back to their relevant field site and released randomly near the centre of experimental areas (Mitchell, 1963b; Greenslade, 1964a). The plastic pots containing the predators were placed in the study site at the point of release and left for five minutes to allow the beetles to acclimatise after transportation. The lids were carefully removed and the containers tilted to encourage the carabids to crawl out. Although time consuming, this method prevented carabids being released in a hyperactive behavioural condition which may have affected their activity and dispersal (Begon, 1979). On several occasions carabids were observed for a few minutes after being released. They generally dispersed quickly and found convenient shelter in which to take cover.

Fourteen samples were made in total (Table 5.3.1). The marking system allowed recaptured carabid predators to be identified to their last day of capture. Results were recorded in a trellis (Southwood, 1966). Males and females of each species were recorded individually as differences have been found in the numbers caught (Chiverton, 1984; Rivard, 1965).

Most of the pitfall traps in Bog field were flooded during a wet period in July, however the traps at Clayton field were unaffected. Only two cases of carabid cannibalism were observed in the pitfall traps and this was not deemed to be affecting the catches of the larger species.

5.3.4 Population density estimates

P.melanarius, *P.madidus*, *P.nigrita*, *H.rufipes*, *L.pilicornis* and some *Amara* species were regularly trapped at Clayton field in June and July.

Table 5.3.1 The number of captured, marked, released and recaptured *P.melanarius* adults in Clayton field in 1993. Day is abbreviated to Dy. Total captured is abbreviated to Tot. cap. and Total released is abbreviated to Tot. rel.

Date	Dy	Tot. cap.	Tot. rel.	Day when last captured 1	4	7	11	14	17	21	25	28	31	35	38	42
15 June	01	3	3													
18 June	04	12	12													
21 June	07	7	7	1	1											
25 June	11	5	5			2										
28 June	14	11	11													
01 July	17	9	9				1		1							
05 July	21	4	4	1												
09 July	25	1	1													
12 July	28	2	2													
15 July	31	0	0													
19 July	35	3	3													
22 July	38	1	1													
26 July	42	4	4													
29 July	45	4	4													1

At Clayton field, seventeen *P.madidus* were caught but only two were recaptured. Seventeen *A.similata* were caught in June but none were recaptured. Eleven *A.plebeja* were caught in June and July but only one was recaptured. Eight *P.nigrita* were caught but only one was recaptured. Twenty two *H.rufipes* were caught but none were recaptured. Sixty six *P.melanarius* beetles were caught, including 31 females and 35 males. Two females and six males were recaptured giving a recapture rate of 12.1 percent. One hundred and sixty four *L.pilicornis* beetles were caught and seventeen were recaptured giving a recapture rate of 10.3 percent. At Bog field, forty six *L.pilicornis* beetles were caught in a seven day period in mid July, however none were recaptured.

The capture and/or recapture of other carabid species were very low. Recapture rates of *P.melanarius* beetles can be high (between 28-35 percent) in experimental plots (Rivard, 1965). In the field, recapture rates of *P.melanarius* can be as low as six percent (Jones, 1979). Recapture rates of 8.5 percent have been used to estimate field population densities of *P.melanarius* (Attah, 1986). The recapture rate of *P.melanarius* beetles in Clayton field was 12.1 percent and therefore density estimates were made for *P.melanarius* beetles. Due to the relatively low number of recaptures in this experiment, the male and female catches were combined. As *L.pilicornis* aggregates in prey patches (e.g. Bauer, 1982) population density estimates were not calculated for this species.

5.3.4.1 Population models

A number of models have been developed which interpret MRR data. The Jackson positive method has been used to estimate the dispersal of *B.lampros* (Mitchell, 1963b) and Bailey's method has been used to estimate the density of *N.brevicollis* in woodland (Penney, 1966). The Lincoln index has been used to estimate carabid populations (Dempster *et al.*, 1959) and populations of *N.brevicollis* in woodland and scrub (Greenslade, 1964a).

The Lincoln index assumes a population is static in mortality, birth rate, immigration and emigration. Other models include immigration, emigration and

survival in the calculation. Jolly's (1965) stochastic model has been used to study the dispersal of carabids (Luff, 1982). However, the choice of model depends on the quality of data. The Fisher-Ford (1947) method has been used when recaptures are low (Begon, 1979; Attah, 1986). Jolly's (1965) model and the Fisher-Ford (1947) model were considered for analysing the data. The Fisher-Ford model has been recommended when data are poor or scanty or recaptures are low (Begon, 1979) as it gives better estimates of survival rates and population size (Begon *et al.*, 1975). Jolly's model can give large overestimates when samples are small (Manley, 1970) and under these conditions the Fisher-Ford model gives more reasonable estimates. Attah (1986) had a low recapture rate for *P.melanarius* beetles in oilseed rape and used the Fisher-Ford (1947) method in preference to Jolly (1965). As the samples were small, the Fisher-Ford model was used to estimate population densities for *P.melanarius* (Begon, 1979; Attah, 1986).

The data were analyzed according to Begon (1979). Data were laid out in a trellis (Table 5.3.1)(Southwood, 1966). Population size was estimated from the modified Petersen estimate by assuming the ratio of marks to total individuals in the day$_i$ sample is the same as in the total population. A summary of the notation can be found in Table 5.3.2.

$$\tilde{N}_i = \frac{(n_i + 1)}{(m_i + 1)} Mi$$

Mi (marks at risk on day i) is not known and must be estimated before Ni can be calculated. Mi is calculated by comparing the days survived by marks in the samples with the days survived by marks in the population. The days survived by marks in the samples was calculated. e.g. on day 17:

$$\sum_j m_{17j} (17\text{-}j) = (1*6) + (1*3) = 9$$

This is made for all days and the observed Total Days Survived (observed TDS) is calculated. A survival rate (ϕ) (=0.885) was calculated by trial and error which gave the estimated Total Days Survived (estimated TDS) as 49. The estimated

Table 5.3.2 Summary of the notation used in population density estimates of *Pterostichus melanarius* in MRR studies (after Begon,1979).

a_i	The number of individuals caught both on day i and i+1
bi	The proportion of the day i+1 population added between days i and i+1
mi	The number of marked individuals caught on day i
mij	The number of marked individuals caught on day i with a day j mark
ni	The number of individuals caught on day i
pi	The proportion of the population captured on day i
qi	The proportion of the day i sample carrying a mark
ri	The number of marked individuals released on day i
yi	The number of individuals marked and released on day i and caught again subsequently
zi	The number of individuals marked before day i, not caught on day i but caught again subsequently.
Ai	The mean age of marks on day i
Bi	The number of additions to the population between days i and i+1
Li	The number of losses from the population between days i and i+1
Mi	The number of marks at risk on day i
Mij	The number of day j marks at risk on day i
Ni	The population size on day i
Ti	The total age of all marks on day i
Wi,Xi Yi,Zi	Mutually exclusive subsets of the day i population
αi,βi γi,δi	The number of immigrations, births, emigrations and deaths respectively in the population between days i and i+1
ϕi	The proportion of the day i population surviving until day i+1; or the chances of an individual in the day i population surviving until day i+1

survival rate (ϕ) was used to calculate a sequence of Mi (Table 5.3.3), which was used to estimate the population (Ni), the number of losses (Li) and the number of additions (Bi) to the population on each sampling day (Table 5.3.4)(e.g. Begon, 1979).

The final densities of *P.melanarius* in Clayton field were calculated by dividing the estimated population size by the area of land sampled (Table 5.3.4). Densities of *P.melanarius* varied between 0.04 to 0.25/m^2 (mean = 0.07, s.d.= 0.06). The highest *P.melanarius* densities occurred at the end of June and the beginning of July.

5.3.5 Discussion

Many species caught in the traps used in the comparative study (section 5.4) were not caught in the MRR traps or were caught in low numbers. Many species which were caught in large numbers in the MRR traps were caught in lower numbers in the traps used in the comparative study. One hundred and sixty four *L.pilicornis* beetles were caught in the MRR traps in June and July at Clayton field and 64 were caught in July at Bog field These numbers conflict with the relatively low numbers recovered from the comparative study traps. The comparative study traps and the MRR traps were placed in similar positions in both fields and at similar distances from field boundaries. Therefore disparities in the catches were due to emigration, immigration, aggregation or dispersal of beetles.

Collembola produce aggregation pheromones (Verhoef *et al.*, 1977) which 'condition' habitats. *L.pilicornis* is able to detect these pheromones and aggregate in Collembola patches (Bauer, 1982). Such prey patchiness may account for the patchy distribution of *L.pilicornis* and disparities between sampling areas in the same field.

Twenty two *H.rufipes* were captured and released in Clayton field but none were recaptured. Low recapture rates have been found in other studies (e.g. Ericson, 1977). In this study, *H.rufipes* was not a suitable species for population estimates.

Table 5.3.3 The observed and estimated days survived by *P.melanarius* adult beetles at Clayton field in 1993. Values calculated from data in Table 5.3.1.

Date	Day (i)	m	{mij (i-j)	Mi	Ai	Ai mi
15 June	1	0	0	0.0	-	-
18 June	4	0	0	2.08	1.00	0
21 June	7	2	9	9.76	3.15	6.3
25 June	11	2	8	10.30	5.83	11.6
28 June	15	0	0	10.60	6.92	0
01 July	18	2	9	15.00	6.39	12.7
05 July	22	1	20	14.70	7.99	8.0
09 July	26	0	0	11.50	10.30	0
12 July	29	0	0	8.64	12.50	0
15 July	32	0	0	7.38	13.10	0
19 July	36	0	0	4.53	17.10	0
22 July	39	0	0	5.22	13.30	0
26 July	43	0	0	3.82	15.20	0
29 July	46	1	3	5.42	10.40	10.4
				Observed 49		Estimated 49

Table 5.3.4 The population density estimate for *P.melanarius* adult beetles at Clayton field in 1993. Ni = total population size on day i. Li = number of losses from the population between days i and i+1. Bi = number of additions to the population between days i and i+1.

Date	Day	Ni (estimate)	Li (estimate)	Bi (estimate)	Density (No./m^2)
15 June	1	-	-	-	-
18 June	4	27	3.11	2.1	0.05
21 June	7	26	2.99	-2.4	0.05
25 June	11	20.6	2.37	109.0	0.04
28 June	15	127	14.63	-63.0	0.25
01 July	18	50	5.75	-7.5	0.10
05 July	22	36.8	4.23	-9.5	0.07
09 July	26	23	2.65	5.57	0.04
12 July	29	25.9	2.98	-16.0	0.05
15 July	32	7.38	0.85	11.6	0.01
19 July	36	18.1	2.08	-5.6	0.03
22 July	39	10.4	1.20	9.86	0.02
26 July	43	19.1	2.19	-3.4	0.04
29 July	46	13.6	1.56	-	-

However, good correlations have been found between *H.rufipes* pitfall catches and population density estimates (Luff, 1982). *H.rufipes* disperses into cereal fields from the field edge (Wallin, 1986) and the low recapture rate of *H.rufipes* in this study may have been due to beetles dispersing into the field away from the sampling area.

The populations of *P.melanarius* at the two study sites were very different. Sixty six *P.melanarius* beetles were caught at Clayton field but none were caught at Bog field. This reflects differences in the carabid fauna at the two sites (section 5.4).

P.melanarius has an even distribution in arable fields (Rivard, 1966), therefore population estimates were considered to be less affected by dispersal or aggregation. However, the accuracy of population estimates depend on the recapture rate and extensive nil-recaptures mean that no satisfactory estimate can be made (Attah, 1986). The recapture rate of *P.melanarius* was low in this study and therefore caution must be used in interpreting results.

P.melanarius was found at densities between 0.04 and 0.25/m^2 in Clayton field. Therefore it occurred at higher densities than in a similar study in oilseed rape in Yorkshire (0.022/m^2)(Attah, 1986), but at lower densities than studies in winter wheat in Sweden (0.73/m^2)(Ericson, 1978) and activity densities in wheat in Germany (1.6/m^2) (Basedows, 1973). *P.melanarius* has also been found at higher densities in woodland (2.5/m^2, Frank, 1967). However, *Pterostichus* species are considered to be woodland inhabitants (Attah, 1986; Wallin, 1986; Wallin and Ekbom, 1988) and may be more abundant in woodland than in open land (Williams, 1959). The population density estimates of *P.melanarius* in arable land in this study compare well with other studies.

Although some *P.melanarius* adults overwinter, most beetles are caught during the summer as new adults emerge. Maximum densities often occur in July and August (Rivard, 1966; Ericson, 1977). At Clayton field, the number of *P.melanarius* caught in the comparative study traps was declining as sampling ended (section 5.4). Therefore, *P.melanarius* densities were unlikely to increase after sampling ended

and the densities estimated in June and July were considered to be maximum field densities. *P.melanarius* were most abundant at Clayton field (section 5.4) but did not occur at densities which reduced slug damage to the chinese cabbage crop in the miniplot experiment (chapter six). However, *P.melanarius* does occur in cereals at densities which reduced slug damage in the miniplot experiment. This is discussed more fully in chapter six. The recapture rate of most carabids was low and the poor capture efficiency of some carabid species in pitfall traps has been documented (Halsall, 1990). The failure to capture a species does not mean it is absent from a field (Jones, 1969).

5.4 Comparative studies of the carabid fauna between three sites and four seasons

5.4.1 Introduction

Beetles with long activity periods, widespread distribution and frequent abundance have good potential as general predators (Luff, 1980). Predators need to be assessed in the field in terms of their spatial and temporal range (Wratten, 1982). These criteria were used to assess slug predators in the three field sites. The aim of the study was to identify well distributed and abundant species with stable populations.

Arable land supports an abundant carabid fauna which occurs under a variety of crops, including sugar beet (Dunning *et al.*, 1975), barley and wheat (Sunderland, 1975; Sunderland and Vickerman, 1980) and oilseed rape (Attah, 1986). Carabids are permanent members of the crop environment (Thiele, 1977). They can utilize many food sources and can persist in crops through periods of low pest density. They are therefore present during periods of pest immigration and population increase (Sunderland, 1975) and remain as a component resisting pest outbreak (Luff, 1982). *H.rufipes* has stable populations over several years (Luff, 1982), it is a field species (Pollard, 1968a) which prefers arable land (Luff, 1980; Wallin, 1985 and 1986). However, not all species are permanent residents of the crop and some species inhabit the crop margins. Species with restricted distributions will have less

of an impact on slugs than more widely distributed species.

The abundance of species can be affected by a number of factors such as soil type (Baker and Dunning, 1975) and humidity (Rivard, 1966). Agricultural land is in constant flux as fields are regularly disrupted by ploughing and pesticide applications which can occur at critical stages in the carabid's life cycle (Vickerman, 1992). Agricultural chemicals, including slug baits are often lethal to carabids (Edwards and Thompson, 1975; Buchs *et al.*, 1989). These factors can all influence the distribution, stability and abundance of carabids.

5.4.2 Experimental procedure

The beetles collected in pitfall traps from the three field sites in 1992 and 1993 for ELISA analysis (chapter four) were used to make comparative estimates in this study. The pitfall traps were plastic beakers measuring 72mm in diameter with a 60mm diameter base. The beakers were 105mm deep. Pitfall traps were sunk into the ground and three-quarters filled with water and a weak detergent solution (e.g. Sunderland, 1975).

On June 15th 1992, twenty pitfall traps were arranged in a five x four grid, five metres apart and thirty metres from the perimeter of Clayton, Square and Bog field (Fig. 4.2.1). The traps were emptied in 1992 and 1993 according to the sampling regime described in section 4.2.3.1.

5.4.3 Results

5.4.3.1 Relative catch/comparison with other studies

Between June 15th 1992 and August 11th 1993, 2184 carabids were captured. The proportion of each species contribution to the catch was calculated. *A.similata* was the most numerous species caught and constituted nearly 30 percent of the entire catch. *H.rufipes* was the second most numerous species (16 percent). *L.pilicornis* was the third most numerous species (12 percent), then *N.brevicollis* (eight percent) and *A.dorsale* (five percent). *P.melanarius* and *P.madidus* constituted 11 percent of

the catch. These seven species constituted 83 percent of the entire carabid catch (Table 5.4.1). The complete carabid catch is presented in Appendix 5.1.

The percentage contribution of each beetle to the catch was compared with five other studies (Table 5.4.2). *A.dorsale, P.madidus* and *H.rufipes* occurred in similar proportions compared with other studies, but larger proportions of *P.melanarius* were caught in other studies. In this study, the large number of *A.similata* caught in 1992, heavily influenced the proportion that the other species contributed to the catch. *A.similata* is infrequently recorded in other studies and when it was rare in this study (1993), the proportion of *P.melanarius* to other species at Square field, compares well with the other studies.

5.4.3.2 Seasonal catch and differences between sites

The data were split up into four equal periods of six weeks: A summer season in 1992 and 1993, an autumn season in 1992 and a spring season in 1993 (Table 4.2.1, chapter four). Some beetles were caught outside the four defined seasons. However, over 94 percent of all of the beetles caught were collected in the four defined seasons.

The carabid catch was not evenly spread over the four seasons (Table 5.4.3)(Fig. 5.1). The effect of vegetation (crop type) on pitfall catches has already been discussed in section 5.2. The two crops in this study (oilseed rape in 1992 and winter wheat from autumn 1992 until August 1993) could be considered as two separate habitats (see Greenslade, 1964b). However, much of the ground under both crops was bare. Consequently, ground vegetation was not thought to be restricting beetle movement which could affect pitfall catches. Therefore comparisons between the two crops over the four seasons were valid.

The weekly beetle catch was calculated for each site, transformed (Log_{10} n+1) to normalise the distributions (Attah, 1986) and used to compare changes in the seasonal catch at each site. One-way ANOVA were used to compare beetle numbers between seasons at the three sites. Significant differences in the seasonal

Table 5.4.1 The proportional contribution of the most frequently occurring species to the summer season catch at each site in 1992 and 1993. An asterisk (*) after the quoted value indicates that fewer than ten specimens were recovered during the season. *C.melanocephalus* is abbreviated to *C.melanoceph.*

	Clayton field		Square field		Bog field	
	1992	1993	1992	1993	1992	1993
A.similata	47.2	0.4*	20.2	1.9*	36.8	5.8*
H.rufipes	24.5	48.1	0.7*	1.9*		
P.melanarius	2.4	11.8	11.5	34.6		
P.madidus	5.1	22.9	0.9*	11.5*		
A.dorsale	7.9	0.7*		1.9*		
L.pilicornis	2.4	5.1*	29.3	15.3*	26.7	5.8*
N.brevicollis	25.7	17.3*	25.7		6.2	23.5*
C.melanoceph.					7.1	41.1*
H.aeneus	1.9	2.2*				

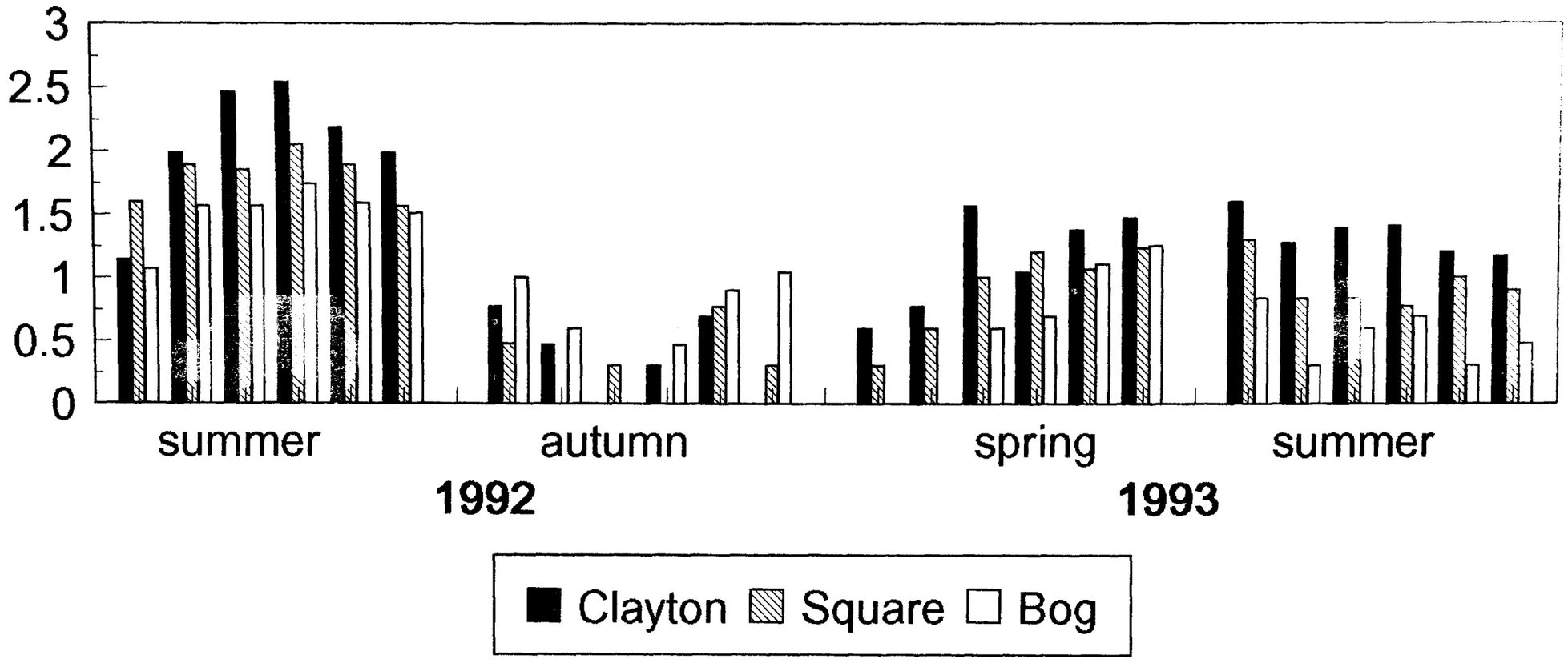
5.4.2 The percentage composition of the entire carabid catch in three fields in Northumberland from 15th June 1992 to 11th August 1993. The percentage catch of the commonest beetle species is compared with five other studies. Baker and Dunning, abbreviated to B & D. Speight and Lawton, abbreviated to S & L, Dixon and Mckinlay, abbreviated to D & Mc. Boiteau, abbreviated to Boit. *C.melanocephalus* is abbreviated to *C.melanoc.* *T.quadristriatus* is abbreviated to *T.quadrist.*

Species	This study	Jones (1976)	B & D (1975)	S & L (1976)	D & Mc (1992)	Boit. (1983)
A.similata	29.85			0.1		
H.rufipes	16.34	<15	12	0.5	0.1	61
L.pilicornis	12.95			1.3		
N.brevicollis	8.05		0.1	0.7	0.1	
P.melanarius	5.95	<15	28	78	66	6
P.madidus	5.35		0.2	0.3	10	
A.dorsale	5.17		2.9	9.6	0.4	
H.aeneus	2.15					
C.melanoc.	1.83					
T.quadrist.	1.14					
P.niger	0.73					

Table 5.4.3 The number of species (Spp.) and the total number (Nos.) of carabids caught in pitfall traps at the three sites in the four defined seasons.

	Summer 92		Autumn 92		Spring 93		Summer 93	
Site	Spp.	Nos.	Spp.	Nos.	Spp.	Nos.	Spp.	Nos.
Clayton	25	976	4	12	17	107	13	133
Square	20	407	5	9	17	55	12	52
Bog	17	208	5	31	10	35	7	17

Fig. 5.1 Distribution of all carabids over four seasons
The seasonal distribution of all carabid beetles in the summer and autumn of 1992 and the spring and summer of 1993.



catch were found at each site (Clayton: $F=20.85$, d.f.$=3,20$, $P<0.0001$; Square: $F=31.03$, d.f.$=3,20$, $P<0.0001$; Bog: $F=8.38$, d.f.$=3,20$, $P<0.001$). Seventy three percent of all the beetles caught were trapped during the summer period of 1992 and the lowest catches were recorded in the autumn season. Catches increased again in the spring of 1993 but the summer catch of 1993 was only 12 percent of the summer catch of 1992. Reductions in the summer catch were 86 percent at Clayton field, 87 percent at Square field and 92 percent at Bog field.

The carabid catch was not evenly distributed over the three sites. The weekly data for each season were used to investigate differences in the carabid catch between the three sites. Significant differences in the carabid catch between the three sites were found in the two summer seasons (summer 1992: $F=3.91$, d.f.$=2,15$, $P<0.05$; summer 1993: $F=26.90$, d.f.$=2,15$, $P<0.0001$). The summer catch at Clayton field in 1992 made up a large proportion of all the beetles caught (Table 5.4.4). A larger number of carabids were caught at Clayton field compared to Square and Bog field in the summer of 1992, spring 1993 and summer 1993 (Table 5.4.3). In the autumn, more carabids were caught at Bog field. In the other three seasons Bog field had the lowest carabid catch.

5.4.3.3 Seasonal catch of the common species

Beetle numbers were considered and thresholds established to identify rare, common and abundant species. In a similar study in oilseed rape, Attah (1986) ignored all species when less than 10 individuals were recovered, as these were considered to be rare species. In this study, species were considered rare when ten or less specimens were caught at a particular site in a season. Species were common when 11-50 specimens were recovered and abundant if 51 or more specimens were recovered. This categorisation of species partly depends on the number of pitfall traps used to make the catches. If more pitfall traps are used then the number of carabids likely to be caught increases and more species may be categorised as common. As this was a comparative study, the categorisation was considered valid. The weekly catches of each species were transformed ($Log_{10}\, n+1$) and ANOVA was used to identify changes in the occurrence of beetles between the

Table 5.4.4 The proportion of beetles caught at each of the sites in the four defined seasons, as a percentage of all of the beetles caught (2168) from June 15th 1992 to August 11th 1993.

	Summer 92	Autumn 92	Spring 93	Summer 93
Clayton	45.01	0.55	4.93	6.13
Square	18.77	0.41	2.53	2.39
Bog	9.59	1.42	1.61	0.78

four seasons.

A.similata

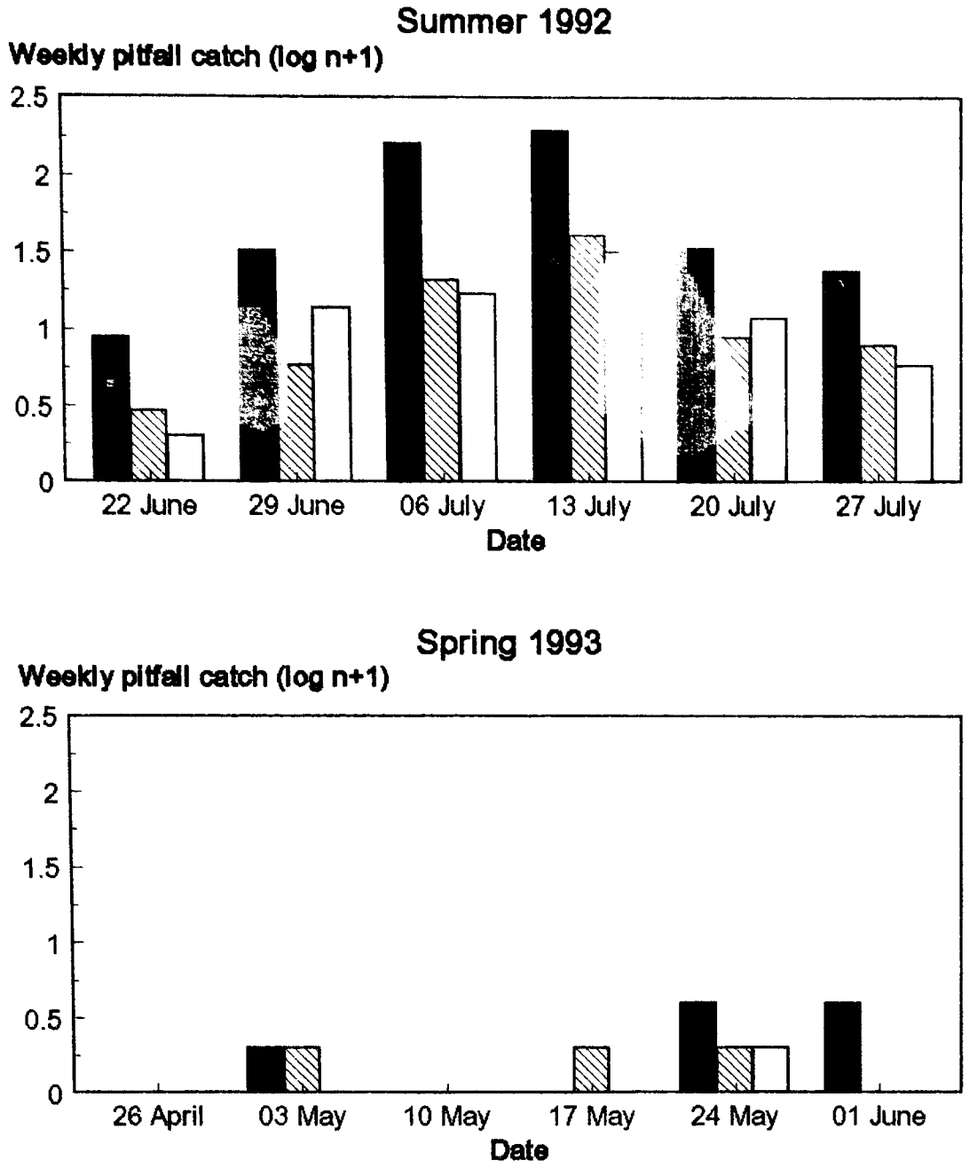
A.similata constituted nearly 30 percent of the entire carabid catch. However, ninety seven percent of the beetles were caught in a six week period in June and July 1992. *A.similata* was not caught during the autumn season, although a few *Amara* specimens were recovered. *A.similata* beetles were caught at very low densities in the spring and summer in 1993 and a total of four *A.similata* beetles constituted the summer catch of 1993 (Fig. 5.2). The weekly seasonal data from each site was combined and significant differences in the occurrence of *A.similata* beetles were found between the four seasons ($F=71.37$, d.f.$=3,68$, $P<0.0001$).

***Pterostichus* species**

The majority of the *Pterostichus* catch consisted of *P.melanarius*, *P.madidus* and *P.niger* beetles. These species were not evenly distributed over the four seasons or between the three sites. At Clayton field, both *P.melanarius* and *P.madidus* were common and *P.madidus* was the dominant *Pterostichus* species. *P.melanarius* was the only common *Pterostichus* species at Square field (Fig. 5.3) and all *Pterostichus* species were rare at Bog field. A single *P.madidus* specimen was recovered in early November from Clayton field and this was the only large carabid caught this late in the year from any of the three sites. A few *P.madidus* and *P.melanarius* beetles were active from early May in 1993 and occurred with increasing frequency in traps through to August (Figs. 5.3 and 5.4). The weekly seasonal data from all three sites were combined and significant differences were found in the occurrence of *P.madidus* beetles between the four seasons ($F=9.16$, d.f.$=3,68$, $P<0.0001$).

At Square field, *P.melanarius* was the only common *Pterostichus* species trapped in both years of the study. No *Pterostichus* beetles were recovered in the autumn season and only a single *P.madidus* and *P.melanarius* beetle were caught in the spring period of 1993 (Figs. 5.3 and 5.4). In the summer season of 1993, fewer *P.melanarius* beetles were caught in the summer season but catches increased in August as sampling ended (see Appendix 5.1). The weekly seasonal data from all

Fig. 5.2 Distribution of Amara similata over four seasons
The seasonal distribution of A.similata in the summer
and autumn of 1992 and the spring and summer of 1993



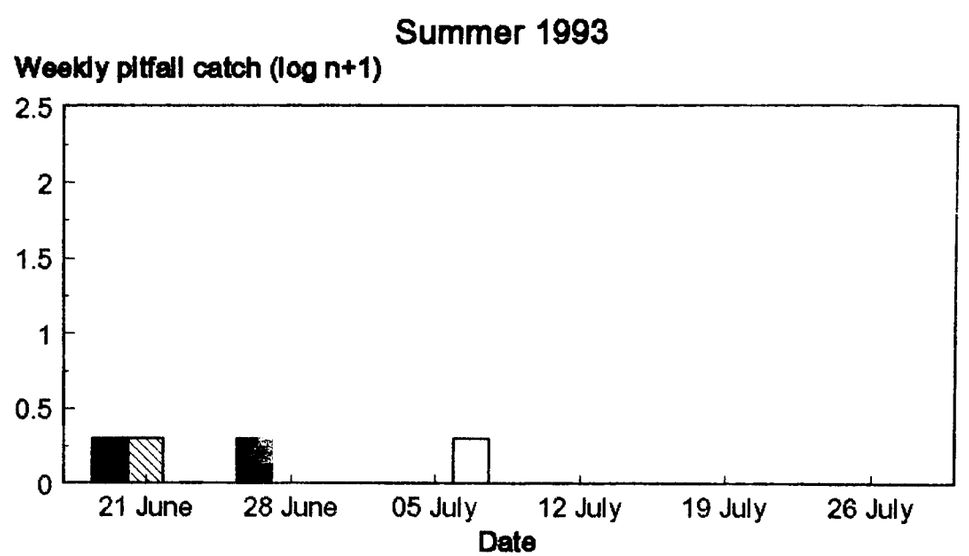


Fig 5.3 Distribution of Pterostichus melanarius over four seasons
The seasonal distribution of P.melanarius in the summer
and autumn of 1992 and the spring and summer of 1993

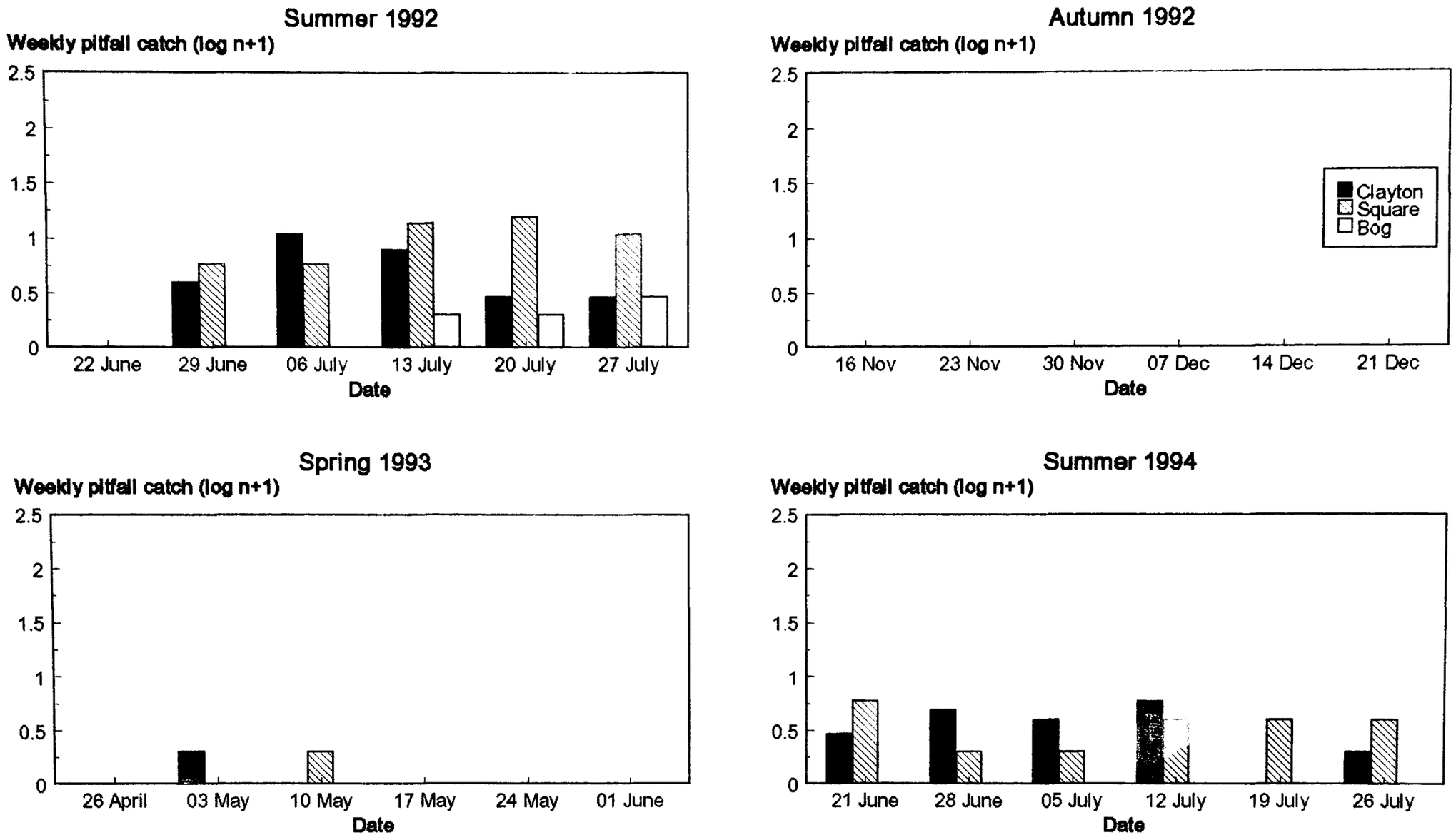
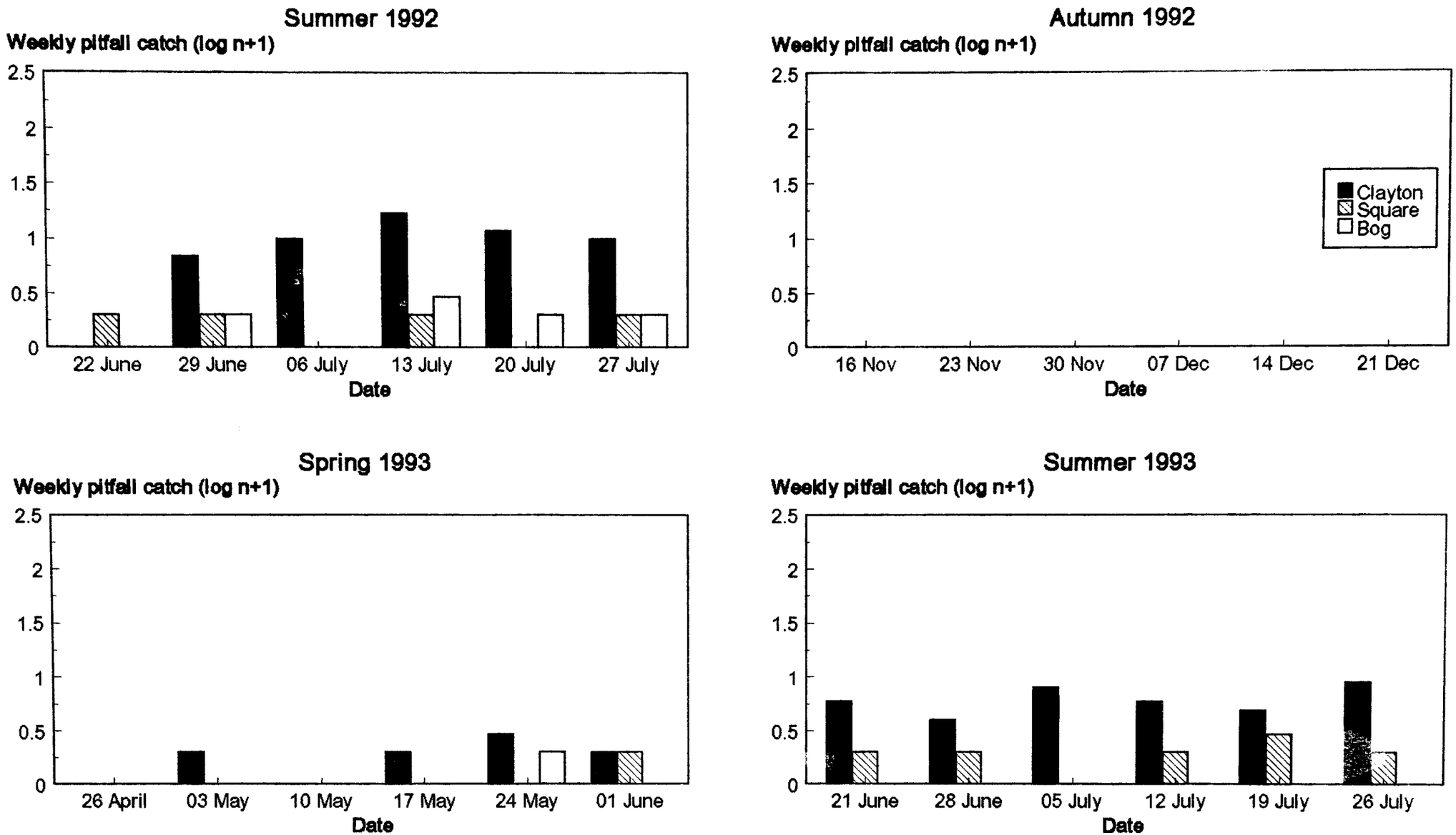


Fig. 5.4 Distribution of Pterostichus madidus over four seasons
The seasonal distribution of P.madidus in the summer
and autumn of 1992 and the spring and summer of 1993



three sites were combined and significant seasonal differences were found in the occurrence of *P.melanarius* beetles between the four seasons ($F=16.18$, d.f.$=3,68$, $P<0.0001$). At Bog field *P.madidus* and *P.melanarius* were caught in equally low numbers in the summer of 1992 (Figs. 5.3 and 5.4). No *Pterostichus* beetles were recovered in the autumn and a single *P.madidus* beetle caught in May was the only large *Pterostichus* beetle caught at Bog field in 1993. A single *P.nigrita* beetle constituted the 1993 summer catch (see Appendix 5.1).

***Harpalus* species**

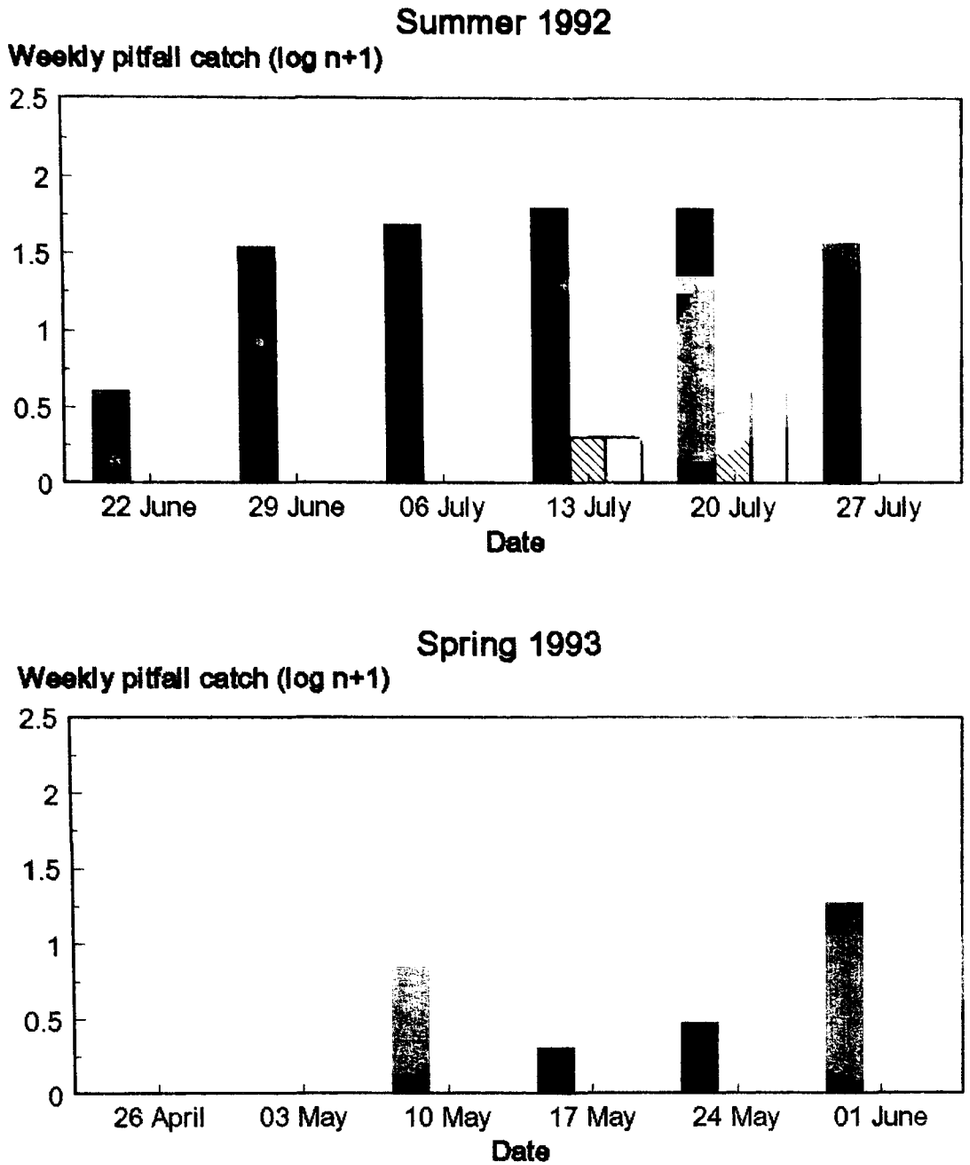
H.rufipes was rare at Square and Bog field and did not occur at Bog field in 1993 (Fig. 5.5). At Clayton field, *H.rufipes* was the second most numerous species in the summer of 1992. *H.rufipes* was not caught in the autumn period. In 1993, *H.rufipes* was recovered from mid-May through to the end of sampling (August) and was the most numerous species at Clayton field. The weekly seasonal data from Clayton field were combined and significant seasonal differences were found in the occurrence of *H.rufipes* beetles between the four seasons ($F=18.96$, d.f.$=3,20$, $P<0.0001$).

H.aeneus was rare at Square and Bog field and was only caught in the summer of 1992 at these two sites. It was common in the summer of 1992 and spring of 1993 at Clayton field and was the first large carabid caught in the spring in 1993 (Fig. 5.6).

A.dorsale

A.dorsale was only abundant at Clayton field. It was the third most numerous species caught at this site in the summer of 1992. A single beetle was caught in the autumn. In 1993, *A.dorsale* was rare at Clayton field and was caught mainly from mid-May to June. *A.dorsale* was the most numerous species caught at Bog field in the spring of 1993 and this was the only common species caught at this site in this year (Fig. 5.7). The weekly seasonal data at Clayton field was used to identify significant differences in the seasonal occurrence of *A.dorsale* beetles ($F=11.82$, d.f.$=3,20$, $P<0.001$).

Fig. 5.5 Distribution of Harpalus rufipes over four seasons
The seasonal distribution of H.rufipes in the summer
and autumn of 1992 and the spring and summer of 1993



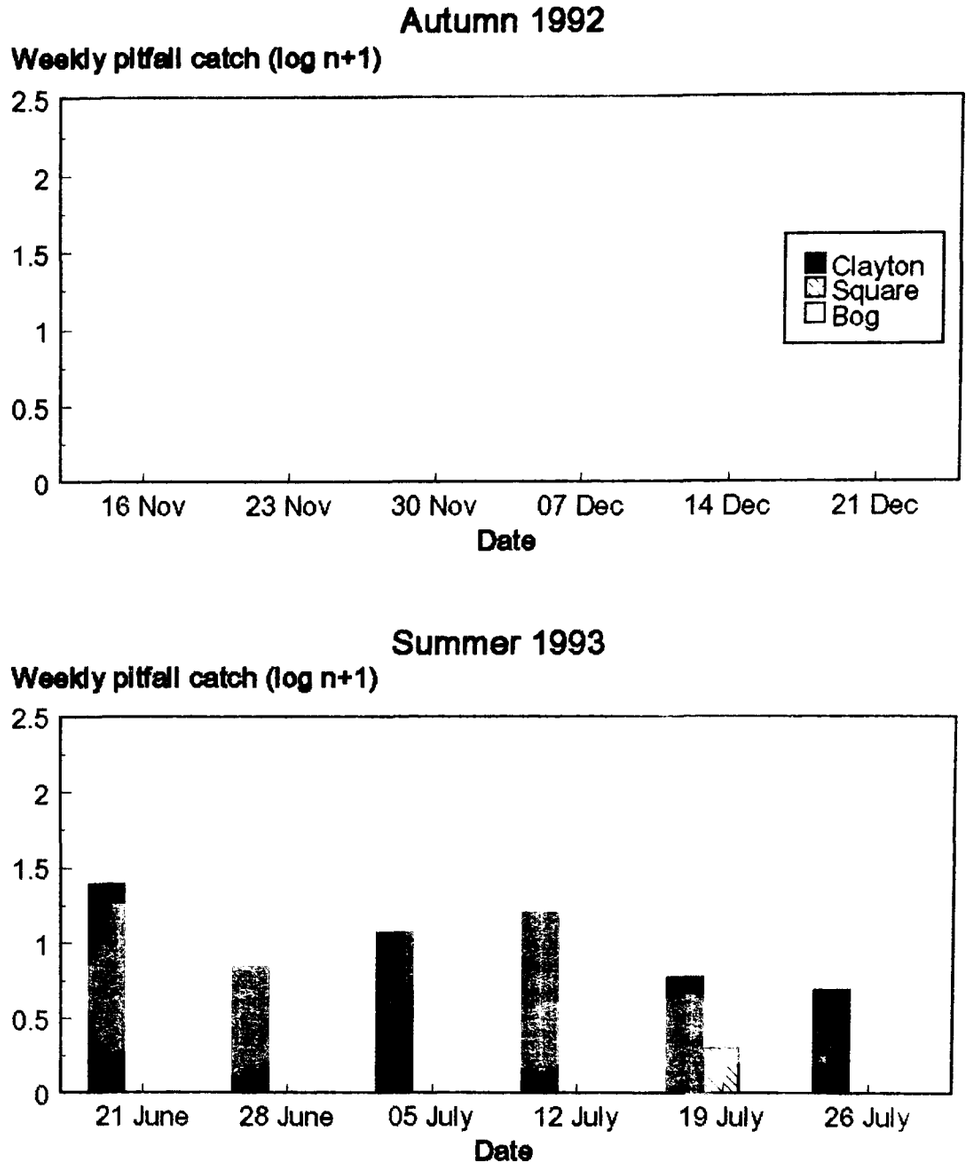
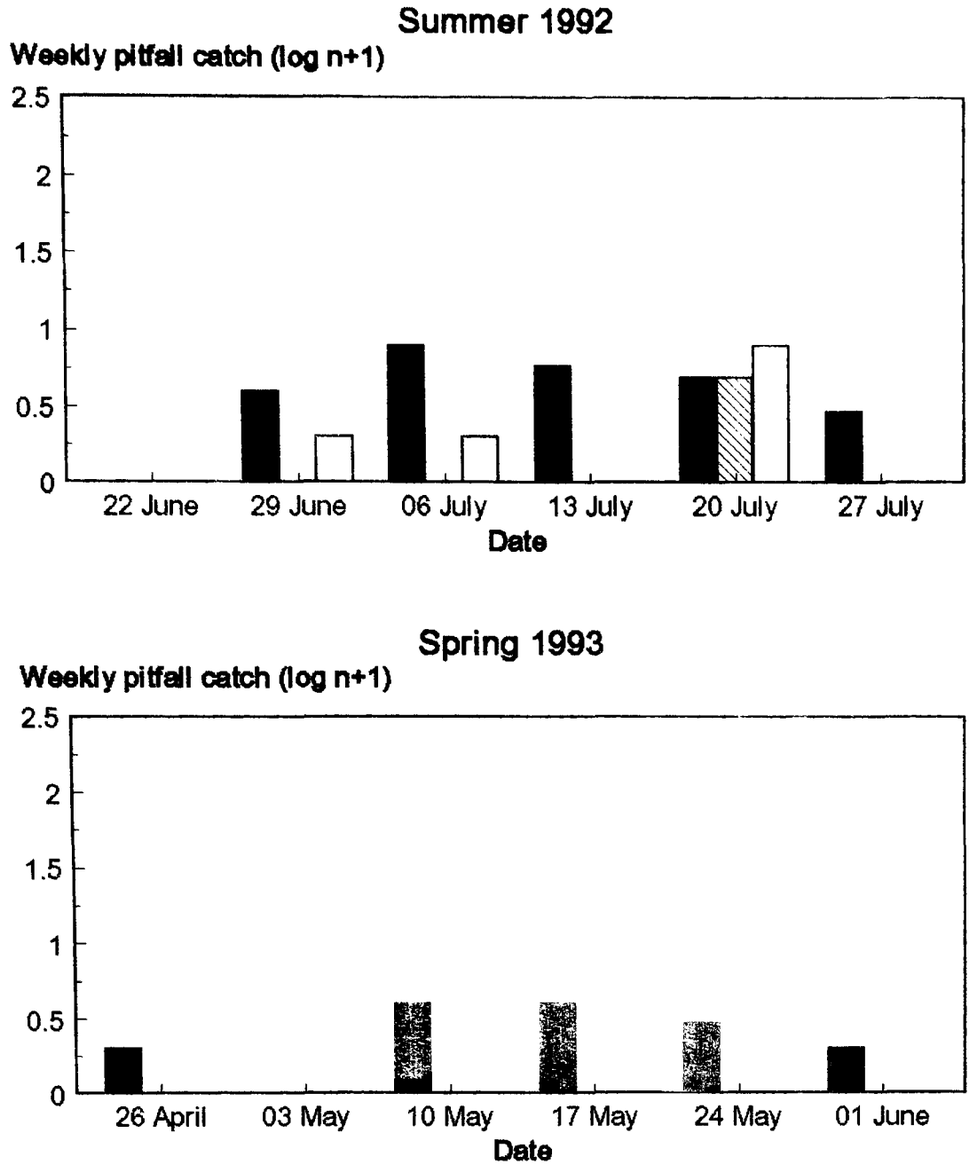


Fig. 5.6 Distribution of Harpalus aeneus over four seasons
The seasonal distribution of H.aeneus in the summer
and autumn of 1992 and the spring and summer of 1993



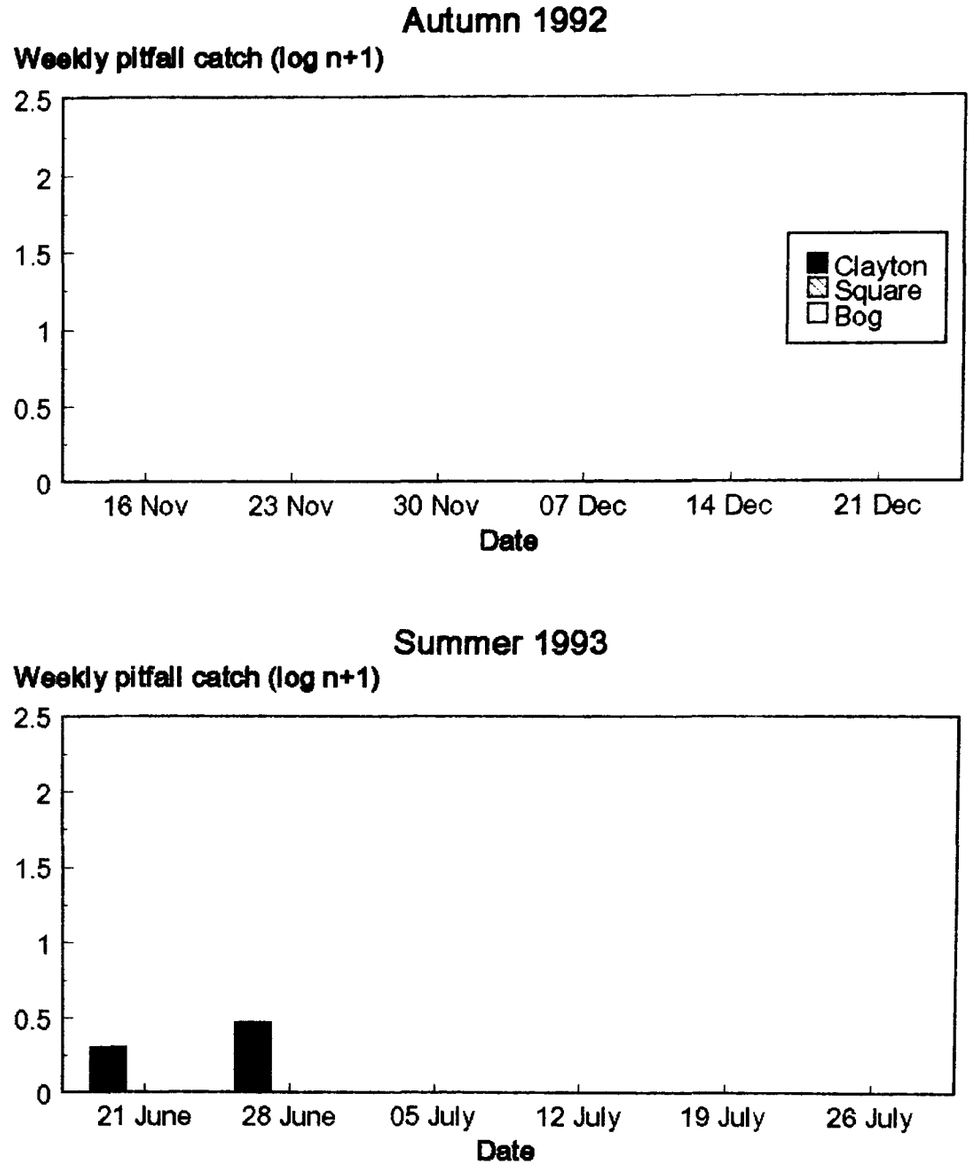
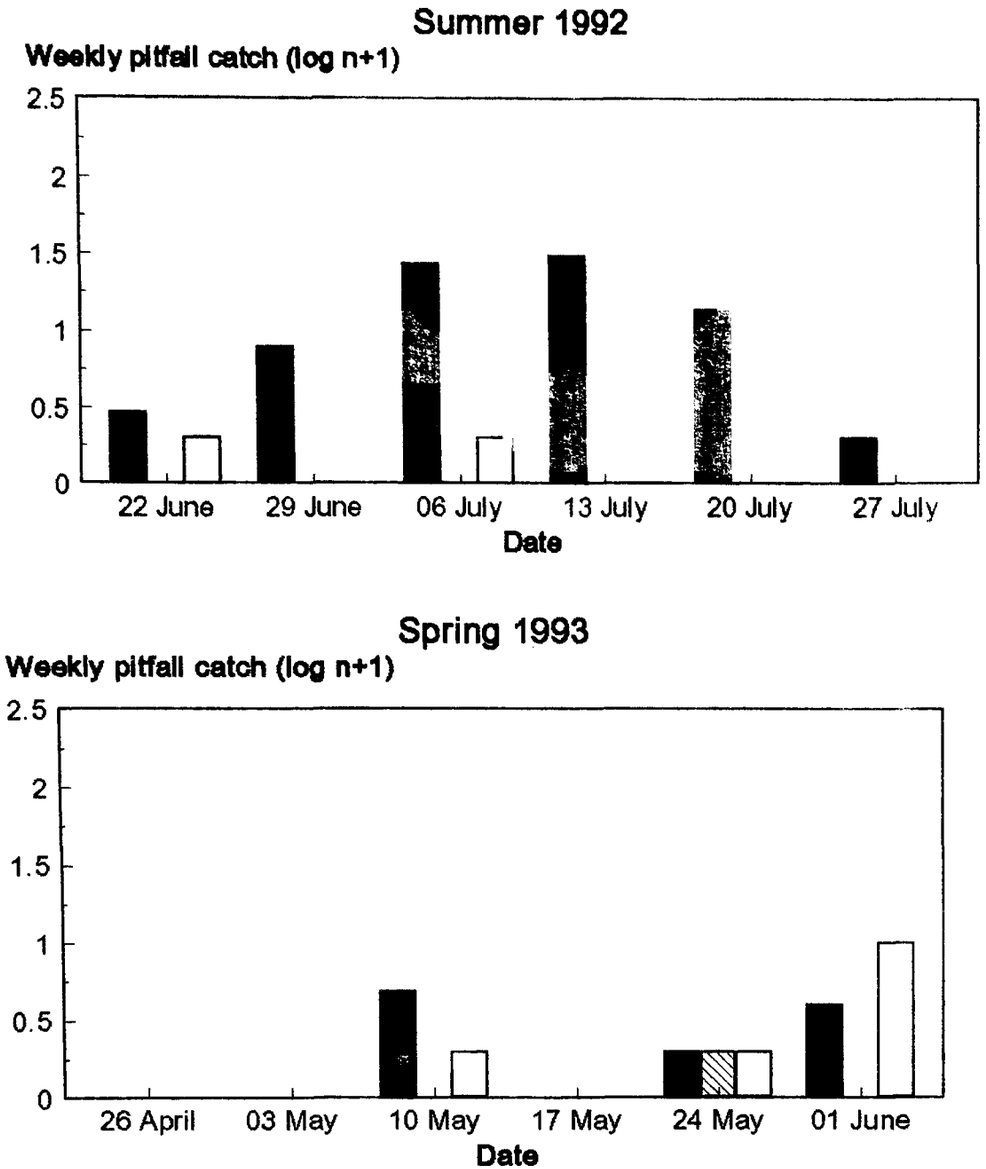
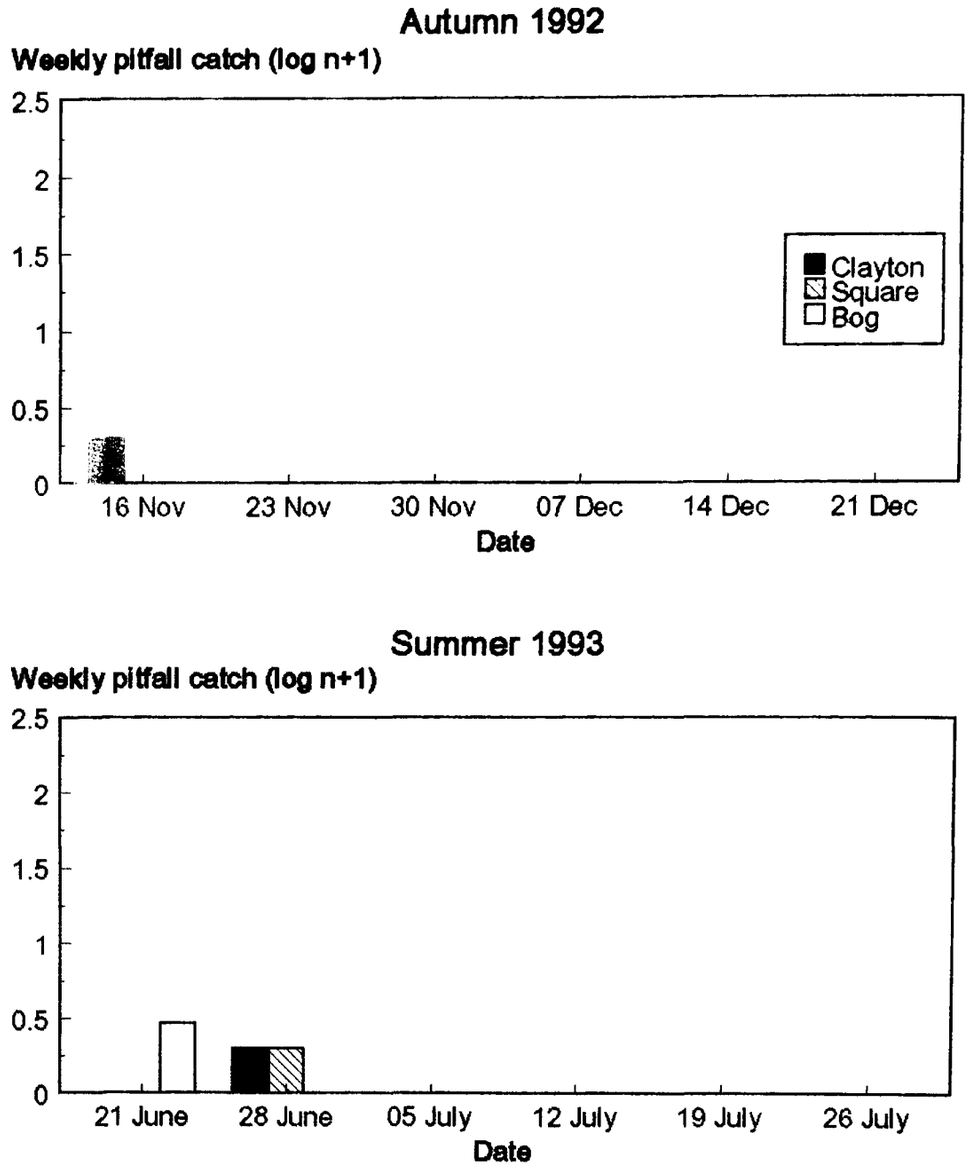


Fig. 5.7 Distribution of Agonum dorsale over four seasons
The seasonal distribution of A.dorsale in the summer
and autumn of 1992 and the spring and summer of 1993





N.brevicollis

N.brevicollis was the second most numerous species caught at Square field in the summer of 1992. It was caught in the autumn and spring at Square and Bog field but was much less numerous in the summer 1993 compared to the summer 1992 (Fig. 5.8). The weekly seasonal data at all three sites were used to identify significant differences in the seasonal occurrence of *N.brevicollis* beetles ($F=9.36$, d.f.$=3,68$, $P<0.0001$).

L.pilicornis

L.pilicornis was the most numerous carabid caught at Square field and the second most numerous carabid caught at Clayton and Bog field in the summer of 1992. No *L.pilicornis* beetles were recovered in the autumn season. In 1993, it was caught in the spring season and was rare in the summer season at all three sites (Fig. 5.9). The weekly seasonal data at all three sites were used to identify significant differences in the seasonal occurrence of *L.pilicornis* beetles ($F=30.65$, d.f.$=3,68$, $P<0.0001$).

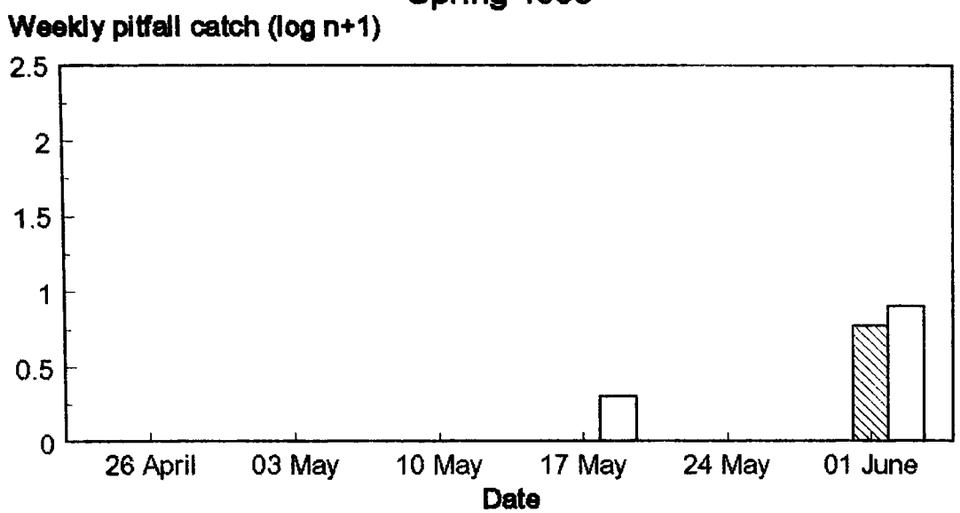
Other carabids

T.quadristriatus was caught almost exclusively in the autumn season (Fig. 5.10) and *Bembidion* species were caught predominantly in the autumn season and spring season of 1993 (Fig. 5.11). Three mollusc specialist species were caught, which included four *C.violaceus* and one *C.caraboides* beetle from Clayton field in 1992. A single *C.caraboides* beetle was caught in Bog field in 1992 and a single *C.problematicus* beetle was caught in Square field in June of 1993. *P.niger* was caught at low densities at Clayton and Square field in both summer periods and a single *A.parallelepipedus* beetle was recovered from Clayton field in the summer of 1992.

5.4.3.4 Stability

In the previous section, all of the carabid species investigated varied in their occurrence according to the season. In this section, the stability of the common summer carabid species were investigated by comparing how numerous they were

Fig. 5.8 Distribution of Nebria brevicollis over four seasons
The seasonal distribution of N.brevicollis in the summer
and sutumn of 1992 and the spring and summer of 1993



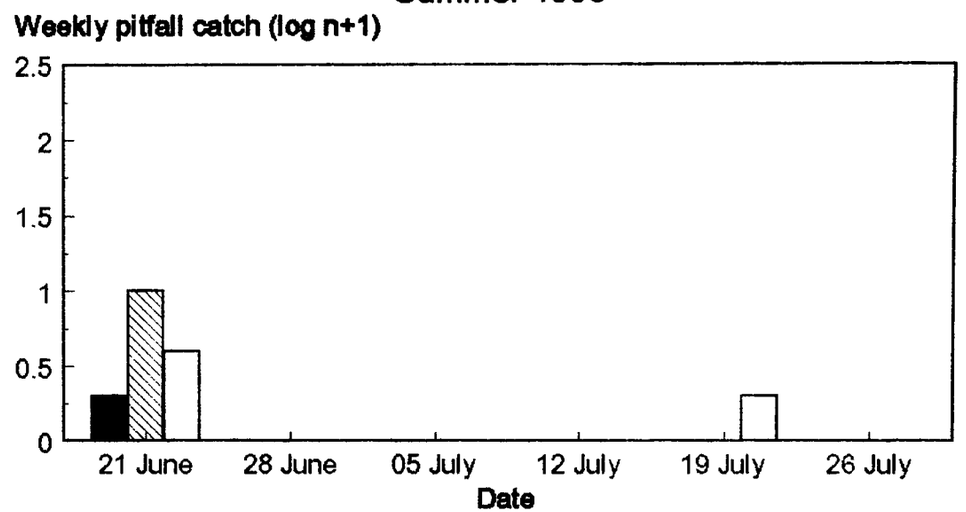
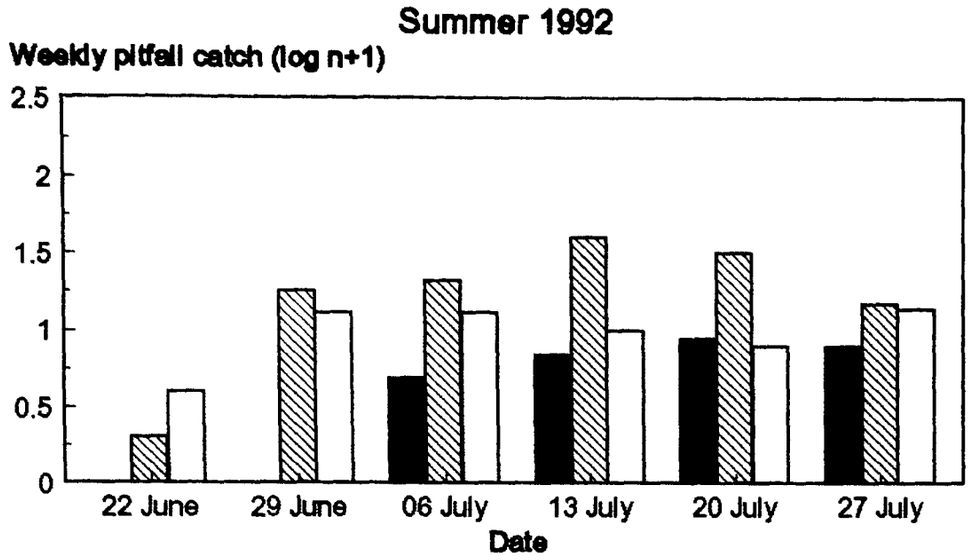
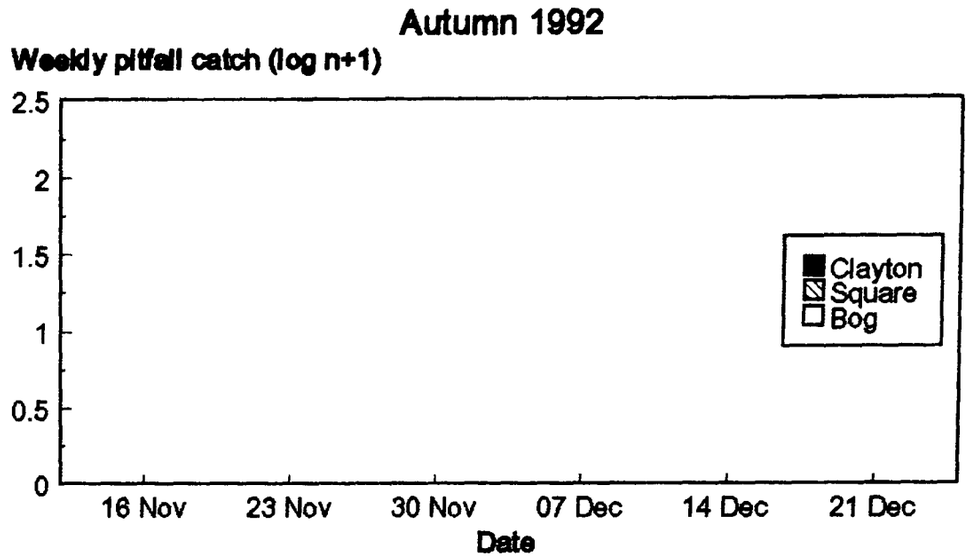
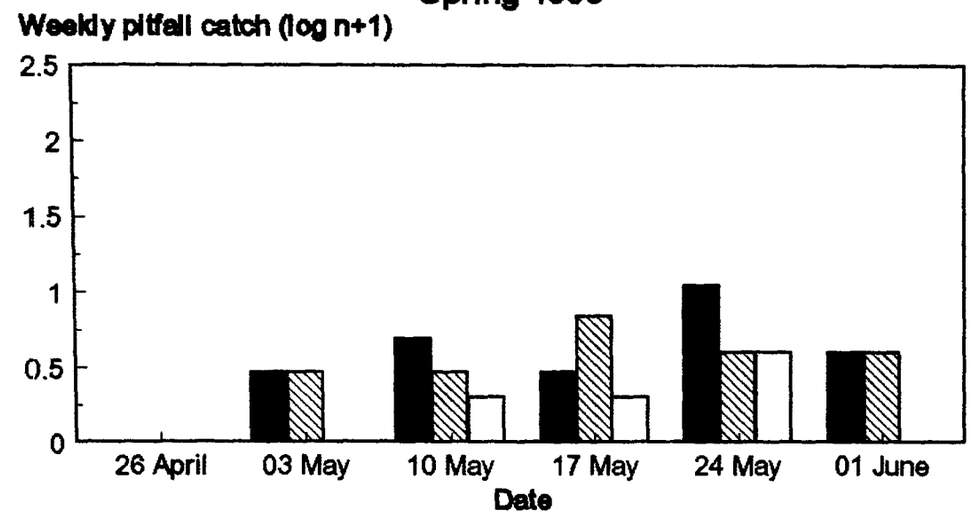


Fig. 5.9 Distribution of Loricera pilicornis over four seasons
The seasonal distribution of L.pilicornis in the summer
and autumn of 1992 and the spring and summer of 1993







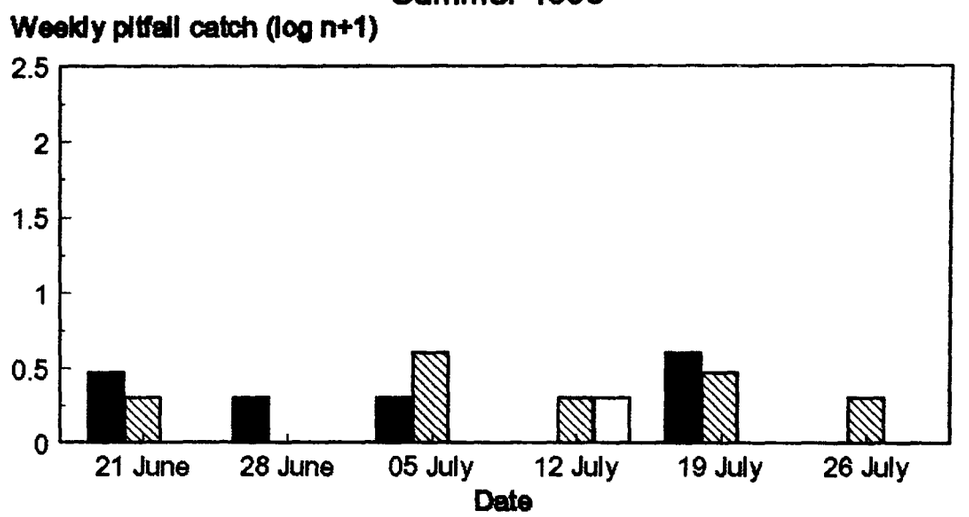


Fig. 5.10 Distribution of Trechus quadristriatus over four seasons
The seasonal distribution of T.quadristriatus in the summer
and autumn of 1992 and the spring and summer of 1993

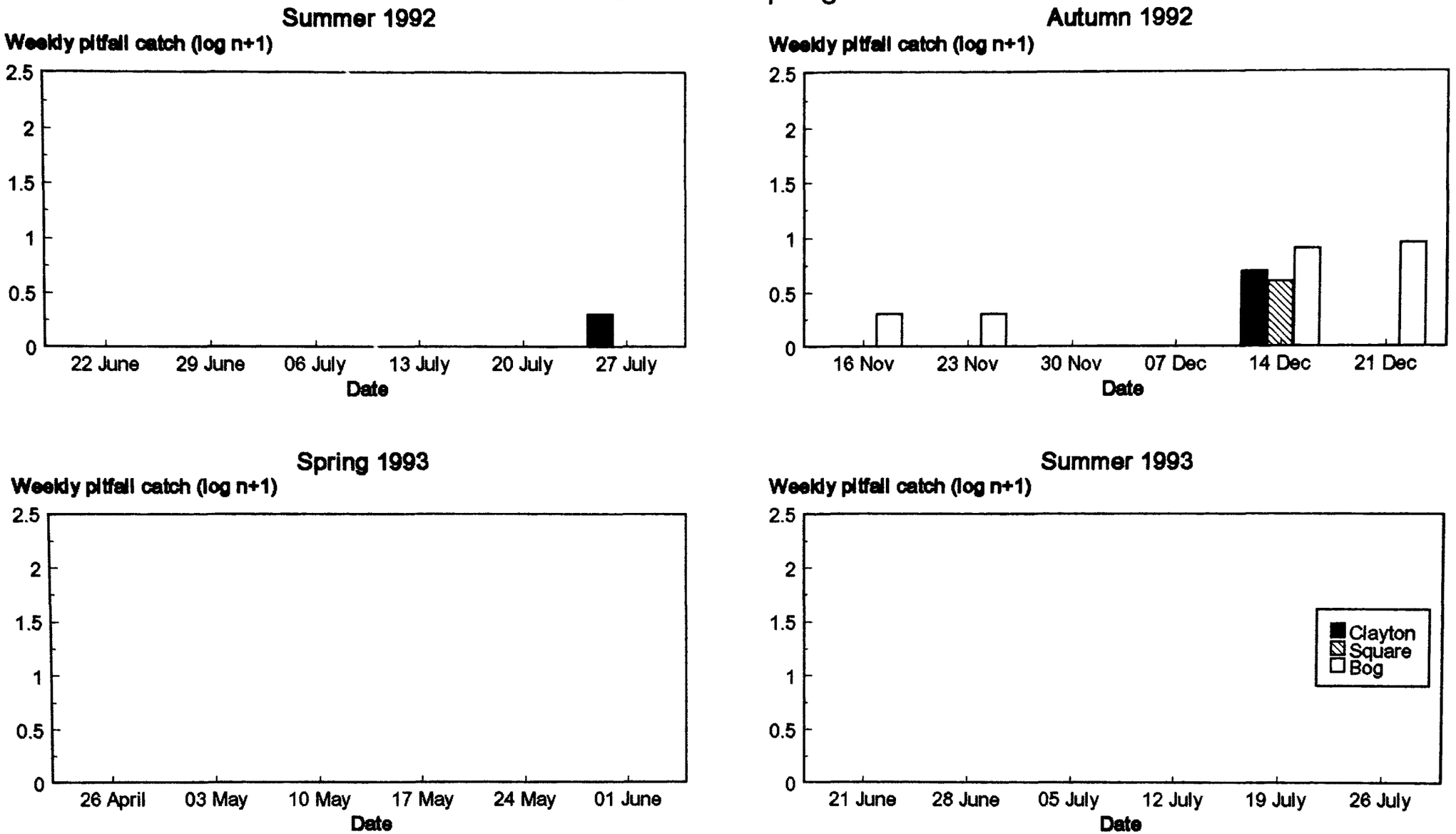
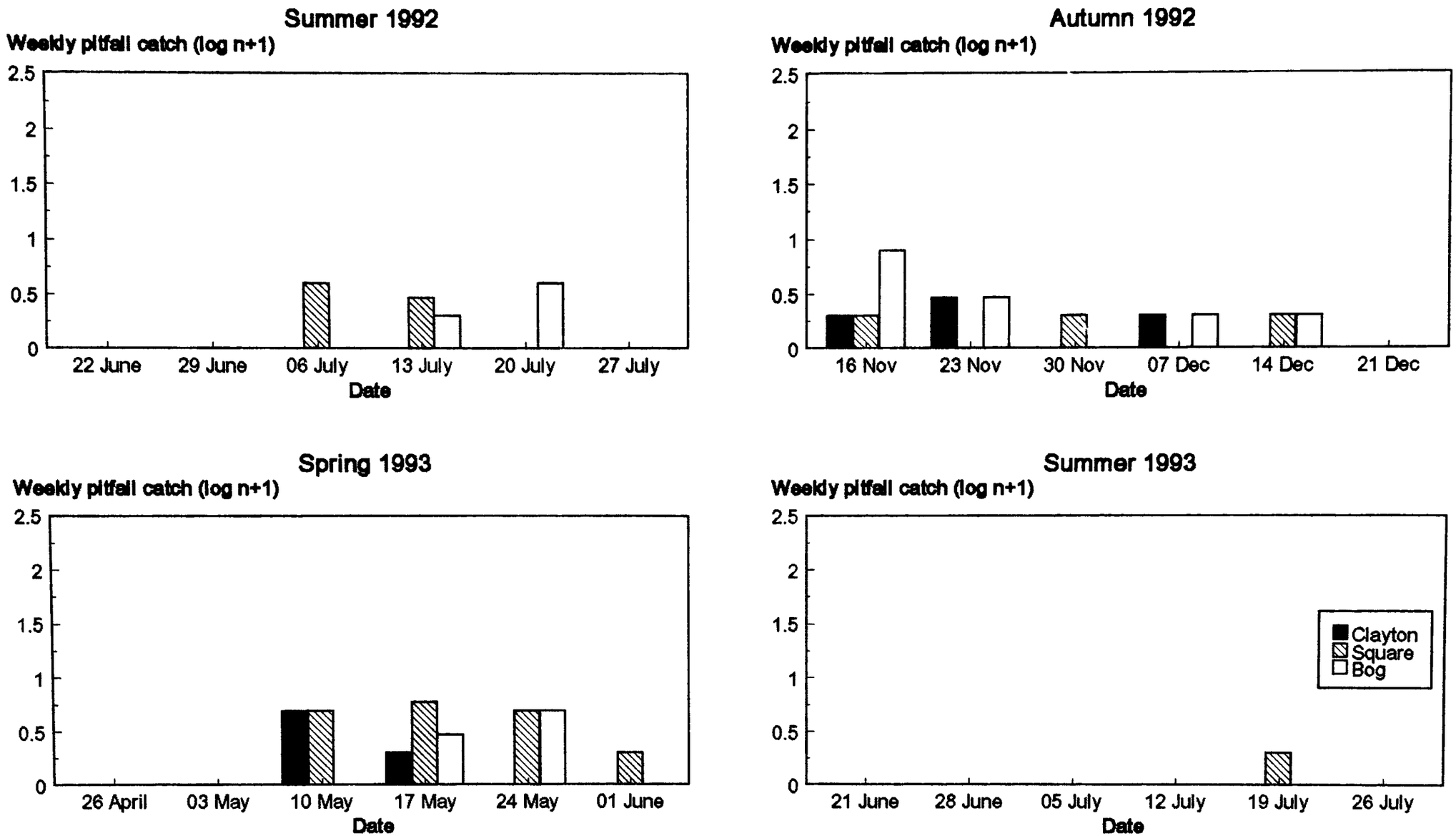


Fig. 5.11 Distribution of Bembidion species over four seasons
The seasonal distribution of Bembidion species in the summer and autumn of 1992 and the spring and summer of 1993



in the two summer seasons.

Clayton field

A.similata dominated the summer catch in 1992 and was rare in the summer of 1993. Similarly, *A.dorsale* was abundant in the summer of 1992 and was rare in the summer of 1993. All of the abundant/common species caught in the summer of 1992 were rare in the summer of 1993, except *P.madidus*, *P.melanarius* and *H.rufipes*. Although fewer of these three species were caught in 1993, they constituted a larger proportion of the catch (Table 5.4.1). *Pterostichus* and *Harpalus* beetles constituted 32 percent of the summer catch in 1992, this increased to nearly 83 percent in 1993.

Square field

In 1992, the summer catch was dominated in ascending order by *L.pilicornis, N.brevicollis*, *A.similata* and *P.melanarius*. In 1993, fewer *P.melanarius* beetles were recovered but this species dominated the summer catch (34 percent) as all the other species were rare. Large *Pterostichus* beetles constituted 46 percent of the summer catch in this year (Table 5.4.1).

Bog field

In 1992, *A.similata* and *L.pilicornis* constituted 64 percent of the summer catch (Table 5.4.1). In 1993, only one specimen of each species were recovered. Large *Pterostichus* beetles and *H.rufipes* were rare at this site in 1992 and did not occur at all in 1993, when the summer data was described by only seventeen beetles. *C.melanocephalus* was the most numerous summer species in 1993 and seven specimens were recovered.

Species

The summer data for each common carabid species was considered separately. The weekly summer catches at each site were combined and Wilcoxon matched-pairs tests were used to compare the numbers of each common carabid species caught between the two summer seasons.

Significant differences in the occurrence of *A.similata* (n=18, T=0, P<0.01), *N.brevicollis* (n=18, T=18, P<0.01) and *L.pilicornis* (n=18, T=5, P<0.01) were found using data from all three sites. Significant differences in the occurrence of *A.dorsale* (n=6, T=0, P<0.05) and *H.rufipes* (n=6, T=1, P<0.05) were found using data from Clayton field. All of these species were more numerous in the summer season of 1992 compared to the summer season of 1993.

Although significantly fewer *H.rufipes* beetles were trapped at Clayton field in the summer of 1993, it was still abundant at this site and was the most numerous carabid caught in 1993. Significantly fewer *A.similata*, *N.brevicollis*, *L.pilicornis* and *A.dorsale* beetles were caught in 1993, but unlike *H.rufipes*, populations of these species were severely reduced.

P.madidus was only abundant at Clayton field and there were no significant difference (P<0.05) in the frequency at which this species was caught between the two summer seasons at this site. *P.melanarius* was common at Clayton and Square field and there were no significant difference (P<0.05) in the frequency at which this species was caught between the two summer seasons at these two sites.

5.4.3.5 Diversity

The number of species present at each site is the simplest and most useful measure of local diversity (Magurran, 1988). This is termed species richness and was used to compare the carabid diversity between the three sites over the four seasons.

The carabid fauna was more diverse in the summer of 1992 at each site, when carabids were also most numerous (Table 5.4.3). The carabid fauna was least diverse in the autumn season at each site when carabids were least numerous. The autumn catch consisted mainly of *T.quadristriatus* and *Bembidion* beetles. Four *N.brevicollis* beetles and a single *P.madidus* beetle caught at the start of November constituted the autumn catch of large/medium sized beetles.

The carabid fauna was less diverse in the summer season of 1993 compared to the

summer season of 1992 at all three sites (Table 5.4.3). In 1993, the carabid fauna was more diverse in the spring season compared to the summer season. Carabids were also more numerous in the spring of 1993 at Square and Bog field compared to the summer season.

Approximately half the species recorded in the summer period of 1992 were recovered in the following summer at all three sites. One additional species was recovered from each site in 1993 which was not present in 1992 (see Appendix 5.1). The carabid fauna at Bog field was less diverse in the summer of both years compared to the other two sites and was particularly impoverished in the summer of 1993 when only seven species were recovered.

5.4.4 Discussion

5.4.4.1 Relative catch/comparison with other studies

A.similata, *H.rufipes*, *L.pilicornis*, *N.brevicollis*, *A.dorsale*, *P.madidus* and *P.melanarius* beetles represented over 83 percent of the catch. The catch was heavily biased by the abundance of *A.similata* in the summer season of 1992 which constituted 30 percent of all beetles caught. Consequently, the relative importance of each species varied according to the season and year. The common species found in this project are comparable with other European studies: *P.melanarius*, *H.rufipes*, *B.lampros*, *P.cupreus*, *A.dorsale*, *Agonum muelleri* (Herbst), *H.aeneus* and *T.quadristriatus* are found in decreasing order of importance in northern Europe (Thiele, 1977).

The abundance of *A.similata* is peculiar to this study. Other species are occasionally abundant in arable land in particular localities. *A.apricaria* constituted 80 percent of the carabid catch in sandy soil in East Anglia (Baker and Dunning, 1975) and *C.nemoralis* is abundant in potato fields in Canada (Boiteau, 1983).

P.melanarius and *P.madidus* are often well represented in other studies and are abundant in agricultural land in Scotland (Gordon and Mckinlay, 1986; Chapman, 1994). *P.melanarius* is abundant in winter wheat and potatoes in Britain (Baker and

Dunning, 1975; Jones, 1976; Speight and Lawton, 1976; Dixon and Mckinlay, 1992). In this study, these two *Pterostichus* species were less numerous than in other studies, but both were common species. *H.rufipes* was abundant at Clayton field, it is abundant in agricultural land in the UK and Canada, under cereals, potatoes and sugar beet (Baker and Dunning, 1975; Jones, 1976; Boiteau, 1983).

5.4.4.2 Seasonal catch and differences between sites

Carabids were most numerous in the spring and summer seasons. In the autumn season, smaller species such as *T.quadristriatus* and *Bembidion* species were active in low numbers. As most large and medium sized beetles were numerous in the spring and summer, these two seasons are the most important for carabid slug predation.

The abundance of carabids in the three fields will influence the degree of slug predation. Carabids were much less abundant at Bog field compared to the other two sites. In addition, slug killing species were less common at Bog field and fed to a lesser extent on slugs (chapter four). The carabid fauna at Bog field was therefore exerting less of an impact on slugs compared to the other two sites. This is discussed in the following section.

5.4.4.3 Seasonal catch of the common species

Many of the carabids found on arable land in this project kill and/or feed on slugs (chapters two and four) and were considered to be exerting some impact on the slug populations in the three fields. The discussion on the abundance of beetle species in this section is based on the findings of this study. Comparisons between this study and other studies on beetle abundance are found in sections 5.4.4.4 and 5.4.4.5. *A.similata* killed slugs in laboratory studies and ate slugs in the field. It was abundant at all three sites but 97 percent of all beetles were caught in one season. Therefore *A.similata* is an occasionally abundant slug predator with a short activity period.

A.dorsale also killed slugs in laboratory studies. It has a longer activity period than

A.similata but was caught mainly at Clayton field where it was abundant in one summer and rare the following summer. Therefore, *A.dorsale* is an occasionally abundant slug predator with a long activity period but localised distribution.

N.brevicollis killed slugs in laboratory studies and ate slugs in the field. Although it was common at Clayton and Bog field, it was only abundant at Square field. It was caught in the autumn and spring periods and has a relatively long activity period. Therefore *N.brevicollis* is an occasionally abundant slug predator with a long activity period which was found at all three field sites.

H.rufipes killed slugs in laboratory studies and ate slugs in the field (chapter four). It was abundant at Clayton field in both summer seasons where it was active from mid May until August when sampling ended. The long activity period, abundance and widespread distribution of *H.rufipes* give it good potential as a general predator (Luff, 1980). In this study, it was a locally abundant slug predator with a long activity period. *H.aeneus* killed slugs in laboratory studies and was the first large carabid to be caught in the spring. Although this predator is active early in the year when few other carabid were active, none of the beetles caught had eaten slugs (chapter four). Therefore, *H.aeneus* is probably of little importance as a slug predator.

P.madidus and *P.melanarius* were the two most frequently caught *Pterostichus* species in this project. Although these two species were caught more frequently in the summer seasons, they had relatively long activity periods. Both species were active in the spring and *P.madidus* was active until November. These two slug predators were distributed unevenly between the field sites. At Clayton field, *P.melanarius* was common and *P.madidus* was abundant. *P.melanarius* was common at Square field and both species were rare at Bog field. Both species are common in agricultural land in Britain (Jones, 1976; Chapman, 1994). They both eat slugs in the field (chapter four) and are potentially very useful slug predators.

P.niger and *A.parallelepipedus* were caught in relatively low numbers in this study.

However, *P.niger* is abundant in other locations and constituted between 32-50 percent of the carabid catch in a study in Poland (Grum, 1959). *P.niger* is also common in cereal fields in Sweden (Wallin, 1985). *A.parallelepipedus* is restricted to the margins of arable land (Pollard, 1968a and 1968b) but it is a voracious slug predator and large numbers feed on slugs in the field (Symondson, 1992). Although these two species are not abundant they are useful slug predators.

Three mollusc specialist species were caught in relatively low numbers at the three sites. These beetles can kill large slugs, have good predation rates, high capture efficiencies and are therefore useful predators. *C.violaceus* was the most frequently caught mollusc specialist and it is a voracious slug predator which always fed on slugs in the field (chapter four). It can make multiple kills in a single night and had the highest predation rate in laboratory studies (section 2.3). Although these species are not abundant, they may have a greater impact on slug populations than smaller, more abundant species.

Factors affecting abundance

Fields within the same locality can have distinct carabid faunas. The abundance of slug killing species is important in determining the overall impact that the carabid population exerts on slug populations at each site: *P.melanarius* and *P.madidus* are both slug predators (chapter four) but both were rare at Bog field which had a low numbers of slug killing species.

Other slug predators were locally distributed. *A.dorsale* was locally abundant in this study and in other studies (e.g. Wallin, 1985). *A.dorsale* overwinters in field hedges (Pollard, 1968a) which may be important in determining its occurrence at a particular site. Pollard (1968a) successfully reduced the abundance of *A.dorsale* by removing the ground flora of an experimental hedge. Hedges contribute to the diversity of species in cultivated areas as they add physical complexity and provide overwintering sites (Pollard, 1968b). The availability of suitable overwintering habitats such as hedges, banks and vegetative characteristics in field boundaries can increase the numbers of overwintering predators, encourage predatory Coleoptera

and increase their abundance on farmland (Sotherton, 1984 and 1985).

These factors may have contributed to the differences in carabid abundance at the three sites in this project. Bog field had an impoverished carabid fauna and was surrounded by arable fields on three sides and a road on the fourth. Clayton field had the most abundant and diverse carabid fauna and was directly adjacent to a large wood and other arable fields. Square field was in close proximity to a large wooded area and adjacent to a small wooded area, pasture and other arable fields.

The importance of woods in providing reserves of/or overwintering sites for predators is widely debated (e.g. Thiele, 1977). The size and location of fields may affect colonisation by carabids and the resulting degree of biological control (Boiteau, 1983). Fields situated in wooded areas are better protected from colorado beetles by carabids than fields in more open areas (Kary, 1970). However, woodland is a poor habitat for overwintering predators and only a few species (e.g. *L.pilicornis*) overwinter in woodland in significantly higher numbers than in other habitats (Sotherton, 1984). The impoverished carabid fauna at Bog field may be due to the relative uniformity of the land surrounding that field in comparison to Clayton and Square field. A wide range of boundary types, such as those at Clayton and Square field, may increase Coleoptera diversity even on an individual farm scale (Beard and Mauremootoo, 1994).

Larval stages of *P.melanarius* utilize fields for their development (Wallin, 1987). The tendency for Bog field to waterlog may have affected the survival of soil dwelling larval stages of these species and other slug predators. Good drainage in raised boundary banks can increase the survival of overwintering beetles (Sotherton, 1985). Other factors such as field aspect and field size may influence beetle abundance (Pollard, 1968b; Wallin, 1985). These factors require intensive rather than extensive studies and were not within the scope of this study.

Crop type

The predatory fauna of oilseed rape is similar in species composition to other

arable crops grown in the UK but relative abundances can depend on crop type (Attah, 1986). In this study, most species were more abundant in oilseed rape compared to winter wheat.

The two crops have distinct microclimate conditions which affect beetle catches. More *P.melanarius, A.dorsale* and *N.brevicollis* beetles are trapped under sugar beet compared to cereals, as the shade given by the vegetation reflects their nocturnal habits and the beetles are active for longer (Pollard, 1968b; Baker and Dunning, 1975). Consequently more beetles are caught. In this study, all three species were caught more frequently under oilseed rape compared to winter wheat. This may have an impact on slug predation, as increased predator activity in a crop where slugs are very active (oilseed rape) will result in more slugs being encountered by the predator (e.g. Frank, 1971).

Some species such as *A.similata* may have distinct habitat preferences. In this study, *A.similata* was abundant in oilseed rape but rare in winter wheat. This confirms Lindroth's (1974) view that *A.similata* is a resident of cruciferous crops. *A.similata* probably disperses to its preferred habitat by flying. Larger beetles disperse by running which is affected by a number of factors including soil surface temperature and density of plant cover (Jones, 1976). Plant cover affects humidity and the numbers of carabids caught (Rivard, 1966). More beetles move into the crop as the growing plants protect the soil surface from extremes of temperature which can cause desiccation of the beetles (Jones, 1976). Mid-crop densities of *A.dorsale* in cereal fields in mid-summer are significantly correlated with weed cover (Coombes and Sotherton, 1986). Root crops with broad leaves and rape offer more shade and greater humidity for beetles than cereals which have linear leaves (Jones, 1979).

In this study, *P.melanarius* was caught more frequently in oilseed rape compared to winter wheat. *P.melanarius* prefers wooded habitats which have moist hiding places (Wallin, 1986; Wallin and Ekbam, 1988). The oilseed rape canopy offers shaded conditions and high humidity which not only reflect the preferred habitat and nocturnal habits of *P.melanarius*, but also reduce the possibility of desiccation

(Attah, 1986).

Humidity can affect populations of alternative prey which carabids exploit. The high humidity under oilseed rape allows large numbers of Collembola to develop early in the year. High numbers are not found in barley until late July (Attah, 1986). Positive correlations exist between the density of macroscopic Isotomidae (Collembola) and the density of predatory Coleoptera in cereal ecosystems (Potts and Vickerman, 1974). Species such as *L.pilicornis* and *N.brevicollis* feed extensively on Collembola (Penney, 1966; Sunderland, 1975; Bauer, 1982). In this study, Collembola were abundant in pitfall traps in oilseed rape but few were found in winter wheat. The reduced availability of Collembola prey may explain the reduced catches of *L.pilicornis* and *N.brevicollis* in 1993.

If few Collembola are available for smaller carabids, the abundance of other species which predate smaller carabids may also be affected. *Pterostichus* species feed on smaller carabids and coleopteran adults are prominent in the diet of *P.melanarius, H.rufipes* and *N.brevicollis* (Mitchell, 1963b; Sunderland, 1975). Low numbers of smaller beetles in winter wheat could account for the reduced numbers of larger beetles caught in 1993. Other dietary factors may be operating: *A.dorsale* is more abundant on barley than oilseed rape as aphids are more abundant on cereal crops and *A.dorsale* prefers aphid prey (Attah, 1986). The availability of alternative foods will alter the density of predators which can prey on slugs (Hunter, 1978).

5.4.4.4 Stability

Predator stability and persistence in arable fields is an important factor which influences the impact that polyphagous predators can exert on pests from year to year. The activity of any species can change between years (Baars, 1979). Ideally, yearly catches should be used to make year to year comparisons of particular species. In this project, stability was assessed as changes in predator numbers between the two years. Data were collected from January to August in 1993, whereas in 1992, data were collected from June to December. Despite the shorter sampling period in 1992, larger numbers of most species were caught in 1992.

The most dramatic change in numbers of a single species occurred with *A.similata* which was abundant in 1992 but rare in 1993. Other authors have found *Amara* species to be more stable (e.g. Baker and Dunning, 1975; Jones, 1976). *A.dorsale* and *N.brevicollis* showed significant changes in the number of beetles caught between the two summers in this study and in other studies (Jones, 1976 and 1979; Attah, 1986).

In this study, *P.madidus, P.melanarius* and *H.rufipes* had relatively stable populations between years. At Clayton field *H.rufipes* was significantly less numerous ($P<0.05$) in the summer of 1993 compared to the summer of 1992 (see 5.4.3.4), but was the most numerous species caught at this site in 1993. All of the other abundant species recorded in 1992 (with the exception of *P.madidus*) were rare the following year. Although fewer *H.rufipes* beetles were caught in 1993, its abundance in both years indicates it has relatively stable populations. Other longer term studies have found *P.melanarius* and *H.rufipes* to be abundant over several years (e.g. Rivard, 1966; Jones, 1979). Caution should be taken when considering the two years field data from this project. However, the relative stability of *P.melanarius*, *P.madidus* and *H.rufipes* agrees well with the literature.

P.melanarius, *H.rufipes* and *P.madidus* have been classified as field species which live independently of the hedge, field boundaries and surrounding uncultivated habitats (Pollard, 1968a, Sotherton, 1984; Wallin, 1985). Therefore the availability of hedgerows, overwintering sites and the many factors which affect the quality of this vegetation are of little importance to these species and may account for their stability.

Predators such as *H.rufipes* are adapted to arable conditions (Briggs, 1965; Luff, 1980) and other factors may be important in determining their stability. Summer factors such as the availability of food may affect adult survival and breeding success (Thomas *et al.*, 1992). *P.melanarius* may invade fields during the summer to exploit the seasonal abundance of arthropods (pests)(Wallin and Ekbom, 1988). This food source is likely to change from year to year depending on crop type,

frequency of pesticide use and the many factors affecting the population dynamics and size of the prey populations.

5.4.4.5 Diversity and distribution of carabids on arable land

The diversity of carabids at a particular site is not a measure of the impact that the carabid fauna can exert on populations of slugs. Many species found in this study were slug predators, but only a few species were abundant, had stable populations, long activity periods and/or were well distributed.

P.melanarius has a wide distribution in the UK and in other countries. It is one of the most abundant carabids of agricultural land in Scotland (Dixon and Mckinlay, 1992; Chapman, 1994), winter wheat in York (Speight and Lawson, 1976) and cultivated land and orchards in Canada (Rivard, 1964 and 1966; Holliday and Hagley, 1978). *H.rufipes* is common in cabbage plots (Coaker and Williams, 1963) and arable land in England (Jones, 1976), Canada (Boiteau, 1983) and northern Europe (Thiele, 1977). Other slug eating carabids are locally abundant in other locations: *A.apricaria* is abundant on sandy soil in East Anglia (Baker and Dunning, 1975) and *B.lampros* is sometimes common in cereals and sugar beet (Baker and Dunning, 1975; Jones, 1976; Wallin, 1985). *P.niger* is common in cereal fields in Sweden (Wallin, 1985) and *C.nemoralis* can be abundant in potato fields in Canada (Boiteau, 1983). However, it is the activity of stable, abundant, well distributed slug predators with long activity periods such as *P.melanarius* and *H.rufipes* which offer the best opportunities as biological control agents.

5.5 Effect of the carabid fauna on slug populations

Population density estimates of *P.melanarius* beetles in Clayton field were compared with densities of *P.melanarius* beetles which reduced slug damage to chinese cabbage plants in the miniplot experiment. This is discussed in chapter six. In Clayton field, *P.melanarius* did not occur at the densities which reduced slug damage in the miniplot experiment. However, *P.melanarius* was only one of several carabid predators which was common in the three fields. The numbers of predators needed to control pests is often difficult to evaluate. Pest populations at two sites

can be reduced comparative to control plots when beetle populations are large at one site and small at the second (Edwards *et al.*, 1979).

The impact that widespread and abundant predators, such as *H.rufipes* and *P.melanarius*, can exert on slug populations will be supplemented by occasionally abundant predators such as *A.similata* and *N.brevicollis*. A third group of large/specialist predators was identified (*A.parallelepipedus*, *C.caraboides*, *P.niger*, *C.violaceus* and *C.problematicus*) which occurred at low densities in this study but which are abundant in other locations. e.g. *P.niger* in cereal fields in Sweden (Wallin, 1985), *C.nemoralis* in potato fields in Canada (Boiteau, 1983). Although they generally occur at low densities, they are often voracious slug predators. Most *A.parallelepipedus* beetles caught in the field feed on slugs (Symondson, 1992). The low densities of these large predators may lead to underestimation of their importance as slug predators.

The seasonal activity of the main pest slug *D.reticulatum* influences its predation by carabid beetles. Slugs are active in the field throughout the year and often cause economic damage in the autumn when carabids are least active. The time of the year at which carabids become inactive and stop feeding changes from year to year (e.g. Dawson, 1965). In this study, carabids were active from April to December. However, the early season predators (*e.g. H.aeneus*) did not eat slugs and the autumn carabid fauna were mainly small species which are limited in their ability to kill slugs.

Carabids are most active and abundant in the spring and summer months when small slugs dominate *D.reticulatum* populations (Hunter, 1968a). Therefore carabids have an opportunity to exert an impact on slug populations and many small carabids can contribute to slug control. *A.aulica*, *A.apricaria*, *A.dorsale*, *A.fuliginosum*, *B.lampros*, *P.strenuus*, *P.nigrita* and *Synuchus nivalis* (Panzer) were all active in the summer months and ate small slugs in laboratory studies (chapter two).

The impact that carabids can exert may vary from year to year depending on the

prevailing weather conditions. Slugs move underground during dry weather in June and July (Glen, 1989; Glen *et al.*, 1982), become inactive and effectively escape predation by carabids (e.g. Luff, 1974). This is clearly a critical period for carabid predation as they are most active during this time. Hot dry summers may seriously diminish the amount of slug predation by carabids. However, some carabid species have long activity periods (e.g. *H.rufipes*) and there is always likely to be some degree of interaction between beetles and slugs in the field.

Wratten (1982) identified the need to determine the temporal and spacial distribution of potential control agents. This study and other work indicate that *P.melanarius* and *H.rufipes* are relatively well distributed, abundant species which predate slugs in the field. This information was used to select *P.melanarius* and *H.rufipes* for use in a miniplot experiment which compared the densities at which beetles reduced slug damage to a chinese cabbage crop with densities of beetles which occurred in the field (section 5.2). The importance of these predators in relation to control programmes is discussed more fully in chapter seven, *7.3 future developments.*

5.6 Conclusions

Carabid population density estimates were made in two fields of winter wheat in the Tyne valley, Northumberland. The capture rates of most species was low and density estimates were only made for *P.melanarius*. The field density of *P.melanarius* and the density at which *P.melanarius* reduced slug damage to a chinese cabbage crop is discussed in chapter six.

The abundance and diversity of the carabid fauna varied between three fields under the same crops over two years. Abundance and diversity also varied within a field over the same period. Factors such as crop type, surrounding land, availability of food, weather conditions, field aspect, soil type and predator life cycles can all affect the year to year abundance of species. These factors all require detailed research which was not within the scope of this study.

Despite the relatively low numbers of carabids recovered in 1993, results from this study show that arable land can support stable populations of some carabid species. Some species such as *A.dorsale* and *N.brevicollis* are localised and only occasionally abundant. Other species such as *A.similata* are more widely distributed, occasionally abundant and may prefer specific crops or soil types. Three species were identified in this project which were relatively abundant in both years of the study under crops of oilseed rape and winter wheat. These were *P.melanarius*, *P.madidus* and *H.rufipes*. Although *H.rufipes* was only abundant at one site in this project, these three species are widely distributed and abundant in the UK and Europe. In addition they have long activity cycles in the field and eat slugs.

Carabid slug predators and slugs are active in agricultural land at the same time. Although carabids are not abundant when slugs cause most economic damage in the autumn, they are abundant in the spring and summer when *D.reticulatum* populations are developing and many small slugs are available. An opportunity exists for carabids to impact mortality on slug populations.

Chapter six

The Miniplot Experiment

6.1 Introduction

Work on the beneficial effects of carabid beetles has mainly been concerned with their impact on populations of aphids (Edwards *et al.*, 1978; Griffiths, 1982; Sopp and Wratten, 1986; Sunderland *et al.*, 1987; Holopainen and Helenius, 1992; Sopp *et al.*, 1992). The impact that carabid beetles exert on populations of slugs has not been widely documented. Some work has been published on the impact of individual carabid species. *S.striatopunctatus* beetles reduced numbers of the slug *L.maximus* in fields of commercially grown daisy-flowers in California (Altieri *et al*, 1982). *A.parallelepipedus* reduced numbers of *D.reticulatum* in plots of lettuce in polythene tunnels in Wales (Symondson, 1989).

Laboratory and ELISA studies conducted in this project have shown that a number of carabid species will kill and eat *D.reticulatum*. *C.nemoralis*, *C.violaceus*, *C.problematicus*, *C.caraboides* and *A.parallelepipedus* are large beetles which readily ate slugs in laboratory studies (chapter two, section 2.3). However these species were found at low densities in the three fields sites in this project (chapter five). Two predation equations (chapter four) indicated that two smaller beetles, *P.melanarius* and *H.rufipes* had a greater impact on slug populations, as they were more abundant in the three fields. The aim of the miniplot experiment described in this chapter, was to produce more information on their potential to reduce slug populations by comparing densities of beetles which reduced slug damage to a chinese cabbage crop with densities of beetles in the field. All beetle species which occurred in oilseed rape during 1992 and fed on slugs (chapter four), were considered for use in the miniplot experiment. Four predator species were used in the experiment. These were; *A.parallelepipedus, P.niger, P.melanarius* and *H.rufipes*.

A.parallelepipedus and *P.niger* are both large predators which have been shown to eat *D.reticulatum* slugs in the laboratory (e.g. Stephenson, 1965). *P.niger* was more

numerous in arable sites in this project than *A.parallelepipedus* and is one of the most frequently caught carabid beetles in cereal fields in Sweden (Wallin, 1985).

P.melanarius and *H.rufipes* were chosen on the basis that they were well represented in arable fields in this study (chapter five) and in other studies (e.g. Wallin, 1985). These two species were found to be abundant every year in an arable field in Hertfordshire (Jones, 1979). *P.melanarius* is the most abundant large ground beetle of agricultural land in north east Scotland (Chapman, 1994) and in pasture in Belgium (Pollet and Desender, 1985). *P.melanarius* is also common in agricultural land in Canada (Rivard, 1966) and both *H.rufipes* and *P.melanarius* feed on slugs in orchards (e.g. Cornic, 1973).

The impact of these four species on populations of *D.reticulatum* was assessed in the miniplot experiment, which allowed comparisons between predator species and beetle densities. The four beetle species are all large carabids and many small carabid species occur in arable land which can kill small slugs (chapter two). The choice of predator partly reflected the size of slug being used in the experiment. Large slugs were chosen because they were easier to sample and monitor. Chinese cabbage plants were used to assess slug damage and large slugs were likely to cause more damage to the plants.

The miniplot experiment reflected elements of the 1992 field survey. The plot was sown with oilseed rape, which acted as a cover crop providing cool humid conditions under which populations of *D.reticulatum* are known to live. In arable situations populations of slugs are known to build up to damaging levels under oilseed rape (e.g. Martin and Kelly, 1986). The rape provided an environment under which populations of *D.reticulatum* maintained continual activity and interacted with predator populations.

6.2 Methods

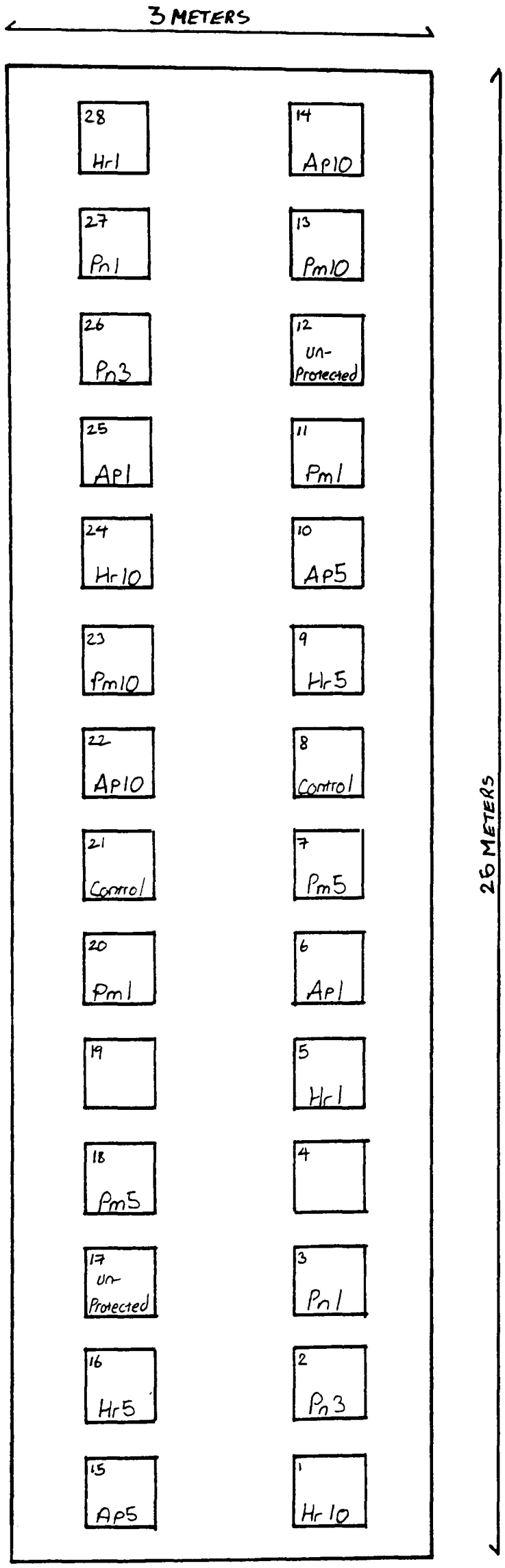
6.2.1 Setting out the experimental area

On April 6th 1993, a 25 x 3 metre plot of land was rotovated in the garden at Close House. The plot was adjacent to a potato crop along one side and a grass path along the other. The grass path was adjacent to a high wall. The plot was sown with undressed oilseed rape, var Lictor (Nickerson seeds Ltd) to provide a cover crop. The recommended commercial sowing rate was used to calculate the amount to be sown in the experimental area. The oilseed rape seeds were weighed out, sown by hand and raked into the soil. The ground was watered after the seeds were sown and watered occasionally for the next two months as the plants became established.

Once the rape was established, metal exclusion barriers were erected within the plot in two rows to create 28 miniplots (Fig. 6.1). The use of inclusion-exclusion barriers is an established method used to evaluate the effects of predators on pest populations, (e.g. Ashby, 1974; Chambers *et al.*, 1983; Chiverton, 1987; Symondson, 1989) and as a means of manipulating predator density (Chiverton, 1986). Edwards *et al.*, (1978) used metal exclusion barriers to contain carabids with aphids in fields of spring wheat.

Each barrier was constructed from a strip of zinc coated steel measuring 250 x 25cm. The steel was folded to form a 62.5 x 62.5cm square and sunk into the ground to a depth of 10 cm. The joint of the square was sealed with a commercial plastic sealant (vallance) which was water repellant, flexible and could be painted over. After the sealant had dried, the top 5cm of the barriers were coated inside and outside with Fluon. Fluon dries to give a low friction plastic surface over which beetles cannot crawl. In previous work at Close House, zinc coated steel barriers have been used to prevent movements of slugs from plot to plot, as they dislike crawling over the metal surface. Symondson (1992) showed that Fluon prevented *D.reticulatum* crawling over plastic in laboratory tests.

Fig. 6.1 Layout of the experimental area in the miniplot experiment. The minplots were arranged in two rows of 14 miniplots. Each miniplot is indicated by a box (□). Ap=*A.parallelepipedus*, Pm=*P.melanarius*, Pn=*P.niger* and Hr=*H.rufipes*. The value indicates the beetle density. e.g. Pm5 indicates that the miniplot contained five *P.melanarius* beetles.



On June 8th, the oilseed rape was damaged by pigeons, therefore a length of chickenwire was placed over the top of the miniplots to protect the plants. This also served to protect beetles and slugs from the attention of birds and other predators.

A single dry pitfall trap was sunk into the soil in each miniplot. A one cm hole was cut in the base of each trap and a piece of metal gauze was glued over the hole. This allowed rainwater to drain out of the traps and prevented the traps from flooding. The lips of the traps were level with the surface of the soil and particular attention was paid to the soil-trap interface. Each trap was positioned in contact with one of the inside walls of the miniplot.

The traps were left open to catch any large carabid predators already present in the miniplots which may have influenced the results. The traps were inspected on three occasions on June 29th, July 1st and July 6th. On these dates, any trapped beetles were noted and removed from the miniplots. A total of ten, thirteen and fourteen carabids were recovered respectively on the three sampling dates. These included five *H.rufipes* and 12 *H.aeneus* beetles. No *Pterostichus* species were caught (see Appendix 6.1). On the final sampling date (July 6th), *L.pilicornis* was the most abundant carabid caught. Therefore carabids were active in the miniplots or immigrating into the miniplots. However, the low densities and generally small species involved were considered acceptable.

A single 15 cm square ceramic tile was placed flat on the soil surface in each miniplot. Slugs use these tiles as daytime refuges and the tiles were used to trap *D.reticulatum* slugs already in the miniplots. The tiles were inspected on June 23rd, June 29th, July 1st and July 6th. On these dates, any slugs were noted and removed from the miniplots. *D.reticulatum* slugs were found in the miniplots (see Appendix 6.2) on all four sampling dates, but the low densities of slugs were considered acceptable.

The oilseed rape plants were inspected twice a week for the presence of

phytophagous invertebrates, which may have attacked the chinese cabbage plants. *P.rapae* (larvae) was the only species found and larvae were removed by hand or by selective pruning of the oilseed rape plants. The miniplots were maintained by pruning oilseed rape plants growing along the inside walls of the plots. This prevented leaves overhanging the barriers and acting as bridges to aid slug emigration and immigration. Plants were cleared for 20cm around the outside walls of the miniplots to create a bare area of soil to discourage slugs immigrating into the plots.

Preliminary work

Several dozen chinese cabbage plants were raised in glasshouses as part of the preparatory work for this experiment. Several hundred *D.reticulatum* slugs were collected from a number of sites around Close House.

The beetles used in the experiment were collected from arable and open land near Close House and kept in culture prior to the experiment. The beetles were maintained between 8 and 20°C with a light/dark cycle of 16:8 hours and fed on a diet of earthworms, blow fly larvae, bran and grass seeds.

The beetle species used in the experiment were assessed to determine whether or not they attacked chinese cabbage plants. Four plant propagators were filled with a sterile compost and a single chinese cabbage plant was planted in each propagator. Each propagator was allocated a beetle species and two beetles were confined with the chinese cabbage plants and incubated for one week at 16°C. None of the beetles attacked the plants.

Two types of controls were used in the miniplot experiment. The first consisted of two miniplots, to which slugs were added at a rate of ten per plot. This control was used to calculate the amount of slug damage to the chinese cabbage in the absence of beetles and is referred to as the 'unprotected' control. The second control consisted of two miniplots without beetles or slugs and this is referred to as the 'blank' control. This control was used to calculate the amount of damage to the

chinese cabbage plants by the residual slug population, slug immigration and other invertebrate. As the miniplots were organised into two rows on the experimental plot (Fig. 6.1), one replicate of each treatment was randomly assigned to a miniplot in each row. Therefore, 22 of the miniplots were assigned to beetle treatments and four of the miniplots were used as controls. Two of the plots were void due to the lack of available *P.niger* specimens.

6.2.2 Experimental procedure

First assessment

On July 9th, the pitfall traps were covered and *A.parallelepipedus*, *P.melanarius* and *H.rufipes* were added to separate miniplots at three different rates: ten, five and one, to simulate different densities of beetles occurring in the field. Unfortunately, only eight *P.niger* beetles were available, therefore this species was added at rates of one and three per miniplot. Therefore each miniplot contained only one beetle species at one density.

On July 16th, three chinese cabbage plants were planted in each miniplot. The chinese cabbage plants were fourteen days old. Before each chinese cabbage plant was planted, it was inspected for insect or slug damage. Plants which had been damaged or exhibited any leaf loss were rejected. Ten *D.reticulatum* slugs were placed in each miniplot excluding the blank miniplots. Slugs weighed between 0.3-0.7g which reflected the range of weights of slugs collected from fields of oilseed rape in July 1992 (Appendix 4.6).

On July 30th, the chinese cabbage plants were removed and the area of eaten leaf (cm^2) was calculated (section 6.2.3). This was referred to as the first assessment. The pitfall traps were opened and the species and number of beetles caught in the traps were noted the following day. The beetles were returned to their plots and the pitfall traps were covered. Beetle numbers were not topped-up to initial densities, as all of the beetles may not have been caught, and any adjustment to their numbers could have increased densities above the initial rates. The number of slugs under each tile in the miniplots was recorded.

Second assessment

On August 6th, a further planting of 14 day old chinese cabbages was made at a rate of three per plot. This determined the slug population active in the miniplots after being exposed to the beetles for three weeks. These plants were removed 14 days later on August 20th, and the area of leaf was calculated in a second assessment. The pitfall traps inside the miniplots were uncovered and the numbers and species of beetles were noted the following day. The beetles were then drowned in a weak detergent solution before being frozen for assessment by an ELISA (chapter 3). The number of slugs under each tile was recorded.

6.2.3 Assessing slug damage

Harvested plants were returned to the laboratory to assess the area of leaf eaten by slugs. Each miniplot was assessed individually. For each miniplot, leaves from the three chinese cabbage plants were stripped from the stem and laid flat on black mounting board. A ruler was placed adjacent to the leaves. The leaves were then photographed using a standard SLR camera loaded with slide film and a diffuse light source.

To assess the area eaten, the processed slide film was placed on top of a back light. A stadium camera was used to project the slide image onto a VDU, linked to an Amiga PC. The area measure facility on a Micromeasure program was calibrated to the ruler image on the VDU, and the area of holes in the leaves and other missing areas of leaf were calculated (see Plates 6.1, 6.2 and 6.3, Appendix 6.3).

6.3 Results

Tests were made to compare the levels of slug damage in terms of area of leaf (cm^2) eaten in the following comparisons:

6.3.1 Slug immigration and emigration

Damage to the chinese cabbage plants occurred in both the unprotected and blank miniplots.

Plate 6.1 Slug damage to a chinese cabbage crop in an unprotected miniplot.

Plate 6.2 Slug damage to a chinese cabbage crop in a miniplot protected by *H.rufipes* beetles at a density of one beetle per miniplot.

Plate 6.3 Slug damage to a chinese cabbage crop in a miniplot protected by *A.parallelepipedus* beetles at a density of five beetles per miniplot.

Damage to plants in the unprotected miniplots occurred at the same level in both assessments, but damage to plants in the blank miniplots was greater in the second assessment. In the first assessment plant damage was greater in the unprotected miniplots but in the second assessment plant damage was greater in the blank miniplots (Fig. 6.2). The differences were not significant. However, the results indicate that slugs were immigrating into or becoming more active in the blank miniplots in the second assessment.

6.3.2 Slug density and plant damage

Data for all four beetle species, all beetle densities and both assessments were combined and used to investigate the relationship between slug density and the area of leaf eaten (plant damage) in a regression analysis. Slug density was measured at the end of both assessments when the chinese cabbage plants were removed from the miniplots. No significant correlation was found between slug density and plant damage in the beetle treatments.

Similar comparisons were made using combined data from the unprotected and blank miniplots. The analysis was strongly affected by the data from miniplot 21 in the second assessment. If this data is omitted from the analysis, a correlation exists between slug density and the damage to the chinese cabbage plants (r^2 = 0.77, d.f.=5, $P<0.05$) (Fig. 6.3).

6.3.3 Slug damage between treatments

Each beetle treatment was considered separately. Chinese cabbage plants in the beetle treatments had lower mean levels of slug damage than the unprotected plants with the exception of *H.rufipes* (10 per miniplot) in the second assessment (Fig. 6.4).

The experimental design presented an analytical problem. Data for three beetle species were available at densities of one, five and ten beetles per miniplot. The data set for *P.niger* was unbalanced as *P.niger* was only assessed at densities of one and three beetles per miniplot. Therefore *P.niger* was excluded from this analysis.

Fig. 6.2 The mean area of leaf eaten in the unprotected and blank miniplots
Data from both assessments are presented. I = standard error.

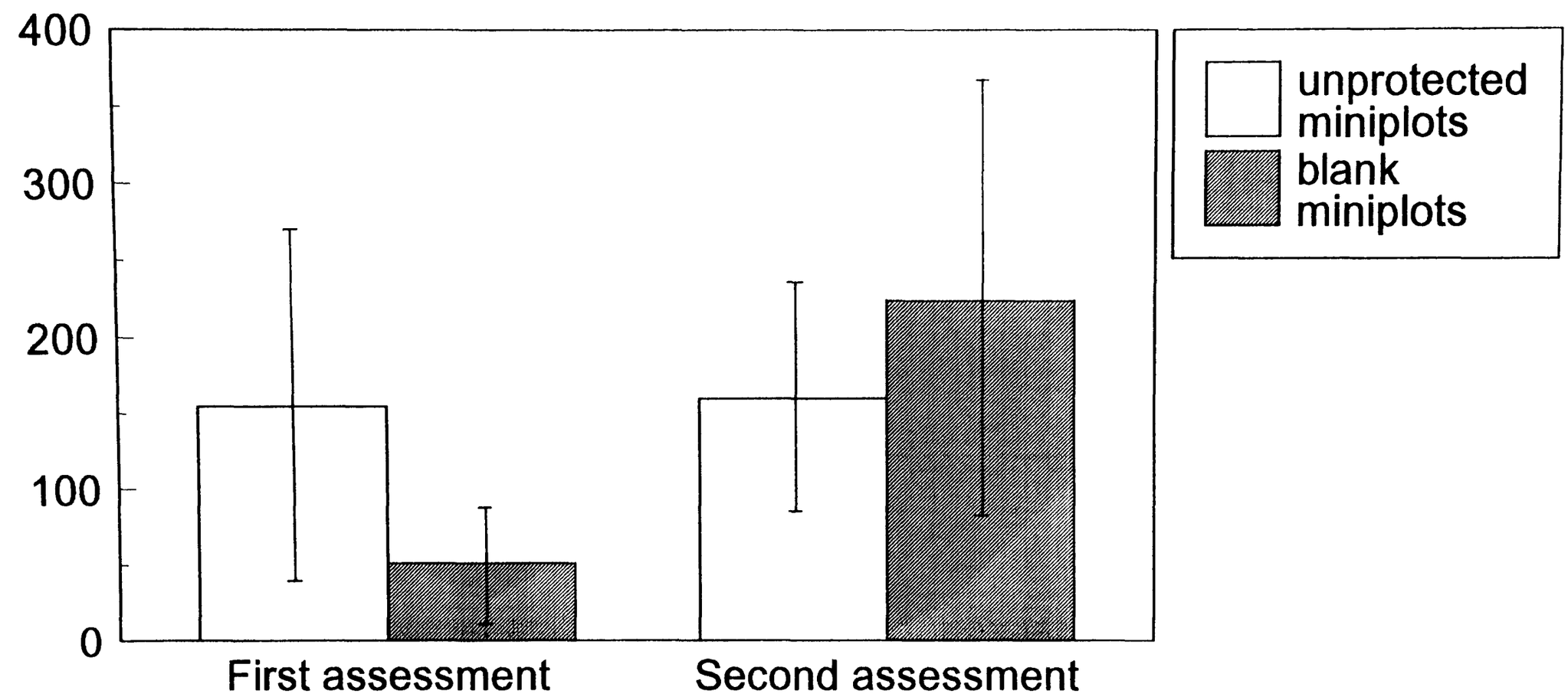


Fig. 6.3 Correlation between slug density and slug damage to chinese cabbage
Data from unprotected and blank miniplots from both assessments are presented.

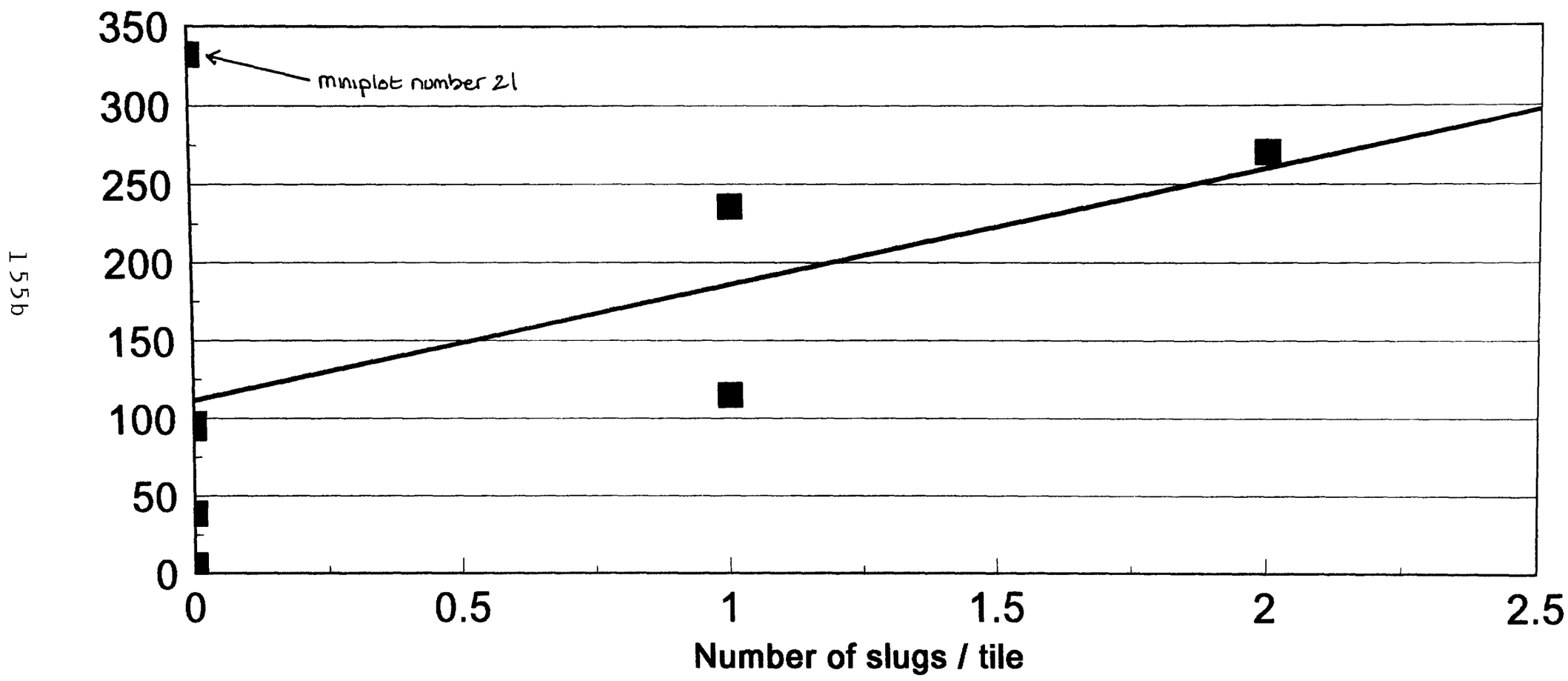
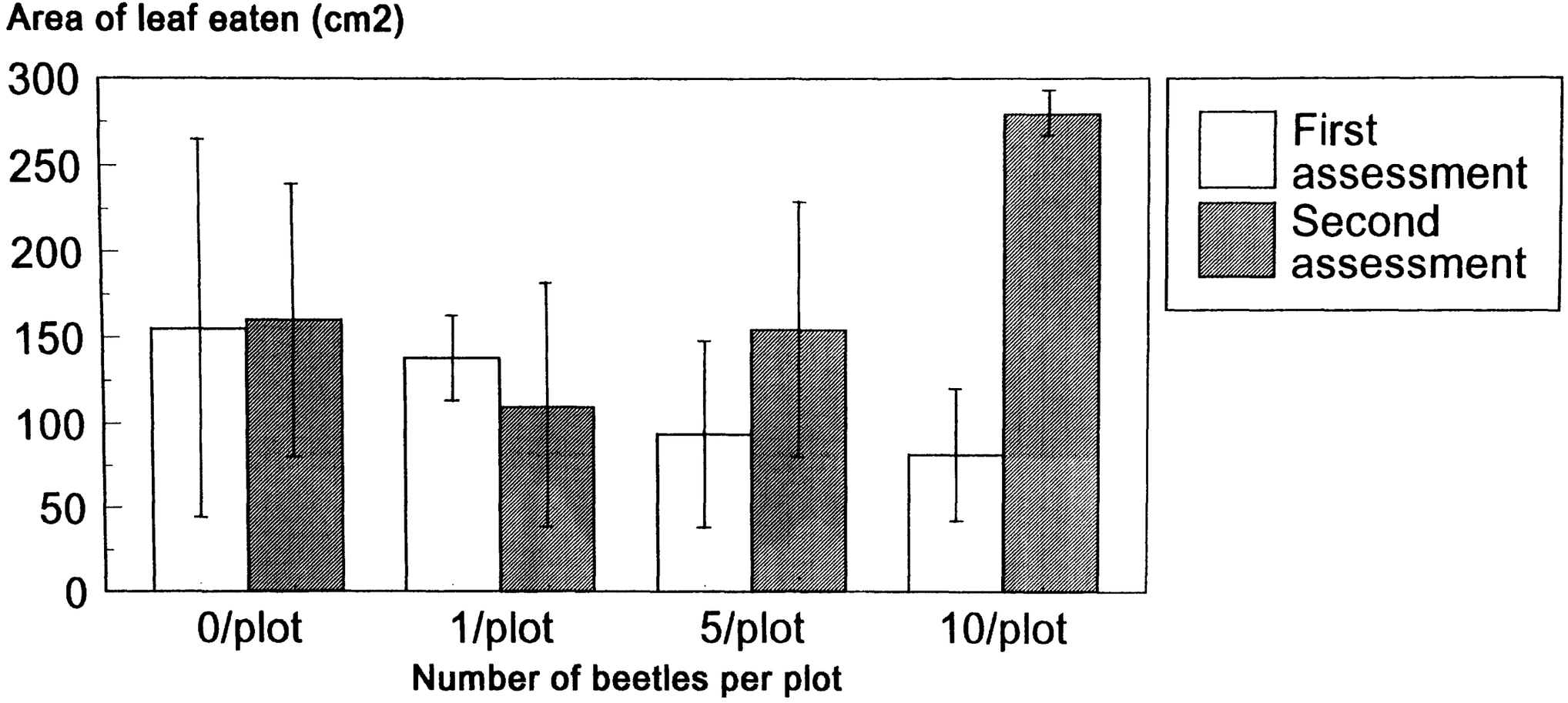


Fig. 6.4 Mean area of leaf eaten in miniplots protected by H.rufipes
The number of beetles per miniplot are indicated on the x-axis. Data from the unprotected miniplots are presented as 0/plot. I = standard error.



A.parallelepipedus, *H.rufipes* and *P.melanarius* were subjected to a three factor ANOVA incorporating species, density and assessment (first and second) as treatments.

Beetle species main effects were significant at $P<0.001$ ($F=15.54$). Significant two way interactions were found between beetle species and assessments ($F=4.35$, $P<0.05$)(Table 6.1). Scheffer's multiple comparison test was used to identify significant differences between beetle species in the second assessment. *H.rufipes* gave significantly less protection in the second assessment ($P<0.05$) and was therefore less effective during the second period. *A.parallelepipedus* was consistently effective and *P.melanarius* was more effective in the second period, but these differences were not significant (see Figs. 6.4-6.6).

Borderline two way interactions were found between beetle densities ($F=2.797$, $P<0.1$). Scheffer's multiple comparison test was used to identify significant differences between beetle densities. At densities of five and ten/miniplot, *A.parallelepipedus* significantly reduced slug damage to below that of *H.rufipes* ($P<0.05$). The large variation in the data affected the detection of treatment differences. However, *A.parallelepipedus* had a considerable effect in reducing slug damage at densities of ten and particularly five beetles per miniplot when slug damage was almost eradicated (see Figs. 6.4-6.6).

Slug damage in treatments containing *P.niger*, *A.parallelepipedus, H.rufipes*, *P.melanarius,* unprotected and blank miniplots were subjected to a two factor ANOVA incorporating species (and controls) and assessment number (first and second) as treatments (Table 6.2). No significant interactions were found due to the large variability in the data.

In both assessments, the lowest levels of slug damage occurred when *A.parallelepipedus* was present at densities of five and ten beetles per miniplot and slug damage was almost eradicated at densities of five beetles per miniplot (Fig. 6.5). *A.parallelepipedus* treatments showed similar levels of damage between the two

Table 6.1 Analysis of variance of a three factorial experiment incorporating predator species (three species), predator density (three densities) and assessment number (first and second). The analysis was performed on the area of chinese cabbage leaf eaten in each miniplot.

Source of variation	Sum of Squares	DF	Mean of Squares	F	Sig F
Main effects					
Species	74032.59	2	37016.27	15.54	.000
Density	7420.53	2	3710.26	1.55	.238
Assessment	1407.50	1	1407.50	0.59	.452
2-way interactions					
Species.Density	26647.51	4	6661.88	2.797	.057
Species.Assessment	20722.33	2	10361.16	4.350	.029
Density.Assessment	6553.20	2	3276.60	1.376	.278
3-way interactions					
Species.Den.Assess.	19568.43	4	4892.10	2.054	0.13
Explained	156352.1	17	9197.18	3.862	.003
Residual	42870.8	18	2381.71		
Total	199222.9	35	5692.08		

Table 6.2 Analysis of variance of a two factorial experiment incorporating predator species (four species, unprotected and blank controls) and assessment number (first and second). The analysis was performed on the area of chinese cabbage leaf eaten in each miniplot.

Source of variation	Sum of Squares	DF	Mean of Squares	F	Sig F
Main effects					
Species	67610.19	5	13522.04	1.632	.225
Assessment	9138.87	1	9138.87	1.103	.314
2-way interactions					
Species.Assessment	27168.97	5	5433.79	0.656	.663
Explained	103918.0	11	9447.09	1.140	.410
Residual	99404.8	12	8283.73		
Total	203322.8	23	8840.12		

Fig. 6.5 Mean area of leaf eaten in miniplots protected by A.parallelepipedus
The number of beetles per miniplot are indicated on the x-axis. Data from the unprotected miniplots are presented as 0/plot. I=standard error.

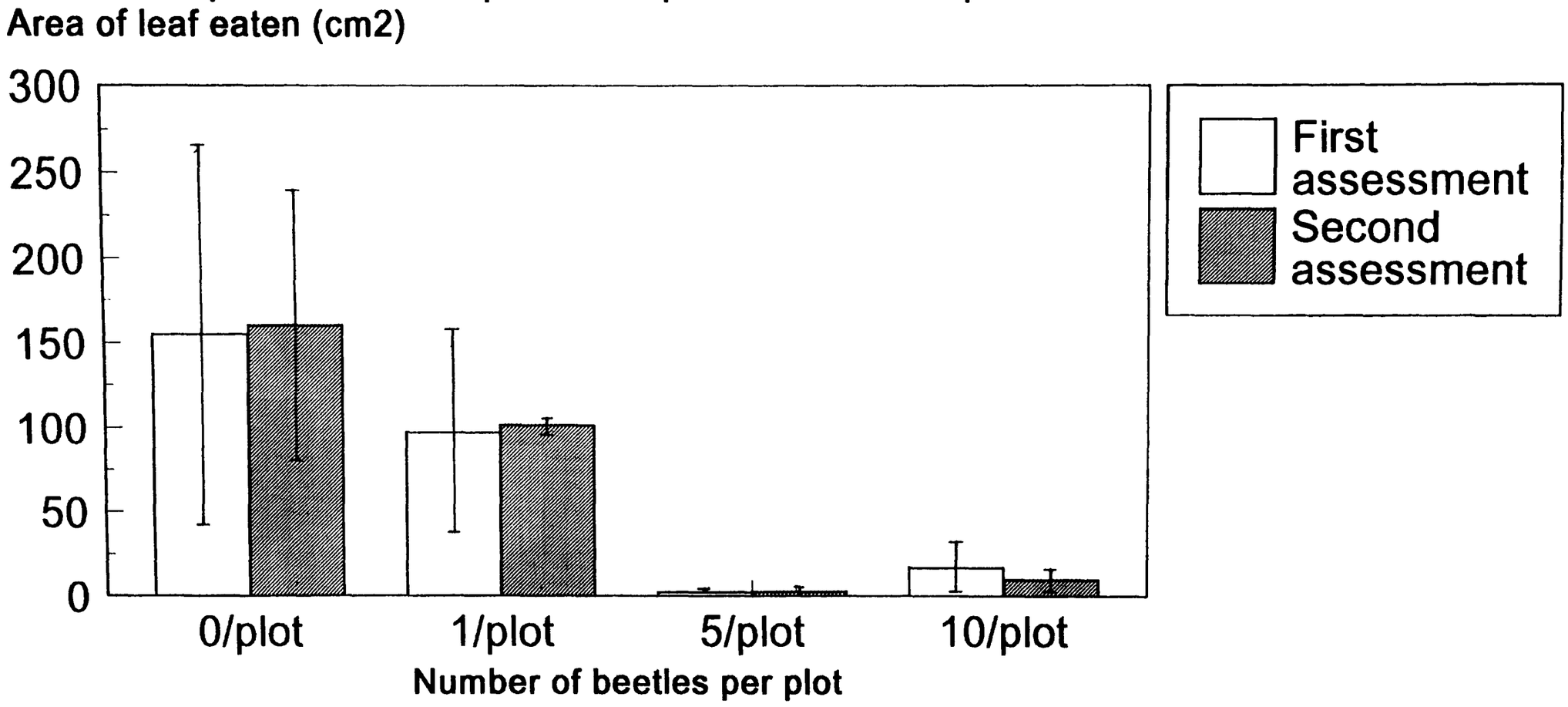
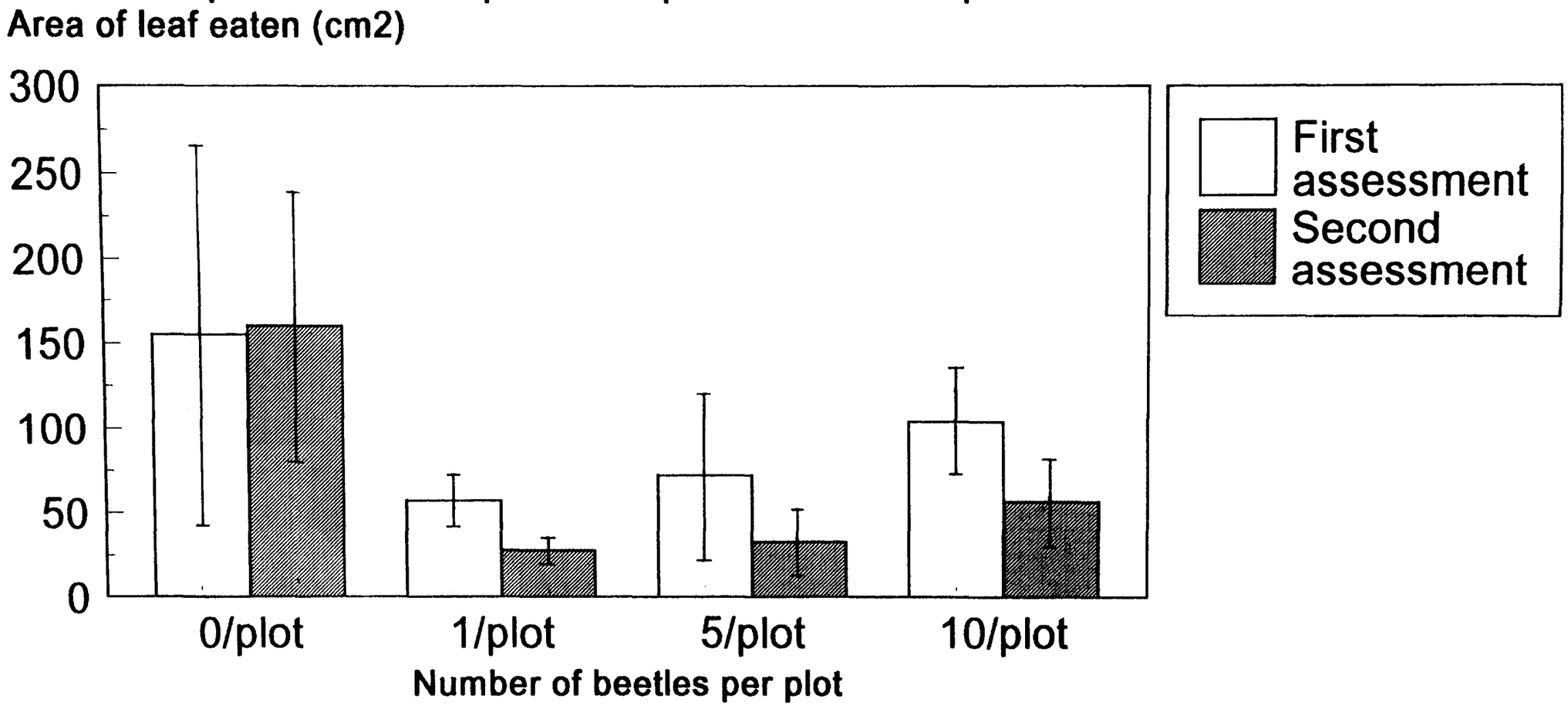


Fig. 6.6 Mean area of leaf eaten in miniplots protected by P.melanarius
The number of beetles per miniplots are indicated on the x-axis. Data from the unprotected miniplots are presented as 0/plot. I=standard error.



assessments at each predator density (Fig. 6.5). Slug damage decreased with increasing densities of *H.rufipes* in the first assessment and a general trend of increasing slug damage with increasing beetle density occurred in the second assessment (Fig. 6.4). *P.melanarius* treatments showed a trend of increasing damage with increasing beetle density in both assessments. However, damage was reduced in the second assessment at all three densities (Fig. 6.6). Finally, *P.niger* treatments showed similar levels of damage between the two assessments (Fig. 6.7).

Beetle catches

The activity of the beetles in the miniplots may have affected the number of slugs attacked and killed. Beetle activity was measured at the end of both assessments as the number of beetles recovered from pitfall traps in the miniplots. Identical proportions of *P.niger*, *A.parallelepipedus* and *P.melanarius* beetles were caught at the end of both assessments but a lower proportion of *H.rufipes* beetles were caught at the end of the second assessment compared to the first assessment. *H.rufipes* beetles were therefore considered to be less active prior to the second assessment (Table 6.3).

An assessment of the gut contents of the beetles caught at the end of the second assessment was made using the ELISA. Three *P.melanarius* beetles and one *A.parallelepipedus* beetle had fed on slugs at the end of the experiment (Table 6.3).

Beetle treatment ranks

Both assessments for each beetle treatment were combined and the mean amount of damage to chinese cabbage plants was used to rank the beetles (Table 6.4). Two of the three *A.parallelepipedus* treatments were highest in the rank and all three *H.rufipes* treatments were lowest in the rank.

Fig. 6.7 Mean area of leaf eaten in miniplots protected by P.niger
The number of beetles per miniplot are indicated on the x-axix. Data from the unprotected miniplots are presented as 0/plot. I=standard error.

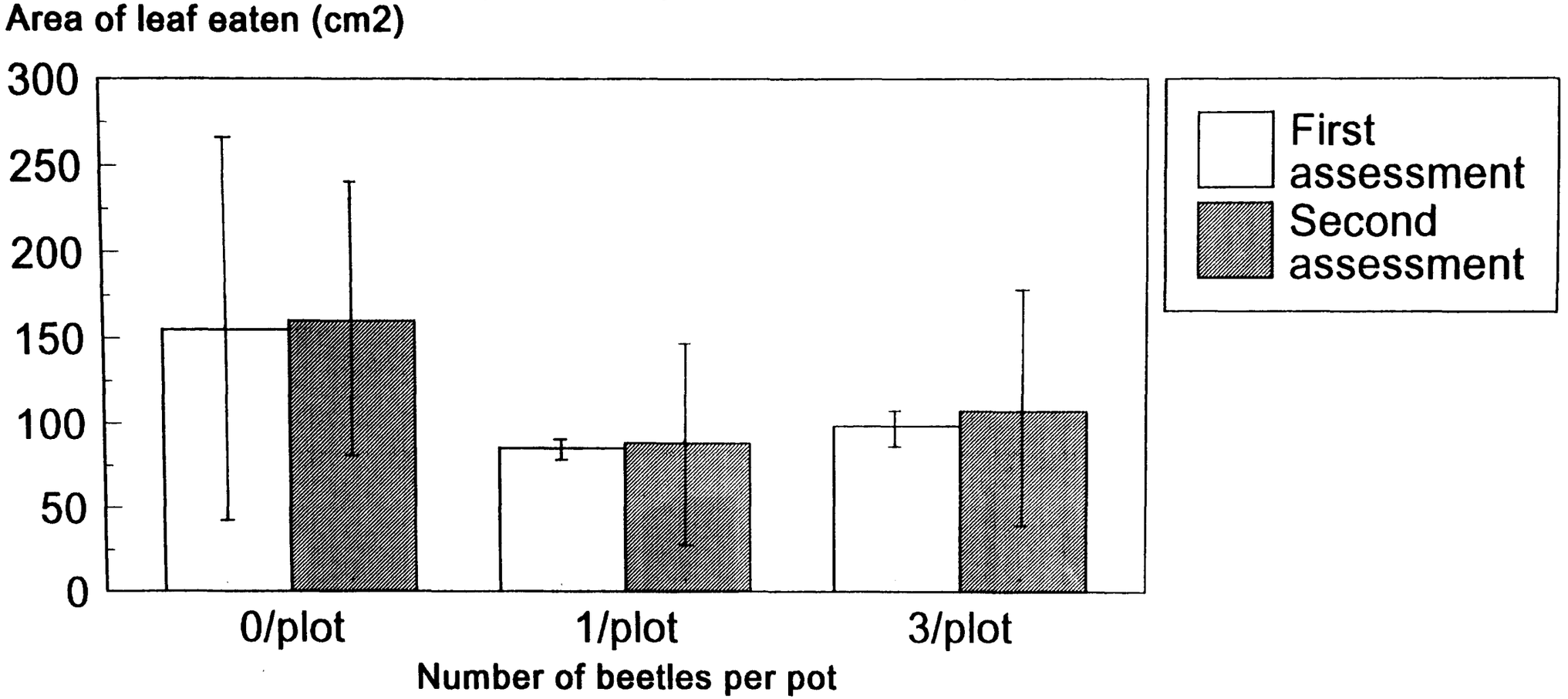


Table 6.3 Recovery of each beetle species after the first and second assessments. The second column indicates the number of beetles released into the miniplots. Columns three and four indicate the number of beetles which were recovered. Columns five and six indicate the proportion (p) of beetles recovered at the end of both assessments as a proportion of initial release of beetles. The final two columns indicate the number of beetles assessed by ELISA and testing positive for slug tissues after the second assessment. *A.parallelepipedus* abbreviated to *A.parallel* and assessment abbreviated to Ass.

		No. of beetles recovered		Proportion (p) beetles recovered		ELISA data	
Species	No.	1st Ass.	2nd Ass.	1st Ass.	2nd Ass.	No. tested	No. +tive
H.rufipes	32	10	4	0.31	0.12	4	0
A.parallel.	32	19	19	0.59	0.59	19	1
P.melanarius	32	21	20	0.65	0.62	15	3
P.niger	8	2	2	0.25	0.25	2	0

Table 6.4 Rank of each beetle treatment. The data from both assessments are combined and the beetle treatments are ranked in terms of the amount of chinese cabbage leaf eaten. Low ranks (e.g. 1, 2) indicate low levels of slug damage and high ranks (e.g. 10, 11) indicate high levels of slug damage.

Rank	Beetle treatment	Leaf damage (cm^2)
1	*A.parallelepipedus* 5/plot	2.46
2	*A.parallelepipedus* 10/plot	13.19
3	*P.melanarius* 1/plot	42.65
4	*P.melanarius* 5/plot	52.52
5	*P.melanarius* 10/plot	80.57
6	*P.niger* 1/plot	86.80
7	*A.parallelepipedus* 1/plot	99.05
8	*P.niger* 3/plot	103.03
9	*H.rufipes* 1/plot	124.10
10	*H.rufipes* 5/plot	124.11
11	*H.rufipes* 10/plot	180.72
	Unprotected plots	157.29

6.4 Discussion

6.4.1 Slug immigration and emigration

The slug damage to the chinese cabbage plants in the blank miniplots indicates that an inactive portion of the slug population were not removed from the miniplots before the experiment started and/or the exclusion barriers were not completely effective at preventing slug immigration into the miniplots. As slug damage increased in the blank miniplots in the second assessment, slugs were either immigrating into the miniplots or becoming more active inside the miniplots

If immigration into the miniplots was occurring, then it can be assumed that emigration was also occurring. This could present problems when interpreting data; low levels of damage may be due to slugs emigrating out of the miniplots rather than beetle predation. For any statistical analysis to be valid, it must be assumed that immigration and emigration are not replacing predation and are occurring with equal frequency in all of the miniplots.

The cover given by the oilseed rape and the chinese cabbage food source, provided a favourable habitat for slugs. Slugs may have been more likely to stay in the miniplots once they had arrived. If slug activity within the miniplots was constant throughout the experiment, then the increased damage in the blank miniplots in the second assessment indicates that immigration was greater than emigration. This is supported by greater amounts of damage occurring in the blank miniplots in the second assessment. There were no other large phytophages found in the miniplots during the investigation. No *P.rapae* caterpillars found on the oilseed rape in the pre-experimental period were found during the assessments and none of the beetle species attacked or damaged the chinese cabbage plants. Since slugs were the only phytophages present and slug immigration was greater than emigration, any reduction in plant damage between the treatments and unprotected miniplots was due to predation on slugs.

6.4.2 Slug density and plant damage

The tile traps in the miniplots were intended to provided daytime refuges for the slugs and to estimate slug densities. Slug density correlated to plant damage in the control miniplots but not in the beetle treatments. The beetles used the tile traps as daytime refuges, which may have discouraged the slugs from using them and caused them to find alternative shelter such as plant leaves. Symondson (1989) found slugs took refuge in lettuce plants.

As slug density was correlated to plant damage in the control miniplots, reduced amounts of plant damage in the beetle treatments were attributed to lower slug densities.

6.4.3 Slug damage between treatments

Inverse correlations have been found between the number of beetles and cereal aphids in plots of wheat (Edwards *et al.*, 1978; Edwards *et al.*, 1979). Similar relationships were expected in this experiment. High beetle densities were expected to give high levels of protection (low levels of plant damage) and low beetle densities were expected to give low levels of protection (high levels of plant damage). This was found with *A.parallelepipedus* which gave good levels of protection at densities of five and ten per miniplot (five and ten/0.39m^2)(see Plate 6.3). *A.parallelepipedus* also gives good levels of protection to lettuce crops at lower densities (six beetles/m^2) and higher slug densities (30/m^2)(Symondson, 1989).

P.niger gave similar levels of protection at both beetle densities (one and three per miniplot). Both densities reduced slug damage compared to the unprotected miniplots. *P.melanarius* reduced plant damage at three densities with the lowest beetle density being the most effective. The reason why *P.melanarius* was more effective at lower densities is unclear. The beneficial effects of this species at the two higher densities may have been offset by slugs immigrating into the miniplots. A number of authors have found female *P.melanarius* beetles consume more food than males (e.g. Sunderland, 1975; Chiverton, 1984; Pollet and Desender, 1985). The relative proportions of male and female beetles in the various miniplots may

have affected the results.

Significantly more slug damage was found in the second assessment at some *H.rufipes* densities. This may be due to a change in feeding behaviour by the beetles. None of the four *H.rufipes* beetles tested at the end of the experiment had fed on slugs. *H.rufipes* may change its diet through the year, it can be carnivorous in the summer and carnivorous and phytophagous in the autumn (Cornic, 1973). However, the increase in slug damage is probably a result of the beetle becoming less active prior to the second assessment. *H.rufipes* has a biennial life cycle, overwintering beetles predominate the catch until July, newly emerged beetles are active from August onwards and the catching rate of old generation beetles falls from mid-August onwards (Luff, 1980). The beetles used in this experiment were old beetles caught at the end of June and were probably becoming less active as the second assessment started on August 14th.

Long activity periods increase the time a predator can exert an impact on pests. *H.rufipes* has a long period of activity from April until November. Old *H.rufipes* beetles are active early in the year until August when newly emerging *H.rufipes* beetles become more active (Luff, 1980). This prolongs the time that this species is active in the field predating slugs.

A.parallelepipedus almost eradicated slug damage at densities of five beetles per miniplot in the first assessment. This indicated that slugs were killed early in the experiment before they had an opportunity to damage the plants. One of the 19 *A.parallelepipedus* beetles tested at the end of the experiment had fed on slugs. This indicates that slugs were not the main constituent of this predators diet during the second assessment and suggests slugs were eaten at an early stage in the experiment.

The level of slug damage were similar between assessments of *P.niger*, indicating a constant level of predation by this species. This is probably because *P.niger* has a long activity period in cereal fields from May until September (Wallin, 1985). Slug

damage was reduced at all densities in the second assessment in the *P.melanarius* treatments. This suggests that this species impact on slugs is not immediate. Three of the 15 *P.melanarius* beetles tested at the end of the experiment had fed on slugs. This indicates that slugs were still an important part of this predators diet. *P.melanarius* has a long period of activity in the field and consequently beetles were still very active at the end of the second assessment (late August). Some *P.melanarius* adults overwinter and are caught in May (Wallin, 1985). New beetles emerge around the end of June and early July (Ericson, 1977) and are often abundant around July and August (e.g. Rivard, 1966). Again, this prolongs the time that this species can predate slugs.

6.4.4 Application to the field

The relative impact that each species will exert on slug populations will depend to some extent on the abundance of each species in arable land. *A.parallelepipedus* occurs at high densities in woodland and has been found at population densities of 5.8 beetles/m^2 in a mixed deciduous copse (Frank, 1967). Miniplot densities of around one beetle per miniplot were more appropriate to field densities of beetles found in arable land in this project. Although *A.parallelepipedus* has been caught in arable land in other studies (e.g. Speight and Lawton, 1976) it is a hedgerow species confined to, or found in proximity to the hedge (Pollard, 1968a). It occurred at extremely low densities in arable land in this project. However, *A.parallelepipedus* is a voracious slug predator and high numbers (up to 92 percent) of *A.parallelepipedus* beetles feed on slugs in the field (Symondson, 1992).

Both *P.melanarius* and *H.rufipes* are field species (Pollard, 1968a). In this project, 349 *H.rufipes* beetles were recovered from Clayton field in 1992 and 1993. In the same period, 44 *P.melanarius* beetles were recovered from the same field (chapter five and Appendix 5.1). Therefore, eight times as many *H.rufipes* beetles were recovered. Both species are slug predators. *P.melanarius* and *H.rufipes* eat slugs in the field (e.g. Cornic, 1973, and chapter four). *P.melanarius* is a voracious predator which kills prey even when satiated (Hagley *et al.*, 1982) and both species attack, kill and eat small *D.reticulatum* slugs (chapter two).

A mark-release-recapture (MRR) programme (chapter five) estimated the population density of *P.melanarius* between 0-0.25 beetles/m^2 in two fields of winter wheat in June and July of 1993. In other studies, it has been found at maximum population densities of 0.73/m^2 in winter wheat in Sweden (Ericson, 1977) and activity densities of 0.6-1.6/m^2 in winter wheat in Germany (Basedow, 1973) and 5.3/m^2 in a strawberry crop in England (Briggs, 1961). The density at which *P.melanarius* reduced slug damage in the miniplot experiment, equates to 2.4 beetles/m^2. Although *P.melanarius* did not occur at such densities in arable fields in this project, it occurs at these densities in other studies. At these densities *P.melanarius* could reduce slug damage to chinese cabbage plants in the field.

The density at which *H.rufipes* reduced slug damage in the miniplot experiment, equates to 2.4 beetles/m^2. Population density estimates were not available for *H.rufipes* in this project, although it was one of the most abundant species at Clayton field in 1992 and 1993. It can occur at activity densities of 7.5/m^2 in the field (Briggs, 1961) and at these densities *H.rufipes* could reduce slug damage to chinese cabbage plants in the field.

A large proportion (43 percent) of *P.niger* beetles feed on slugs in the field (Tod, 1973) and this species attacks and kills large *D.reticulatum* slugs (chapter two). *P.niger* was less numerous than *P.melanarius* and *H.rufipes*, but occurred at all three field sites (chapter five). *P.niger* reduced slug damage at densities of 2.4 beetles/m^2 and can occur at population densities of 1.3/m^2 in the field (Grum, 1959). At these densities *P.niger* would not reduce damage to chinese cabbage plants in the field.

Chinese cabbage plants were used in this experiment to assess the relative effectiveness of the different beetle treatments in controlling surface active slugs. The various beetle species and densities should not be taken as critical field densities needed to control slugs in the field. *P.melanarius* is a good candidate species for controlling slugs. It was ranked highly, it reduced slug damage in miniplots at low densities and it was one of the most numerous and regularly caught predators on arable land in this project and in other projects (e.g. Rivard, 1966;

Jones, 1979; Pollet and Desender, 1985). In the field it can occur at densities which reduced slug damage in the miniplot experiment. Conversely, *H.rufipes* had a low rank and was less effective at reducing slug damage. However, it is one of the most abundant carabids on arable land (e.g. Jones, 1979), it was more numerous than *P.melanarius* in this study (chapter five) and a larger proportion of *H.rufipes* beetles fed on slugs in the field compared with *P.melanarius* beetles (chapter four).

In the field, the availability of alternative food may affect the extent to which predators feed on slugs. *P.melanarius* efficiency as a predator of codling moth larvae is greatly affected by the abundance of other large alternative prey (Hagley *et al.*, 1982). The relatively low proportion of *P.melanarius* beetles feeding on slugs in the field in this project (chapter four), indicates that they were exploiting other prey. Therefore *H.rufipes,* which were less effective in the miniplot experiment, may be a more important slug predator.

The size of the slugs used in this investigation also needs to be considered. Large slugs were chosen on the basis of the sizes of slugs collected from oilseed rape crops in 1992. Large beetles are more able to attack and kill large slugs. By virtue of its size, *A.parallelepipedus* is able to deal with the size of slugs used in the experiment. The other three predator species may have found the largest experimental slugs difficult to handle. In the field, *D.reticulatum* slugs exist in mixed age groups and small slugs dominate the *D.reticulatum* population on arable land in the summer (Hunter, 1968a). If smaller slugs were used in this investigation, the four predator species may have given similar levels of control.

This study has shown that four carabid species reduced slug damage to a chinese cabbage crop in a miniplot experiment. Two of these species, *H.rufipes* and *P.melanarius*, are field species (Pollard, 1968a) which are common and abundant on all types of agricultural land (e.g. Rivard, 1966; Baker and Dunning, 1975; Jones, 1976 and 1979). Both species eat slugs in the field (this project and Cornic, 1973). In addition, other beetle species occur in arable land which are beneficial. Laboratory investigations in this project (chapter two) indicated that a number of

carabids, smaller than the ones used in the miniplot experiment, attacked and ate small slugs. In the field, many predator species are acting to reduce slug numbers.

The densities of beetles needed to control pest populations in the field are not known. Edwards *et al.*, (1979) found that beetle populations reduced aphid numbers at two sites, where beetle populations were large at one site and small at the second site. The densities of *P.melanarius* and *H.rufipes* beetles which reduced slug damage in this study, occur at similar or higher densities in arable land. These are both very useful species which have the potential to make an impact on populations of slugs. Any impact on pest populations is useful and may ultimately contribute towards economic control (Luff, 1983).

6.5 Conclusions

Four carabid species assessed in this chapter reduced slug damage to chinese cabbage plants in a miniplot experiment. The beetle densities at which plant damage was reduced varied between the species. *A.parallelepipedus* gave the best protection to the plants at densities of five per miniplot. This species occurs at much lower densities in arable land. *P.melanarius* and *H.rufipes* are found in arable land at densities which reduced slug damage in the miniplot experiment.

The effectiveness of the beetles species at protecting plants may change over the season according to the beetles life cycle. Of the four beetles assessed, *P.melanarius* and *H.rufipes* were the most important species found in arable land in this project. They are frequently abundant and are widely distributed. *P.niger* was found at lower densities in this project, but can be abundant in arable land. *P.niger*, *P.melanarius* and *H.rufipes* all overwinter as adults and have long periods of activity. *H.rufipes* in particular has a long activity cycle which increases the time this species can exert an impact on populations of slugs.

Chapter seven

Discussion

7.1 Project aims and objectives

This project aimed to identify carabid predators of slugs in arable land and assess their relative importance. This was achieved by following a scheme outlined by Wratten (1982) and Sunderland (1987). Five areas of study were investigated and they are discussed below. A general discussion follows which considers the role of carabids in agriculture and their use in Integrated Pest Management (IPM) programmes.

7.1.1 Laboratory investigations of slug predation and beetle behaviour

Beetle size and slug predation (section 2.2)

The objective of this study was to identify which small, medium and large sized beetles caught in arable land killed and ate slugs. In previous studies, large generalist beetles and mollusc specialists have been investigated as slug predators (e.g. Bless, 1977). However, many beetles which occur in arable land are small and medium sized generalist species. Predators were divided into three size classes and their ability to predate hatchling slugs was assessed.

Slugs can be difficult prey for carabids to attack and their tough mucus coated skin may deter generalist beetles (Pakarinen, 1994). However, many generalist beetles were able to overcome the mucus defence and kill slugs. A relationship was found between slug predation and beetle size. More large and medium sized species (large = 10mm and over, medium = 7.1-9.9mm) killed slugs compared to small species (small = 7.0mm or less). Other studies have found similar correlations between beetle size and slug consumption (e.g. Tod, 1973).

Slugs, like other prey, may eventually grow to sizes which effectively protect them from attack by many medium and large sized generalist beetles (e.g. Dempster,

1967; Frank, 1971). However, when slugs hatch, they weigh a few micrograms and are vulnerable to attack by a wide range of carabid predators. Further work may identify prey size thresholds for carabid beetles and slugs, such size thresholds are already recognised in other carabid prey (Dempster, 1967; Frank, 1971; Ernsting and Van der Werf, 1988).

Slugs exposed to beetles were more likely to die than slugs used in the controls, even when predation was eliminated. Some small carabids did not eat slugs, but exposure to the beetles increased slug mortality. The interactions between beetles and slugs could lead to rejection of the slug as prey, but the death of the slug. Therefore, postmortem investigations such as ELISA which only measure the mass of slugs eaten, may underestimate the impact that beetles exert on slugs in the field as they do not yield information on the numbers of prey attacked, killed or injured.

Activity cycles and predation rates (section 2.3)

The objectives of this study were to identify compatible activity cycles between slugs and five model slug predators, identify predation rates, foraging times, handling times and capture efficiencies. These are important components of any predator-prey relationship.

The amount of predator-prey contact depends on the activity of the predator and prey. Slugs are normally nocturnal and therefore any potential slug predator must be nocturnal in order to interact with the slug population. All of the five large predators studied were nocturnal and have comparable activity cycles with slugs. Many of the species which fed on slugs in the laboratory studies (section 2.2) are nocturnal in grasslands (Luff, 1978). Therefore, many carabids have comparable activity cycles to slugs which allow interaction in the field and these species have an opportunity to predate slugs in arable land.

Foraging time and capture efficiencies

Luff (1983) described several criteria for an ideal predator. These included a high voracity (many prey eaten), high attack rate (capture efficiency) and short handling

time. These criteria were used to assess five model carabid predators, including three mollusc specialists and two generalist predators.

The capture efficiencies of the specialists were much higher than the generalists and this reflects the degree of specialisation of *Carabus* and *Cychrus* species on molluscs. The three mollusc specialists captured a slug approximately every second encounter. The capture efficiencies of the two generalists were much lower; *A.parallelepipedus* one in ten and *P.niger* one in 50. However, at least one beetle of each species investigated predated a slug within the first few contacts. Other work support the results from this study and confirm that large generalists do attack slugs (e.g. Bless, 1977; Loreau, 1984).

The two generalists spent longer periods of time foraging for slugs than the three specialists. Long foraging times in the field will tend to bring the predator into contact with other prey species which may be predated at the expense of predating slugs. Some generalist predators may find slugs difficult to handle and ignore them altogether if other prey are available (Pakarinen, 1994). As most field carabids are generalist, this may have a serious impact on slug predation. However, large generalists readily feed on slugs (Loreau, 1984) and large numbers eat slugs in the field (Symondson, 1992).

Handling time

All of the predators have long handling times (over 20 minutes). The two generalists have shorter handling times than the three specialists which digest their prey pre-orally and spent up to three hours feeding on one slug. The long handling time partly reflects the large size of the experimental slugs and short handling times for such large prey can not be expected.

Predation rates

The predation rates of the five predator species ranged from 0.26 to 0.43 slugs killed/night. The predation rates of generalists and specialists were fairly similar, but specialists generally expressed higher rates: *A.parallelepipedus* expressed the

lowest predation rate and *C.violaceus* expressed the highest predation rate. The predation rate depends on the voracity of the predator and the relatively low predation rates partly reflect the large size of the experimental slugs. It is probable that satiation occurred after each slug meal. There was variation within species, as some beetles made multiple kills on a single night and one *C.violaceus* beetle was particularly voracious and killed three slugs in one night. *C.violaceus* was active in Clayton field throughout July. With a predation rate of 0.43 slugs/night, each *C.violaceus* beetle could kill thirteen large slugs in this month at this site.

None of the predators expressed all the criteria of an ideal predator, i.e. continually feeding, killing many prey and short handling time (Luff, 1983). These criteria have been used to assess predators of much smaller agricultural pests such as aphids and cabbage root fly. The low predation rates in this study were partly due to the large size of the experimental slugs. Multiple kills and short handling times cannot be expected by predators which may quickly become satiated by such large prey. In agroecosystems, slugs exist in mixed age groups and small slugs can dominate the slug population (Hunter, 1968a). Therefore the predation rates of carabids on slugs may be much higher than the rates found in this study.

Orientation to slugs (section 2.4)

In this study, the orientation to slug mucus was investigated in three generalist and four specialist slug predators. Seven locomotory (walking) categories were identified and used to assess the orientation of beetles to slug mucus.

Both generalist and specialist beetles significantly changed their walking behaviour when encountering slug mucus on the soil and five of the seven species investigated showed significant responses. Slug mucus arrested beetle locomotion. The predators spent more time stationary investigating the soil, foraged longer, covered greater distances, walked slower and turned more frequently on soil covered by slug mucus than on soil in control areas. Four species, including the generalist *P.madidus*, exhibited specialised klinokinetic, orthokinetic or klinotactic movement. This type of behaviour was considered to be a general response to all types of prey

but is a desirable trait which increase the likelihood of a predator encountering a slug in the field.

7.1.2 Identification of predators in the field

Chapter 3 and section 4.2

An Enzyme-linked immunosorbent assay (ELISA) was successfully developed which was used to identify slug antigens in the guts of carabids caught in the field. The ELISA detected slug remains in the guts of a number of species. The high antigen decay rates in some species meant that slug predation was probably underestimated. This is particularly true for predators which had eaten a small slug meal. ELISA data only measures the mass of slug ingested and does not fully describe the numbers of slugs attacked, killed or injured (see section 2.2)

The degree of slug predation differed between the three field sites. These differences partly arise due to differences in the carabid fauna at the three sites (e.g. more slug feeding species at a particular site), size of individual slugs and the degree of scavenging.

In some species, the proportion of beetles feeding on slugs depended on the site from which the beetle was recovered. At Square field, the proportion of *N.brevicollis* feeding on slugs was significantly correlated with slug activity density. *P.melanarius* also fed on slugs on the two occasions when slugs were most numerous. The efficiency of any predator depends on the quantity of other prey which are available (Holling, 1961) and the level of slug predation by *P.melanarius* may reflect the abundance of other prey (e.g. Pollet and Desender, 1985). All those factors which affect slug activity (humidity, temperature) and the phenology and abundance of other alternative prey will influence the impact that carabids have on slug populations. The relatively large weight of individual slugs at Bog field may explain why *A.similata* beetles did not feed on slugs at this site when similar proportions fed on slugs at the other two sites.

Predation equations and ranks were used to assess slug predators. The results must be interpreted with caution. The two predation equations used in this project (Dempster and Rothschild) gave very different measurements of slug predation. This is due to the emphasis placed on the different components in the equations. Equally, beetle ranks should be interpreted with caution. All species with low activity densities had low ranks. However, large species with low activity densities and low ranks may have a greater impact on slug populations than smaller, more abundant species which may scavenge dead slugs.

Predation and scavenging

The measurement of the extent of scavenging was not within the scope of this project. However, beetles ate dead macerated slug tissues in the laboratory (chapter three) and it is likely that many generalist and specialist mollusc predators scavenge dead slugs to some extent (e.g. Loreau, 1984), as they scavenge any decaying organic material. Partially consumed slugs are exposed to scavenging to a number of predators which might not normally be able to attack and kill slugs (Frank, 1971). Large slug killing species were rare at Bog field and these species are likely to enhance the number of slug corpses which scavengers feed from. This may explain why *A.similata* beetles did not feed on slugs at Bog field. The high proportion of *A.similata* beetles which fed on slugs at the other two sites may be due to scavenging dead slugs.

Scavenging and predation habits need to be assessed when selecting beetles for control programmes. Video tape behavioural studies may yield valuable evidence which may support a species predatory role.

7.1.3 Abundance of slug eating predators

Section 5.3 and 5.4

The objectives of these two studies were to estimate carabid population density in two arable fields in June and July of 1993 and assess the distribution, abundance and stability of carabid species.

Carabids were less numerous in the summer season of 1993 compared with the summer of 1992. The recapture rates of beetles in the mark-release-recapture (MRR) study were low and population density data were only available for *P.melanarius* beetles. MRR studies are inappropriate for certain species (e.g. Ericson, 1977) or during periods of beetle emigration or immigration. The population densities of *P.melanarius* and their effect on slug damage are discussed in section 7.1.5 below.

Beetle abundance and species richness varied from field to field and from year to year. The three fields showed the same pattern of beetle abundance and diversity relative to each other over the two summers. Species such as *P.melanarius*, *H.rufipes*, *P.madidus* had relatively stable populations but populations of *A.similata* and *A.dorsale* fluctuated considerable between the years. The species composition of arable land may not vary substantially from year to year, but the abundance of some species may vary considerably (e.g. Boiteau, 1983). Factors such as crop type, surrounding land, food, field aspect, weather, soil type and pesticide / herbicide use may all affect year to year abundance.

Many of the larger species and mollusc specialists occurred at low densities in arable land. The large Pterostichini slug predator *A.parallelepipedus* was rare in arable fields in this project and is probably restricted to the margins of arable land (Pollard, 1968a). *A.similata* was the most numerous beetle in oilseed rape in the summer of 1992 but was very rare the following summer in winter wheat. This species may prefer brassicae crops (e.g. Lindroth, 1974) and this may account for its population fluctuation between years. Other *Amara* species show similar habitat preferences (e.g. Baker and Dunning, 1975).

Agricultural land is an ephemeral habitat and some species prefer this habitat. *H.rufipes* is well adapted to arable land (Luff, 1982). *P.melanarius* utilises abundances of food in arable fields during the summer and for larval development (Wallin, 1986). *H.rufipes* was abundant in one of three fields and *P.melanarius* and *P.madidus* were relatively common in two of the three fields. Populations of

P.madidus, *P.melanarius* and *H.rufipes* were relatively stable in this study and they all ate slugs in the field. These three useful slug predators are well distributed and are abundant in agricultural land in Britain, Russia and Canada (Rivard, 1966; Baker and Dunning, 1975; Soboleva-Dokuchayeva, 1975; Jones 1976 and 1979; Boiteau, 1983; Dixon and Mckinlay, 1992; Chapman, 1994).

Other slug predators are only locally abundant. *P.niger* was the third most frequently trapped Pterostichini in this study but was much less numerous than *P.melanarius* and *P.madidus*. However, *P.niger* has a broad habitat distribution and moves between woods and arable fields (Wallin and Ekbom, 1988). It is numerous in pine forests (Grum, 1959) and common in spring barley in Sweden and potato fields in Scotland (Wallin, 1985; Dixon and Mckinlay, 1992).

Four mollusc specialists species (*Carabus* and *Cychrus*) occurred at low densities in the field sites. However, *Carabus* species are abundant in agricultural land in other studies. These are very useful slug predators which have high capture efficiencies and a strong orientation to slug mucus. *C.violaceus* is a field inhabiting species (Pollard, 1968a) and was the most frequently caught mollusc specialist in this project. This is a voracious slug predator which may inflict an impact on slug populations even at low beetle densities.

In this project, three fields were monitored for two summers, a spring and winter season. This relative short monitoring is unlikely to reveal the full extent of the carabid fauna of the fields.

7.1.4 Predator density manipulation and slug damage

Chapter six

The objective of this study was to determine the effectiveness of four beetle species in reducing slug damage to a chinese cabbage crop. The experiment assessed three densities of three beetle species and two densities of one beetle species. The densities of beetles which reduced slug damage were compared with densities of

beetles occurring in the field (section 5.3). These densities were not intended to be used as critical field densities required to give slug control in arable fields. However, the relative merits of each species could be evaluated.

All four beetle species reduced slug damage to the chinese cabbage plants relative to the unprotected miniplots, but the differences were not significant ($P<0.05$). *A.parallelepipedus* gave the best protection to the chinese cabbage plants and virtually eradicated slug damage at densities of five beetles/miniplot (or five beetles/0.62m^2). However, *A.parallelepipedus* occurs in arable land at lower densities, only one beetle was recovered in this study and in other studies (e.g. Speight and Lawton, 1976). This species is restricted to the margins of arable land (Pollard, 1968a) which will limit its impact on field populations of slugs.

Population density estimates from the MRR study were only available for *P.melanarius* beetles. In this study, *P.melanarius* did not occur at densities which reduced slug damage to the chinese cabbage crop, but it does occur in arable land at activity densities which reduced slug damage (e.g. Briggs, 1961). *P.niger* occurred at relatively low activity densities (section 5.4), but it is abundant/common at other locations (Wallin, 1985; Dixon and Mckinlay, 1992) and may be locally important as a slug predator. *H.rufipes* was much more numerous in arable land than the other three species (section 5.4) and higher proportions fed on slugs compared to *P.melanarius* and *P.niger* (section 4.2). *H.rufipes* and *P.melanarius* are common and abundant on all types of agricultural land and feed on slugs in arable crops (section 4.2) and in orchards (Cornic, 1973). They also attack a number of other agricultural pests and are useful species which have the potential to make an impact on populations of slugs.

Large slugs were used in the experiment to enable slug populations to be monitored more effectively. All four beetle species may have found the largest experimental slugs difficult to handle. The beetle species may have given similar levels of control and higher levels of control had smaller slugs been used. Small slugs can dominate the slug population in arable land during the summer (Hunter, 1968a) and the

impact of these beetle species on slug populations may be underestimated. In agroecosystems many carabid species occur which attack slugs and they all have the potential to contribute to slug control. However, in the field a variety of alternative prey exist and these may be predated by carabids at the expense of slug predation.

7.1.5 Summary

The project has identified and assessed a number of slug predators which are found in arable land. Some small and many medium and large sized carabids attack, kill and eat slugs in the field and carabids can be considered as beneficial slug predators. Burn (1992) found carabids reduced slug numbers and/or activity in plots in cereals but could only postulate which carabid species were responsible. In this project, an ELISA was developed which identified a number of carabid slug predators. However, the impact that carabids exert on slugs is only partially determined. Four species reduced slug damage to a crop of chinese cabbage in the miniplot experiment and all four species and many other species have a potential to predate slugs in arable land. Predation of slugs in the field will be influenced by a number of factors (Holling, 1961):

1. Prey density.
2. Predator density.
3. Characteristics of the environment (e.g. abundance / variety of alternative prey).
4. Characteristics of prey (e.g. defence mechanisms).
5. Characteristics of the predator (e.g. attack techniques).

All of these factors are important when considering carabid predators of slugs. Slugs have an effective defence strategy (mucus) which may deter many predators. However, some beetle species have developed attack strategies to overcome the mucus defence (Pakarinen, 1994). In this project, the mollusc specialist species and many generalist beetles overcame the mucus defence and killed slugs. Both types of predator orientated to slug mucus.

The abundance of predators in the field will affect their impact on slugs. Widely distributed and abundant predators are more important than predators with restricted ranges and low abundance. In this study, only *P.melanarius*, *P.madidus* and *H.rufipes* were relatively abundant/common in both years of the study. The mollusc specialist *C.violaceus* occurred at low densities in this project but it is a voracious slug predator with a high predation rate. This species may have an impact on slug populations even at low beetle densities.

Prey density is important in determining the effect of predation. *H.rufipes* only eats aphids at high densities when damage to cereal plants has already been inflicted (Loughridge and Luff, 1983). Slugs generally cause damage in the winter when carabids are largely inactive. In this project, carabids and slugs were active throughout the summer months and carabids have an opportunity to predate slugs and reduce slug populations. The impact that beetles can exert on slugs during this period may change from year to year depending on those factors which affect slug density and activity. Dry summers can cause slugs to shelter underground and under these conditions, surface active carabids may have little impact on slug populations.

The diet of polyphagous predators reflects the relative abundance of other prey (e.g. Kiritani *et al.*, 1972) and polyphagous predators 'switch' from one prey to another depending on the respective abundance of the prey (e.g. Loughridge and Luff, 1983). *P.melanarius* switches feeding from one prey to other prey as they become more abundant (Pollet and Desender, 1985) and the abundance and variety of alternative prey is an important factor which will affect slug predation.

7.2 Carabids role in agriculture

Carabids are one of the most abundant group of ground arthropods in agricultural land (e.g. Baker and Dunning, 1975; Speight and Lawton, 1976; Boiteau, 1983). In earlier reports, carabids were occasionally cited as agricultural pests (e.g. Mclachlan, 1897; Briggs, 1961) but contemporary work has shown that they attack a number of noxious species and they are now perceived as beneficial inhabitants of

agroecosystems.

Carabids are found in strawberry crops (e.g. Briggs, 1961), under brassicas and oilseed rape (e.g. Attah, 1986) and are often abundant under cereals (e.g. Jones, 1976 and 1979). They are found in sugar beet (Baker and Dunning, 1975), pasture, clover, alfalfa (Rivard, 1966), corn (Rivard, 1966; Whitfield and Showers, 1987) and potatoes (Dixon and Mckinlay, 1992). Carabids contribute to pest control in woodland as well as arable land. *A.parallelepipedus, P.melanarius, P.cupreus* and *P.madidus* are important predators of oak-defoliating winter moth pupae (Frank, 1967) and *P.melanarius* and *H.rufipes* eat slugs in apple orchards (Cornic, 1973).

The status of carabids as predators of arable pests has grown in the last twenty years as the full extent of their diets has been revealed. They are known to feed on a variety of agricultural pests including cereal aphids (e.g. Sunderland and Vickerman, 1980; Griffiths, 1982; Holopainen and Helenius, 1992) and cabbage root fly in cabbage and cauliflower crops (e.g. Coaker, 1965). *H.rufipes* is an important predator of *P.rapae* (Dempster, 1967). *P.melanarius* attacks leatherjackets (Chapman, 1994) and other *Pterostichus* and *Harpalus* species are important in controlling the European corn borer in the USA (Whitford and Showers, 1987).

Carabids are economically beneficial (Soboleva-Dokuchayeva, 1975) and may completely eradicate pests in some fields (e.g. Frank, 1971). Mollusc specialists species may suppress the numbers of harmful molluscs (Larochelle, 1972) and control slugs in the field (Poulin and O'Neil, 1969) and in commercial fields (Altieri *et al.*, 1982). In Europe and North America, generalist species are the most abundant carabids in agricultural land (Jones, 1976 and 1979; Whitford and Showers, 1987). Generalist beetles eat molluscs in the field (Davies, 1953; Dawson, 1965; Cornic, 1973; Tod, 1973; Luff, 1974) and have been used to reduce slugs damage to lettuce crops (Symondson, 1992).

This project has identified carabid beetles which eat slugs in arable fields under

commercial production of winter wheat and oilseed rape. Some of these species are abundant under oilseed rape. This may have considerable commercial implications: Slug populations build up under crops of oilseed rape (Martin and Kelly, 1986). Oilseed rape is often followed by crops of winter wheat (Stephenson and Bardner, 1976; Glen, 1989) which is then attacked by slugs and can result in germination failure in the autumn. Any impact that carabids can exert on slug populations under oilseed rape may reduce the impact that slugs exert on subsequent crops.

7.3 Future developments

Carabids are abundant in arable land and are largely perceived as beneficial. They are often grouped together with other polyphagous predators and all are considered to be beneficial in terms of pest control. This is not correct as larger carabids may attack smaller species (Finch, 1993) and the abundance of one group of predators may have a marked effect on the density of others (Sunderland *et al.*, 1994).

Up to eighty percent of carabid species (including all of the common species) found on eight conventional and organic farms in Iowa have been cited as possible biological control agents (Dritschilo and Wanner, 1980). However, certain criteria need to be established to determine if beetles are suitable for IPM programmes. The relative abundance and distribution of a beneficial species is an important factor. Many slug specialists occur in arable land but generally at low densities. Rivard (1964) thought only 12 of 159 carabid species caught on agricultural land in Canada were sufficiently numerous to warrant investigation as to their possible use as control agents. Key species need to identified that have a beneficial effect in pest control (Finch, 1993). The pesticide industry need to know which natural enemies are important so that appropriate products can be developed (Brown, 1989).

Many criteria have been suggested to assess beneficial species including the season of activity, field penetration, response to prey heterogeneity, feeding behaviour and consumption rate, the extent of disruption by agricultural practices (Wratten *et al.*, 1984) and dispersal data (Coombes and Sotherton, 1986).

Two criteria used to assess predators in this study were, the predation of the target pest in the field (ELISA results) and abundance and stability between sites and years. Two beneficial species identified using these criteria were *H.rufipes* and *P.melanarius*. Both species fed on slugs in arable fields, both were relatively abundant/common and stable between years. In addition, *P.melanarius* was relatively common at two of the three sites. Both species have long activity periods and are known to be important predators of other agricultural pests, such as leatherjackets, aphids and *P.rapae* (Dempster, 1967; Edwards *et al.*, 1978; Jepson, 1989; Chapman, 1994).

Pterostichus and *Harpalus* species have a long season of activity (e.g. Luff, 1982), they are found on arable land, they are widely distributed and often abundant (Speight and Lawton, 1976; Jones 1976 and 1979; Wallin, 1985; Dixon and Mckinlay, 1992). Particular species within the two genera dominate at different localities or in different countries. *Pterostichus chalcites* Say is the most numerous *Pterostichus* species in Iowa and Illinois (Hsin *et al.*, 1979; Dritschilo and Wanner, 1980; Whitford and Showers, 1987). *P.vernalis* can represent up to 19 percent of the carabid catch in pasture and 58 percent of the catch in pasture edge in Belgium (Desender *et al.*, 1981). *P.melanarius* and *P.madidus* dominate the carabid fauna in arable land in Scotland (Dixon and Mckinlay, 1992; Chapman, 1994) and *P.melanarius* is generally the most numerous *Pterostichus* caught in Britain (Baker and Dunning, 1975; Jones, 1979), Ontario (Rivard, 1964) and Canada (Boiteau, 1983).

H.rufipes is generally the most numerous *Harpalus* species in Britain (Baker and Dunning, 1975; Jones, 1979) and is abundant in Canada (Boiteau, 1983). *Harpalus pensylvanicus* Degeer is often the most numerous member in the United States (e.g. Hsin *et al.*, 1979) and *H.pensylvanicus*, *Harpalus compar* Lec. and *Harpalus erraticus* Say are abundant in Canada (Rivard, 1964 and 1966).

Harpalus and *Pterostichus* genera have many desirable attributes which suit biological control programmes. They inhabit a diversity of agroecosystems, are

relatively abundant and are tolerant of many insecticides (Whitford and Showers, 1987). They are therefore affected to a lesser extent by agrochemicals.

Habitat management

Natural control can only be maximised if favourable habitats are created which ensure that predators exist at high densities (Altieri and Letourneau, 1982; Poehling, 1989). Undersowing swedes with clover can increase the activity density of carabids (Armstrong and McKinlay, 1994) and occasionally microhabitat manipulation has been used as a tool to increase predator numbers. The success of *S.striatopunctatus* in reducing densities of the molluscs *L.maximus* and *H.aspersa*, depended on suitable refuge being provided for the beetles (Altieri *et al.*, 1982).

On a large scale, such microhabitat manipulation are impracticable and studies of the total agroecosystem are needed to determine elements essential for maintaining optimum levels of key species (Finch, 1993). Differences in abundance have been found between organic and conventional farms. Carabids can be seven times more abundant on organic farms compared to conventional farms in the same locations and have up to twice the number of species (Dritschilo and Wanner, 1980). The preservation and improvement of natural habitat and land surrounding the crop is important for maintaining predator numbers and needs to be assessed (Coombes and Sotherton, 1986).

The quality of crop edges and hedgerow may benefit overwintering populations of natural enemies as they provide refuges for many species (Sotherton, 1985; Coombs and Sotherton, 1986). The degradation of hedgerow may lead to a reduction in the recruitment of predators and their subsequent impact on pests. However, the importance of these sites is widely debated (e.g. Thiele, 1977). The maintenance of boundary variation may be more important in preserving and enhancing farmland biodiversity (Beard and Mauremootoo, 1994). Other factors, such as predator survival and breeding success may ultimately determined the number of species available to colonise field boundaries (Thomas *et al.*, 1992) and field boundary quality may then be of minor importance in the year to year population dynamics

of polyphagous predators.

Two key slug predators identified in this project (*P.melanarius* and *H.rufipes*) have both been classified as field species (Pollard, 1968a) with no association with field edges. *Harpalus* species and *P.melanarius* migrate from uncultivated land to fields for food, reproduction and larval development (Wallin, 1985; Whitford and Showers, 1987). The quality of hedgerow is of little importance to these field species (Pollard, 1968a). However, the abundance and diversity of beetles can change considerably even in localised areas: In this study, the *Pterostichus* and *Harpalus* fauna at Bog field was impoverished compared to the other two sites.

Pesticides

The use of broad spectrum insecticides may exacerbate pest problem (e.g. Vickerman and Sunderland, 1977; Vickerman, 1992) by killing non-target arthropods which feed on agricultural pests. Spraying non-selective insecticide over large areas may induce the increase of pest numbers (Basedow, 1973). There is a general consensus for the need to develop biological pest control methods and to reduce chemical inputs into agriculture (Sunderland *et al.*, 1994).

The broad spectrum insecticides fonofos, parathion and phorate are extremely toxic to predatory beetles (Edwards and Thompson, 1975). Apart from causing acute toxicity, some pesticides can have a more subtle effects on beetle numbers. *P.cupreus* beetles which feed on aphids contaminated with the aphicide pirimicarb are unable to build up fat reserves needed for egg production (Wallin *et al.*, 1992). Full insurance pesticide regimes have long term effects on springtail (collembolan) populations which are important in the diet of carabids. Any reduction in the number of springtail will have a negative effect on populations of carabid predators (Frampton *et al.*, 1992).

Molluscicides also have a direct effect on beetle populations. Methiocarb and metaldehyde are the two most frequently used molluscicides in Great Britain (Davis *et al.*, 1992). Methiocarb has a 100 percent mortality rate on some carabid species

(Buchs *et al.*, 1989). In the field, carabid numbers can be locally reduced following treatment by methiocarb (Kennedy, 1990).

In integrated IPM programmes, insecticides need to be used which have a minimal impact on beneficial species. *P.chalcites* is the most abundant species in agricultural land in Illinois and Iowa (Dritschilo and Wanner, 1980) and is more susceptible to carbofuran and terbufos than to dieldrin, owing to the species developing resistance to dieldrin after many years of exposure (Hsin *et al.*, 1979). The authors recommended that carbofuran and terbufos should be applied with great care to avoid reducing populations of *P.chalcites* and other carabid species.

Results from the SCARAB study (a follow up to the Boxworth project), have shown that generally autumn and winter applied broad spectrum insecticides have the most adverse effects on beneficial invertebrates (Cilgi and Frampton, 1994). The SCARAB study found herbicides and fungicides had no substantial effects on beneficial invertebrates although herbicides are known to affect the quality of overwintering sites (Pollard, 1968a) which may be important in predator recruitment.

As the beneficial effects of carabid beetles are realised, more concerted attempts are being made to gather data and assess the effects of pesticides on carabids and other predatory arthropods (Davis, 1968; Powell *et al.*, 1985; Asteraki *et al.*, 1992; Theiling and Croft, 1992; Burn 1992). The mortality of pesticides on some species such as *P.melanarius* has already been investigated (Luff *et al.*, 1990). Although a great deal of toxicity data has been collected, studies are difficult to compare due to the variety of techniques used (Brown, 1989).

It is unlikely that insecticides will be developed which exclusively kill the target pest due to economic considerations in the agrochemical industry (van Emden, 1989). The use of lowered dose rates for physiologically selective pesticides offer the best chance of reducing loss on non-target organisms (Poehling, 1989). A number of recent programmes have been developed which attempt to ensure compatible use

of chemical and biological methods. Programmes which help select pesticides which are less toxic to the most important natural enemies of pests in a crop are particularly useful (Hassan, 1989).

The International Organisation for Biological Control, West Palaearctic Regional Section (IOBC/WPRS) have worked towards standard testing procedures for pesticides on a series of beneficial organisms in different countries (Hassan, 1989). A recent workshop brought together the IOBC, the Beneficial Arthropod Regulatory Testing Group (BART) and the European and Mediterranean Plant Protection Organisation with the Council of Europe (EPPO/CoE) for consensus on the risk assessment for non target arthropods by pesticides for regulatory and IPM purposes (Barrett *et al.*, 1994). However, methodologies need to be developed to help assess the effects of pesticides on non-target invertebrates (Jepson, 1989).

The improvement of cultural techniques and more selective use of pesticides could help farmers decrease pesticide applications and contribute to IPM programmes. The long term benefits of IPM might outweigh the short term economic and environmental costs of present day pest control methods (Coaker, 1987). Although robust IPM programmes for agricultural pests such as slugs are unlikely to be developed in the near future, it is sensible to exploit naturally occurring predators rather than relying exclusively on pesticides (Poehling, 1989).

References

Allen, A.A. (1953). Predatory behaviour of an *Amara* (Col., Carabidae). *Entomologist's Monthly Magazine. 89:* 236.

Allen, W.R. and Hagley, E.A.C. (1982). Evaluation of immunoelectrophoresis on cellulose polyacetate for assessing predation of Lepidoptera (Tortricidae) by Coleoptera (Carabidae) species. *Canadian Entomologist 114:* 1047-1054.

Altieri, M.A. and Letourneau, D.K. (1982). Vegetation management and biological control in agroecosystems. *Crop Protection. 1:* 405-430.

Altieri, M.A., Hagen, K.S., Trujillo, J. and Catagirone, L.F. (1982). Biological control of *Limax maximus* and *Helix aspersa* by indigenous predators in a daisy field in central coastal California. *Acta OEcologia Applicata 3*: 387-390.

Armstrong, G. and Mckinlay, R.G. (1994). Undersowing brassicas with clover to increase the activity of carabid beetles. *Brighton Crop Protection Conference - Pests and Diseases 1994.* pp1175-1180. British Crop Protection Council, Thornton heath.

Ashby, J.W. (1974). A study of arthropod predation of *Pieris rapae* L. using serological and exclusion techniques. *Journal of Applied Ecology. 11*: 419-425.

Asteraki, E.J., Hanks, C.B. and Clements, R.O. (1992). The impact of two insecticides on predatory ground beetles (Carabidae) in newly-sown grass. *Annals of Applied Biology. 120*: 25-39.

Attah, P.K. (1986). *The insect pests and polyphagous arthropod predators associated with crops of oilseed rape in North Yorkshire and Humberside.* Unpublished Ph.D. thesis, University of York.

Aubrook, E.W. (1949). *Amara familiaris* Duft. (Col., Carabidae) feeding in flower heads. *Entomologist's Monthly Magazine. 85*: 44.

Ayre, K. (1995). *Coboldia fuscipes* Meigen (Diptera: Scatopsidae) feeding on slug eggs. *Entomologist's Record and Journal of Variation. 107*: 175-177.

Baars, M.A. (1979a). Catches in pitfall traps in relation to mean densities of carabid beetles. *Oecologia. 41*: 25-46.

Baars, M.A. (1979b). Patterns of movement of radioactive carabid beetles. *Oecologia. 44*: 125-140.

Bailey, N.T.J. (1952). Improvements in the interpretation of recapture data. *Journal of Animal Ecology. 21*: 120-127.

Baker, A.N. and Dunning, R.A. (1975). Some effects of soil type and crop density on the activity and abundance of the epigeic fauna, particularly Carabidae, in sugar-beet fields. *Journal of Applied Ecology. 12*: 809-818.

Barnes, H.F. and Weil, J.W. (1945). Slugs in gardens: Their numbers, activities and distribution. Part 2. *Journal of Animal Ecology. 14*: 71-105.

Barrett, K.L., Grandy, N.J., Hassan, S. and Oomen, P. (1994). Pesticide regulatory testing procedures with beneficial arthropods: Recommendations arising from the SETAC-ESCORT workshop. *Brighton Crop Protection Conference - Pests and Diseases - 1994*. pp 661-668. British Crop Protection Council, Thornton heath.

Basedow, Th. (1973). Der Einflu*B*epigaischer Raubarthropoden auf die Abundanz phytophager Insekten in der Ayrarlandschaft. *Pedobiologia. 13*: 410-422.

Bauer, T. (1982). Predation by carabid beetle specialised for catching Collembola. *Pedobiologia. 24*: 169-179.

Beard, R.C.W. and Mauremootoo, J.R. (1994). The biodiversity of Coleoptera overwintering in arable field boundaries. *Brighton Crop Protection Conference - Pests and Diseases - 1994*. pp 943-944. British Crop Protection Council, Thornton heath.

Beer, G.J. (1989). Levels and economics of slug damage in potato crops 1987-1988. *Slugs and Snails in World Agriculture*, pp 101-105. Ed; I. Henderson. BCPC Mono. 41, 295-300. British Crop Protection Council, Thornton heath.

Begon, M. (1979) Investigating animal abundance. *Capture-recapture for biologists*. Edward Arnold.

Begon, M., Milburn, O. and Turner, D. (1975). Density estimates of *Drosophila* in Southern England. *Journal of Natural History. 9*: 315-320.

Bett, J.A. (1960). The breeding season of slugs in gardens. *Proceedings of the Zoological Society of London. 135*: 559-568.

Bless, Von R. (1977). Untersuchungen zur Frage des Rauber-Beute-Verhaltnisses von Carabiden und Gastropoden. *Anzeiger Schadlingskund Pflanzenschutz, Umweltschutz. 50*: 55-57.

Boer, P.J. Den. (1977). Dispersal power and survival. Carabids in a cultivated counrtyside. *Miscellaneous papers, Landbouwhoogeschool, Wageningen.14*: 1-190.

Boiteau, G. (1983). Activity and distribution of Carabidae, Arachinida and Staphylinidae in New Brunswick potato fields. *Canadian Entomologist. 115*: 1023-1030.

Borden, J.H. (1977). Behavioural responses of Coleoptera to pheromones, allomones and kairomones. *Chemical control of insect behavior. Theory and application*. pp 169-200. Ed; Shorey, H.H and Mckelvey, J.J. Jr. John Wiley and Sons.

Boreham, P.F.L. and Ohiagu, C.E (1978). The use of serology in evaluating invertebrate prey-predator relationships: A review. *Bulletin of Entomological Research. 68*: 171-194.

Briggs, J.B. (1961). A comparison of pitfall trapping and soil sampling in assessing populations of two species of ground beetles (Col: Carabidae). *Annual Report East Malling Research Station 1960*. p108-112.

Briggs, J.B. (1965). Biology of some ground beetles (Col., Carabidae) injurious to strawberries. *Bulletin of Entomological Research. 56*: 79-93.

Brown, R.A. (1989). Pesticides and non-target terrestrial invertebrates: An industrial approach. *Pesticides and Non-target invertebrates*. Ed; Jepson, P.C. Intercept, Dorset.

Bryan, K.M. and Wratten, S.D. (1984). The responses of polyphagous predators to prey spatial heterogeneity: aggregation by carabid and staphylinid beetles to their cereal aphid prey. *Ecological Entomology. 9*: 251-259.

Buchs, W., Heimbach, U. and Czarnecki, E. (1989). Effects of snail baits on non-target carabid beetles. 1989 BCPC Mono. No. 41 *Slugs and Snails in World Agriculture*. pp 245-253. Ed; Henderson, I.F. British Crop Protection Council, Thornton heath.

Burn, A.J. (1982). The role of predator searching efficiency in carrot fly egg loss. *Annals of Applied Biology. 101*: 154-159.

Burn, A.J. (1992). Interactions between cereal pests and their predators and parasites. *Pesticides, cereal farming and the environment*. P.W. Greg-Smith, G.K. Frampton and A.R. Hardy (Eds), pp110-131. HMSO, London.

Burrows, P.M.; Scott, S.W.; Barnett, O.W. and McLaughlin, M.R. (1984). Use of experimental designs with quantitative ELISA. *Journal of Virological Methods. 8*: 207-216.

Calver, M.C. (1984). A review of ecological applications of immunological techniques for diet analysis. *Australian Journal of Ecology. 9*: 19-25.

Campion, N. (1961). The structure and function of the cutaneous glands in *Helix aspersa*. *Quarterly Journal of Microscopic Science. 102*: 195-216.

Chambers, R.J., Sunderland, K.D., Wyatt, I.J and Vickerman, G.P. (1983). The effects of predator exclusion and caging on cereal aphids in winter wheat. *Journal of Applied Ecology. 20*: 209-224.

Chapman, P.A. (1994). Control of leatherjackets by natural enemies: The potential role of the ground beetle *Pterostichus melanarius*. *Brighton Crop Protection Conference - Pests and Diseases - 1994*. pp 933-934. British Crop Protection Council, Thornton heath.

Cherrill, A.J. and Begon, M. (1989). Predation on grasshoppers by spiders in sand dune grasslands. *Entomologia Experimentalis et Applicata. 50*: 225-231.

Chiverton, P.A. (1984). Pitfall-trap catches of the carabid beetle *Pterostichus melanarius*, in relation to gut contents and prey densities, in insecticide treated and untreated spring barley. *Entomologia Experimentalis et Applicata. 36*: 23-30.

Chiverton, P.A. (1986). Predator density manipulation and its effects on populations of *Rhopalosiphum padi* (Hom: Aphidae) in spring barley. *Annals of Applied Biology. 109*: 49-60.

Chiverton, P.A. (1987). Effects of exclusion barriers and inclusion trenches on polyphagous and aphid specific predators in spring barley. *Journal of Applied Entomology. 103*: 193-203.

Cilgi, T. and Frampton, G.K. (1994). Arthropod populations under current and reduced-input pesticide regimes: Results from the first four treatment years of the MAFF 'SCARAB' project. *Brighton Crop Protection Conference - 1994 - Pests and Diseases.* pp 653-660. British Crop Protection Council, Thornton heath.

Clark, M.F and Adams, A.N. (1977). Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. *Journal of General Virology. 34*: 475-483.

Coaker, T.H. and Williams, D.A. (1963). The importance of some Carabidae and Staphylinidae as predators of the cabbage root fly, *Erioischia brassicae* (Bouche). *Entomologia Experimentalis et Applicata. 6*: 156-164.

Coaker, T.H. (1965). Further experiments on the effect of beetle predators on the number of the cabbage root fly, *Erioischia brassicae* (Bouche), attacking brassicae crops. *Annals of Applied Biology. 56*: 7-20.

Coaker, T.H. (1987). Cultural methods: the crop. *Integrated Pest Management*. Ed. A.J. Burn, T. Coaker and P. C. Jepson. Academic Press, London. pp 69-82.

Coombes, D.S. and Sotherton, N.W. (1986). The dispersal and distribution of polyphagous predatory Coleoptera in cereals. *Annals of Applied Biology. 108*: 461-474.

Cornic, J.F. (1973). Etude du regime alimentaire de trois especes de Carabiques et de ses variations en verger de pommiers. *Annales de la Societe Entomologique de France 9*: 69-87.

Crawford-Sidebotham, T.J. (1972). The influence of weather upon the activity of slugs. *Oecologia. 9*: 141-154.

Crook, N.E. and Sunderland, K.D. (1984). Detection of aphid remains in predatory insects and spiders by ELISA. *Annals of Applied Biology. 105*: 413-422.

Crowell, H.H. (1967). Slug and snail control with experimental poison baits. *Journal of Economic Entomology. 60*: 1048-1050.

Crowson, R.A. (1955). *The natural classification of the families of Coleoptera.* London.

Dainton, B.H. (1954). The activity of slugs. I The induction of activity by changing temperatures. *Journal of Experimental Biology. 31*: 165-181.

Davies, M.J. (1953). The contents of the crops of some British carabid beetles. *Entomologist's Monthly Magazine. 89*: 18-23.

Davis, B.N.K. (1968). The soil macrofauna and organochlorine insecticide residues at twelve agricultural sites near Huntingdon. *Annals of Applied Biology. 61*: 29-45.

Davis, R.P., Thomas, M.R., Garthwaite, D.G and Bowen, H.M. (1992). Arable farm crops in Great Britain 1992. *Pesticide usage survey report 108.*

Dawson, N. (1965). A comparative study of the ecology of eight species of Fenland Carabidae (Coleoptera). *Journal of Animal Ecology. 34*: 299-314.

Dempster, J.B.; Richards, O.W. and Waloff, N. (1959). Carabidae as predators on the pupal stage of the Chrysomelid beetle, *Phytodecta olivacea* (Forster). *Oikos. 10*: 66-70.

Dempster, J.P. (1967). The control of *Pieris rapae* with DDT. I The natural mortality of the young stages of *Pieris*. *Journal of Applied Ecology. 4*: 485-500.

Dennison, D.F. and Hodkinson, I.D. (1983). Structure of the predatory beetle community in a woodland soil ecosystem. I Prey selection. *Pedobiologia. 25*: 109-115.

Desender, K., Maelfait, J.P., D'Hulster, M. and Vanhercke, L. (1981). Ecological and faunal studies on Coleoptera in agricultural land. I Seasonal occurrence of Carabidae in the grassy edge of a pasture. *Pedobiologia. 22*: 379-384.

Deyrup-Olsen, I. and Martin, A.W. (1982). Surface exudation in terrestrial slugs. *Comparative Biochemistry and Physiology. 72c*: 45-51.

Digweed, S.C. (1993a). Selection of terrestrial gastropod prey by Cychrine and Pterostichini ground beetles (Coleoptera: Carabidae). *The Canadian Entomologist. 125*: 463-472.

Digweed, S.C. (1993b). Detection of mucus producing prey by *Carabus nemoralis* Muller and *Scaphinotus marginatus* Fischer (Coleoptera: Carabidae). *Proceedings of the Entomological Society of Alberta. 39*: 8-9.

Dijk, Th.S. van. (1973). The age-composition of populations of *Calathus melanocephalus* L. analysed by studying marked individuals kept within fenced sites. *Oecologia. 12*: 213-240.

Dixon, P.L and Mckinlay, R.G. (1992). Pitfall trap catches of and aphid predation by *Pterostichus melanarius* and *Pterostichus madidus* in insecticide treated and untreated potatoes. *Entomologia Experimentalis et Applicata. 64*: 63-72.

Dritschilo, W. and Wanner, D. (1980). Ground beetle abundance in organic and conventional corn fields. *Environmental Entomology. 9*: 629-637.

Dub, M. (1971). Movements of *Microtus arvalis* and a method of estimating its numbers. *Zoologicke Listy. 20*: 1-14.

Dunning, R.A., Baker, A.N. and Windley, R.F. (1975). Carabids in sugar beet crops and their possible role as aphid predators. *Annals of Applied Biology. 80*: 125-128.

Duthoit, C.M. (1964). Slugs and food preferences. *Plant Pathology. 13*: 73-78.

Edgar, W. (1970). Prey and feeding behaviour of adult females of the wolf spider *Pardosa amentata* (Clerck). *Netherlands Journal of Zoology. 20*: 487-491.

Edwards, C.A. and Thompson, A.R. (1975). Some effects of insecticide on predatory beetles. *Annals of Applied Biology. 80*: 132-135.

Edwards, C.A., Parsons, N., George, K.S. and Heilbroon, T.D. (1978). Carabids as predators of cereal aphids. *Annual Report of Rothamsted Experimental Station for 1977.* Part 1. pp 101.

Edwards, C.A., Sunderland, K.D. and George, K.S. (1979). Studies on polyphagous predators of cereal aphids. *Journal of Applied Ecology. 16*: 811-823.

Ericson, D. (1977). Estimating population parameters of *Pterostichus cupreus* and *P.melanarius* (Carabidae) in arable fields by means of capture-recapture. *Oikos. 29*: 407-417.

Ericson, D. (1978). Distribution, activity and density of some Carabidae (Coleoptera) in winter wheat fields. *Pedobiologia. 18*: 202-217.

Ernsting, G., Jager, J.C., van der Meer, J and Slob, W. (1985). Locomotory activity of a visually hunting carabid beetle in response to non-visual prey stimuli. *Entomologica Experimentalis et Applicata. 38*: 41-47.

Ernsting, G. and van der Werf, D.C. (1988). Hunger, partial consumption of prey and prey size preference in a carabid beetle. *Ecological Entomology. 13*: 155-164.

Evans, M.E.G. (1967). Notes on the feeding of some predaceous beetles of the woodland floor. *The Entomologist. 100*: 300-303.

Evans, M.E.G. and Forsythe, T.G. (1985). Feeding mechanisms, and their variation in form, of some adult ground-beetles (Coleoptera: Caraboidea). *Journal of Zoology, London. (A). 206*: 113-143.

Fenlon, J.S and Sopp, P.I. (1991). Some statistical considerations in the determination of thresholds in ELISA. *Annals of Applied Biology. 119*: 177-189.

Ffrench-Constant, R.H. and Devonshire, A.L (1987). A multiple homogeniser for rapid sample preparation in immunoassays and electrophoresis. *Biochemical Genetics. 7/8*: 493-499.

Fichter, B.L and Stephen, W.P. (1981). Time related decay in prey antigens ingested by the predator *Podisus maculiventris* (Hemiptera; Pentatomidae) as measured by ELISA. *Oecologia. 51*: 404-407.

Fichter, B.L and Stephen, W.P. (1984). Time related decay of prey antigens ingested by arboreal spiders as detected by ELISA. *Environmental Entomology. 13*: 1583-1587.

Finch, S. (1993). Integrated pest management of the cabbage root fly and the carrot fly. *Crop Protection. 12*: 423-430.

Fisher, R.A. and Ford, E.B. (1947). Spread of a gene in natural conditions in a colony of the moth *Panaxia dominula* (L). *Heredity. 1*: 143-174.

Frampton, G.K., Langton, S.D., Greig-Smith, P.W. and Hardy, A.R. (1992). Changes in the soil fauna at Boxworth. *Pesticides, cereal farming and the environment*. P.W. Greg-Smith, G.K. Frampton & A.R. Hardy (Eds), pp110-131. HMSO, London.

Frank, J.H. (1967). The insect predators of the pupal stage of the winter moth, *Operophtera brumata* (L.)(Lepidoptera: Hydriomenidae). *Journal of Animal Ecology. 36*: 375-389.

Frank, J.H. (1971). Carabidae (Coleoptera) as predators of the red-backed cutworm (Lepidoptera: Noctuidae) in central Alberta. *Canadian Entomologist. 103*: 1039-1049.

Giller, P.S. (1984). Predator gut state and prey detectability using electrophoretic analysis of gut contents. *Ecological Entomology 9*: 157-162.

Glen, D.M. (1989). Understanding and predicting slug problems in cereals. 1989 BCPC Mono. No. 41 *Slugs and Snails in World Agriculture*. pp 253-262. Ed; Henderson, I.F. British Crop Protection Council, Thornton heath.

Glen, D.M., Lyons, C.H., Milsom, N.F. and Wiltshire, C.W. (1982). Ecology, behaviour and control of slugs in cereal crops. *Long Ashton Report for 1982.* p58-60.

Glen, D.M., Wiltshire, C.W. and Milsom, N.F. (1988). Effects of straw disposal on slug problems in cereals. *Aspects of Applied Biology 17, Environmental Aspects of Applied Biology Part 2*, 173-179.

Glen, D.M., Milsom, N.F. and Wiltshire, C.W. (1989). Effects of seed-bed conditions on slug numbers and damage to winter wheat in a clay soil. *Annals of Applied Biology. 115*: 177-190.

Glen, D.M., Jones, H. and Fieldsend, J.K. (1990). Damage to oilseed rape seedlings by the field slug *Deroceras reticulatum* in relation to glucosinolate concentration. *Annals of Applied Biology. 117*: 197-207.

Gordon, P.L. and Mckinlay, R.G. (1986). Dispersal of ground beetles in a potato crop; a mark-release study. *Entomologia Experimentalis et Applicata. 40*: 104-105.
Gould, H.J. (1961). Observations on slug damage to winter wheat in East Anglia, 1957 to 1959. *Plant Pathology. 10*: 142-146.

Gould, H.J. (1965). Observations on the susceptibility of maincrop potato varieties to slug damage. *Plant Pathology. 14*: 109-111.

Gradwell, G.R. (1954). Feeding habits of two carabid beetles. *Entomologist's Monthly Magazine. 90*: 231.

Greenslade, P.J.M. (1963). Daily rhythms of locomotory activity in some Carabidae (Coleoptera). *Entomologia Experimentalis et Applicata. 6*: 171-180.

Greenslade, P.J.M. (1964a). The distribution, dispersal and size of a population of *Nebria brevicollis* (F.), with comparative studies on three other Carabidae. *Journal of Animal Ecology. 33*: 311-333.

Greenslade, P.J.M. (1964b). Pitfall trapping as a method for studying populations of Carabidae (Coleoptera). *Journal of Animal Ecology. 33*: 301-310.

Greenstone, M.H. (1989). Foreign exploration for predators: A proposed new methodology. *Environmental Entomology. 18*: 195-200.

Griffiths, E. (1982). The carabid *Agonum dorsale* as a predator in cereals. *Annals of Applied Biology. 101*: 152-154.

Griffiths, E., Wratten, S.D. and Vickerman, G.P. (1985). Foraging by the carabid *Agonum dorsale* in the field. *Ecological Entomology. 10*: 181-189.

Grum, L. (1959). Seasonal changes of activity of the Carabidae. *Ekologia Polska. A7*: 255-268.

Gruntal, S.Y. and Sergeyeva, T.K. (1989). Food relations characteristics of the beetles of the genera *Carabus* and *Cychrus*. *Zoologicheskii Zhurnal. 58*: 45-51.

Hagley, E.A.C., Holliday, N.J. and Barber, D.R. (1982). Laboratory studies of the food preferences of some orchard carabids (Coleoptera: Carabidae). *Canadian Entomologist. 114*: 431-437.

Halsall, N.B. (1990). *Time-lapse video studies of invertebrate foraging behaviour with special reference to Carabidae.* Unpublished Ph.D. thesis, University of Southampton.

Halsall, N.B. and Wratten, S.D. (1988). The efficiency of pitfall trapping for polyphagous predatory Carabidae. *Ecological Entomology. 13*: 293-299.

Hartree, E.F. (1972). Determination of protein: A modification of the lowry method that gives a linear photometric response. *Analytical Biochemistry. 48*: 422-427.

Hassan, S.A. (1989). Testing methodologies and the concept of the IOBC/WPRS working group. *Pesticides and Non-target invertebrates*. Ed. Jepson, P.C. Intercept, Dorset.

Hassell, M.P. and May, R.M. (1974). Aggregation of predators and insect parasites and its effect on stability. *Journal of Animal Ecology. 43:* 567-594.

Heck, F.C.; Williams, J.D. and Pruett, J. (1980). Interpretation of spectrophotometric absorbace values to define results of Enzyme-linked immunosorbent assays. *Journal of Clinical Microbiology. 11*: 398-401.

Hengeveld, R. (1980a). Polyphagy, oligophagy and food specialisation in ground beetles (Coleoptera, Carabidae). *Netherlands Journal of Zoology. 30*: 564-584.

Hengeveld, R. (1980b). Food specialisation in ground beetles: An ecological or a phylogenetic process ? (Coleoptera, Carabidae). *Netherlands Journal of Zoology. 30*: 585-594.

Herbert, C.N; Edwards, S.; Bushnell, S.; Jones, P.C. and Perry, C.T. (1985). Establishment of a statistical base for use of ELISA in diagnostic serology for infectious bovine rhinorracheitis. *Journal of Biological Standardisation. 13*: 243-253.

Holliday, N.J. and Hagley, E.A.C. (1978). Occurrence and activity of ground beetles (Coleoptera: Carabidae) in a pest management apple orchard. *The Canadian Entomologist. 110*: 113-119.

Holling, C.S. (1961). Principals of insect predation. *Annual Reviews in Entomology. 6*: 163-182.

Holopainen, J.K. and Helenius, J. (1992). Gut contents of ground beetles (Col., Carabidae), and activity of these and other epigeal predators during an outbreak of *Rhopalosiphum padi* (Hom., Aphididae). *Acta Agriculturae Scandinavica, Section B, Soil and Plant Science. 42*: 57-61.

Hsin, C-Y, Sellers, L.G and Dahm, P.A. (1979). Seasonal activity of carabids and the toxicity of Carbofuran and Terbufos to *Pterostichus chalcites*. *Environmental Entomology. 8*: 154-159.

Hunter, P.J. (1966). The distribution and abundance of slugs on an arable plot in Northumberland. *Journal of Animal Ecology. 35*: 543-557.

Hunter, P.J. (1967). The effect of cultivations on slugs of arable land. *Plant Pathology. 16*: 153-156.

Hunter, P.J. (1968a). Studies on slugs of arable ground. II Life cycles. *Malacologia. 6*: 379-389.

Hunter, P.J. (1968b). Studies on slugs of arable ground. III Feeding habits. *Malacologia. 6*: 391-399.

Hunter, P.J. (1968c). Studies on slugs of arable ground. I sampling methods. *Malacologia. 6*: 369-377.

Hunter, P.J. (1969). An estimate of the extent of slug damage to wheat and potatoes in England and Wales. *National Agricultural Advisory Service, Quarterly Review. 85*: 31-37.

Hunter, P.J. (1978). *Slugs - a study in applied ecology*. Ed. Fretter, V. and Peake, J. Academic Press. pp 271-286.

Hunter, P.J., Symonds, B.V and Newell, P.F. (1968). Potato leaf and stem damage by slugs. *Plant Pathology. 17*: 161-164.

Hunter, P.J. and Runham, N.W. (1972). Slugs: a world problem. *Tropical Science. 13*: 191-197.

Ingram, M.W. (1946). Mollusk food of the beetle *Scaphinotus interruptus* (Men.). *Bulletin of the Southern California Academy of Sciences. 45*: 34-36.

Jepson, P.C. (1989). The temporal and spacial dynamics of pesticide side-effects on non-target invertebrates. *Pesticides and Non-target invertebrates*. Ed. Jepson, P.C. Intercept, Dorset.

Jolly, G.M. (1965). Explicit estimates from capture-recapture data with both death and immigration, stochastic model. *Biometrica. 52*: 225-247.

Jones, M.G. (1969). Some reflections on quantitative distribution and control of plant nematodes. *Nematodes of Tropical Crops.* Ed; Peachey, J.E. Farnham Royal Common Agricultural Bureau.

Jones, M.G. (1975). The predators of the wheat-bulb fly. *Annals of Applied Biology. 80*: 128-130.

Jones, M.G. (1976). The carabid and staphylinid fauna of winter wheat and fallow on a clay with flint soil. *Journal of Applied Ecology. 13*: 775-791.

Jones, M.G. (1979). The abundance and reproductive activity of common Carabidae in a winter wheat crop. *Ecological Entomology. 4*: 31-43.

Kary, J. (1970). Elimination of the Colorado beetle (*Leptinotarsa decemlineata* Say) by Carabidae. *Bulletin International de l'Academic polonaise des Sciences at des Lettres. 18*: 397-400.

Kelly, J.R and Martin, T.J. (1989). Twenty one years experience with methiocarb bait. 1989 BCPC Mono. No. 41 *Slugs and Snails in World Agriculture.* pp 131-145. Ed; Henderson, I.F. British Crop Protection Council, Thornton heath.

Kennedy, P.J. (1990). *Effects of molluscicides on the abundance and distribution of ground beetles (Col: Car) and other invertebrates.* Unpublished Ph.D. thesis, University of Bristol.

Kiritani, K., Kawahara, S., Sasaba, T. and Nakasyi, F. (1972). Quantitative evaluation of predation by spiders on the green rice leafhopper, *Nepholettix cineticeps* Uhler, by a sight-count method. *Reseraches in Population Ecology. 13*: 187-200.

Kloet, G.S and Hincks, W.D. (1945). *A check list of British insects.* Buncle and Co. Ltd.

Laing, J. (1937). Host-finding by insect parasites. I Observations on the finding of hosts by *Alysia manducator, Mormoniella vitripennis* and *Tichogramma evanescens. Journal of Animal Ecology. 6*: 298-317.

Laing, J. (1938). Host-finding by insect parasites. II The chance of *Tichogramma evanescens* finding its hosts. *Journal of Experimental Biology. 15*: 281-302.

Larochelle, A. (1972). Notes on the food of Cychrini (Coleoptera: Carabidae). *The Great Lakes Entomologist. 5*: 81-83.

Lilley, R., Hardie, J., Powell, W. and Wadhams, L.J. (1994). The aphid sex pheromone: A novel host location cue for the parasitoid *Praon volucre*. *Brighton Crop Protection Conference - Pests and Diseases - 1994.* pp 1157. British Crop Protection Council, Thornton heath.

Lindroth, C.H. (1974). *Handbooks for the identification of British Insects. Vol IV part 2*: Coleoptera Carabidae. Royal Entomological Society of London.

Loreau, M. (1984). Etude experimentale de L'alimentation de *Abax ater* Villers, *Carabus problematicus* Herbst et *Cychrus attenuatus* Fabricius (Coleoptera, Carabidae). *Annales de la Societe r. zoologique de Belgique. 114*: 227-240.

Loughridge, A.H. and Luff, M.L. (1983). Aphid predation by *Harpalus rufipes* (Degeer)(Coleoptera: Carabidae) in the laboratory and field. *Journal of Applied Ecology*. *20*: 451-462.

Lovei, G.L.; Monostori, E. and Ando, E. (1985). Digestion rate in relation to starvation in the larva of a carabid predator *Poecilus cupreus*. *Entomologia Experimentalis et Applicata. 37*: 123-127.

Luff, M.L. (1973). The annual activity pattern and life cycle of *Pterostichus madidus* (F.)(Col. Carabidae). *Entomologica Scandinavica. 4*: 259-273.

Luff, M.L. (1974). Adult and larval feeding habits of *Pterostichus madidus* (F.) (Coleoptera; Carabidae). *Journal of Natural History. 8*: 403-409.

Luff, M.L. (1975). Some features influencing the efficiency of pitfall traps. *Oecologia. 19*: 345-357.

Luff, M.L. (1978). Diel activity patterns of some field Carabidae. *Ecological Entomology. 3*: 53-62.

Luff, M.L. (1980). The biology of the ground beetle *Harpalus rufipes* in a strawberry field in Northumberland. *Annals of Applied Biology. 94*: 153-164.

Luff, M.L. (1982). Population dynamics of Carabidae. *Annals of Applied Biology. 101*: 164-170.

Luff, M.L. (1983). The potential of predators for pest control. *Agriculture, Ecosystems and Environment. 10*: 159-181.

Luff, M.L. (1987). Biology of polyphagous ground beetles in agriculture. *Agricultural Zoology Reviews. 2*: 237-278.

Luff, M.L., Clements, R.O. and Bale, J.S. (1990). An integrated approach to assessing effects of some pesticides in grassland. *1990 Brighton Crop Protection Conference - Pests and Diseases. 1*: pp 143-152. British Crop Protection Council, Thornton heath.

Madden, J. (1968). Behavioural responses of parasites to the symbiotic fungus assiciated with *Sirex noctillio* F. *Nature. 218*: 189-190.

Magurran, A.E. (1988). *Ecological diversity and its measurement*. Croom Helm Ltd. London.

Manly, B.F.J. (1970). A simulation study of animal population estimation using the capture-recapture method. *Journal of Applied Ecology. 7*: 13-39.

Martin, T.J. and Kelly, J.R. (1986). The effect of changing agriculture on slugs as pests of cereals. *Proceedings 1986 British Crop Protection Conference 2.* pp 411-424. British Crop Protection Council, Thornton heath.

Mattinson, G. (1965). A study of antigens in adult and embryonic tissues of the slug *Deroceras*. *Canadian Journal of Zoology. 43*: 1-12.

McIver, J.D. (1981). An examination of the utility of the precipitin test for evaluation of arthropod predator-prey relationships. *Canadian Entomologist. 113*: 213-222.

Mclachlan, R. (1897). *Harpalus ruficornis*, F., Destructive to ripe strawberries. *Entomologist's Monthly Magazine. 33*: 171-172.

Meijer, J. (1974). A comparative study of the immigration of carabids (Coleoptera, Carabidae) into a polder. *Oecologia. 16*: 185-208.

Meijer, J. (1975). Carabid (Coleoptera; Carabidae) migration studies with laboolbeniales (Ascomycetes) as biological tags. *Oecologia. 19*: 99-103.

Mellanby, K. (1961). Slugs at low temperatures. *Nature. 189*: 944.

Miller, M.C. (1981). Evaluation of enzyme-linked immunosorbent assay of narrow- and broad-spectrum anti-adult Southern pine beetle serum. *Annals of the Entomological Society of America. 74*: 279-282.

Mills, N.J. (1982). Satiation and the functional response: a test of a new model. *Ecological Entomology. 7*: 305-315.

Mitchell, B. (1963a). Ecology of two carabid beetles, *Bembidion lampros* (Herbst) and *Trechus quadristriatus* (Schrank). I Life cycles and feeding Behaviour. *Journal of Animal Ecology. 32*: 289-299.

Mitchell, B. (1963b). Ecology of two carabid beetles, *Bembidion lampros* (Herbst) and *Trechus quadristriatus* (Schrank). II Studies on populations of adults in the field, with special reference to the technique of pitfall trapping. *Journal of Animal Ecology. 32*: 377-392.

Moens, R. (1982). Observations au sujet de la predation de *Zonitoides nitidus* Muller sur *Lymnaea truncatula* Muller. *Bulletin D'Ecologie. 13*: 273-282.

Moens, R and Vase, J. (1986). La predation de *Zonitoides nitidus* (Muller) et autres *Zonitides* sur les oeufs Gasteropodes. *Bulletin des recherches Agronomiques Gembloux. 21*: 161-172.

Mols, P.J.M. (1979). Motivation and walking behaviour of the carabid beetle *Pterostichus coerulescens* L. at different densities and distribution of the prey. *On the evolution of behaviour of carabid beetles. Miscellaneous papers. No. 18*, 185-198. Landbouwhogeschool, Wageningen. Ed; Den Boer, P.J. Veenman and Zonen, Wageningen.

Murdoch, W.W. (1966). Aspects of the population dynamics of some marsh Carabidae. *Journal of Animal Ecology. 35*: 127-156.

Newell, P.F. (1966). Time lapse photography to record nocturnal behaviour of slugs. *Medical and Biological Illustration. 16*: 146-159.

Nentwig, W. and Wissel, C. (1986). A comparison of prey lengths among spiders. *Oecologia. 65*: 595-600.

Obrtel, R. (1971). Number of pitfall traps in relation to the structure of the catch of soil surface Coleoptera. *Acta Entomologia Bohemoslavaca. 68*: 300-309.

Pakarinen, E. (1994). The importance of mucus as a defence against carabid beetles by the slugs *Arion faciatus* and *Deroceras reticulatum*. *Journal of Molluscan Studies. 60*: 149-155.

Pearson, R.G. and White, E. (1964). The phenology of some surface-active arthropods of moorland country in North Wales. *Journal of Animal Ecology. 33*: 245-253.

Penney, M.M. (1966). Studies on certain aspects of the ecology of *Nebria brevicollis* (F.) (Coleoptera, Carabidae). *Journal of Animal Ecology. 35*: 505-512.

Pickavance, J.R. (1970). A new approach to the immunological analysis of invertebrate diets. *Journal of Animal Ecology. 39*: 715-724.

Poehling, H.M. (1989). Selective application strategies for insecticides in agricultural crops. *Pesticides and Non-target invertebrates*. Ed; Jepson, P.C. Intercept, Dorset.

Pollard, E. (1968a). Hedges III. The effect of removal of the bottom flora of a hawthorn hedgerow on the Carabidae of the hedge bottom. *Journal of Applied Ecology. 5*: 125-139.

Pollard, E. (1968b). Hedges IV. A comparason between the Carabidae of a hedge and field site and those of a woodland glade. *Journal of Applied Ecology. 5*: 649-657.

Pollet, M. and Desender, K. (1985). Adult and larval feeding ecology in *Pterostichus melanarius* Ill (Coleoptera, Carabidae). *Mededelingen van de Fakulteit Landbouwwertenschappen Rijksuniversiteit (Gent), 50/2b.* 581-594.

Port, C.M. and Port, G.R. (1986). The biology and behaviour of slugs in relation to crop damage and control. *Agricultural Zoology Reviews. 1:* 255-299.

Potts, G.R. and Vickerman, G.P. (1974). Studies in the cereal ecosystem. *Advances in Ecological Research.* 8: 107-197.

Poulin, G and O'Neil, L.C. (1969). Observations sur les predateurs de la limace noire *Arion ater* (L.)(Gasteropodes, Pulmones, Arionides). *Phytoprotection. 50*: 1-6.

Powell, W., Dean, G.J. and Bardner, R. (1985). Effects of pirimicarb, dimethoate and benomyl on natural enemies of cereal aphids in winter wheat. *Annals of Applied Biology. 106*: 235-242.

Quicke, D.L. (1987). Orbweb weaving spiders preying on slugs. *Proceedings and Transactions of the British Entomology and Natural History Society. 20*: 90.

Quick, H.E. (1960). British slugs (Pulmonata: Testacellidae, Arionidae, Limacidae). *Bulletin of the British Museum (Natural History), Zoology. 6*: 103-226.

Ragsdale, D.W.; Larson, A.D. and Newsom, L.D. (1981). Quantitative assessment of the predators of *Nezara viridula* eggs and nymphs within a soybean agroecosystem using an ELSIA. *Environmental Entomology. 10*: 402-405.

Rivard, I. (1964). Carabid beetles (Coleoptera: Carabidae) from agricultural lands near Belleville, Ontario. *The Canadian Entomologist. 96*: 517-520.

Rivard, I. (1965). Dispersal of ground beetles (Coleoptera: Carabidae) on soil surface. *Canadian Journal of zoology. 43*: 465-473.

Rivard, I. (1966). Ground beetles (Coleoptera: Carabidae) in relation to agricultural crops. *The Canadian Entomologist. 98*: 189-195.

Robinson, W.H. (1965). Biology of a phorid feeding on slug eggs. *Bulletin of the Entomological Society of America. 11*: 155.

Robinson, W.H. and Foote, B.A. (1968). Biology and immature stages of *Megaselia aequalis*, a phorid predator of slug eggs. *Annals of the Entomological Society of America. 61*: 1587-1594.

Rollo, C.D. (1982). The regulation of activity in populations of the terrestrial slug, *Limax maximus*(Gastropoda, Limacidae).*Researches on Population Ecology. 24*: 1-32.

Rothschild, G. (1966). A study of a natural population of *Conomelus anceps* Germar (Homoptera: Delphacidae) including observations on predation using the precipitin test. *Journal of Animal Ecology. 35*: 413-434.

Sandness, J.N and McMurtry, J.A. (1972). Prey consumption behaviour of *Amblyseius largoensis* in relation to hunger. *Canadian Entomologist. 104*: 461-470.

Shankey, J.H.P. (1949). Observations on food, enemies and parasites of British harvest-spiders (Arachnida, Opiliones). *Entomologist's Monthly Magazine. 85*: 246.

Sly, J.M.A. (1984). *Pesticide usage survey report 35. Arable farm crops and grass 1982.* Ministry of Agriculture, Fisheries and Food, London.

Smith, G.S., Allison, J.C.S. and Pammenter, N.W. (1994). Bio-assay study of the response by a parasitoid to frass and feeding substrates of its host, the stalk borer *Eldana Saccharina. Annals of Applied Biology. 125*: 439-446.

Soboleva-Dokuchayeva, J.J. (1975). Determination of the role of carabids in agrobiological communities by the serological method. *Zhurnal Obshchei Biologii. 36*: 749-760.

Sopp, P.I. (1987). *Quantification of predation by polyphagous predators on Sitobian avenae (F.) (Homoptera: Aphidae) in winter wheat using ELISA*. Unpublished Ph.D. thesis, University of Southampton.

Sopp, P.I. and Wratten, S.D. (1986). Rates of consumption of cereal aphids by some polyphagous predators in the laboratory. *Entomologia Experimentalis et Applicata. 41*: 59-73.

Sopp, P.I. and Sunderland, K.D. (1989). Some factors affecting the detection period of aphid remains in predators using ELISA. *Entomologia Experimentalis et Applicata. 51*: 11-20.

Sopp, P.I., Sunderland, K.D., Fenlon, F.S. and Wratten, S.D. (1992). An improved quantitative method for estimating invertebrate predation in the field using an Enzyme-linked immunosorbent assay(ELISA).*Journal of Applied Ecology. 29*:295-302.

Sotherton, N.W. (1984). The distribution and abundance of predatory arthropods overwintering on farmland. *Annals of Applied Biology. 105*: 423-429.

Sotherton, N.W. (1985). The distribution and abundance of predatory Coleoptera overwintering in field boundaries. *Annals of Applied Biology. 106*: 17-21.

South, A. (1965). Biology and ecology of *Agriolimax reticulatus* (Mull) and other slugs: spatial distribution. *Journal of Animal Ecology. 34*: 403-417.

Southwood, T.R.E. (1966). *Ecological methods. With particular reference to the study of insect populations*. Methuen and co Ltd.

Speight, M.R. and Lawton, J.H. (1976). The influence of weed-cover on the mortality imposed on artificial prey by predatory ground beetles in cereal fields. *Oecologia. 23*: 211-223.

Spradbery, J.P. (1970). Host finding by *Rhyssa persuasoria* (L.) an Ichneumonid parasite of Siricid woodwasps. *Animal Behaviour. 18*: 103-114.

Stephenson, J.W. (1965). Biology of slugs. *Rothamsted Experimental Station Report for 1964.* pp 187-189.

Stephenson, J.W. and Knutson, L.V. (1966). A resume of recent studies of invertebrates associated with slugs. *Journal of Economic Entomology. 59*: 356-360.

Stephenson, J.W. and Bardner, R. (1976). Slugs in agriculture. *Rothamsted Experimental Station Report 1976, Part 2.* pp 167-187.

Sunderland, K.D. (1975). The diet of some predatory arthropods in cereal crops. *Journal of Applied Ecology. 12*: 507-515.

Sunderland, K.D. and Sutton, S.L. (1980). A serological study of arthropod predation on woodlice in a dune grassland ecosystem. *Journal of Animal Ecology. 49*: 987-1004.

Sunderland, K.D. and Vickerman, G.P. (1980). Aphid feeding by some polyphagous predators in relation to aphid density in cereal aphids. *Journal of Applied Ecology. 17*: 389-396.

Sunderland, K.D. (1987). A review of methods of quantifying invertebrate predation occurring in the field. *Acta Phytopathologica et Entomologica Hungarica 22*: 13-34.

Sunderland, K.D.; Crook, N.E.; Stacey, D.L. and Fuller, B.J. (1987). A study of feeding by polyphagous predators on cereal aphids using ELISA and gut dissection. *Journal of Applied Ecology. 24*: 907-933.

Sunderland, K.D., Ellis, S.J., Weiss, A., Topping, C.J. and Lang, S.J. (1994). The effects of polyphagous predators on spiders and mites in cereal fields. *1994 Brighton Crop Protection Connference - Pests and Diseases.* pp 1151-1156. British Crop Protection Council, Thornton heath.

Sunderland, K.D. (1988). Quantitative methods for detecting invertebrate predation occurring in the field. *Annals of Applied Biology. 112*: 201-224.

Sutula, C.L.; Gillet, J.M.; Morrissey, S.M. and Ramsdell, C. (1986). Interpreting ELISA data and establishing the positive-negative threshold. *Plant Disease. 70*: 722-726.

Symondson, W.O.C. (1989). Biological control of slugs by carabids. 1989 BCPC Mono. No. 41 *Slugs and Snails in World Agriculture*, pp 295-300. Ed; Henderson, I.F. British Crop Protection Council, Thornton heath.

Symondson, W.O.C. (1992). *Biological control of slugs by carabid beetles.* Unpublished Ph.D. thesis, University of Wales college of Cardiff.

Symondson, W.O.C. and Liddell, J.E. (1993a). The development and characterisation of an anti-haemocyanin antiserum for the detection of mollusc remains within carabid beetles. *Biocontrol Sci. Technol. 3*: 261-275.

Symondson, W.O.C. and Liddell, J.E. (1993b). A monoclonal antibody for the detection of Arionid slug remains in carabid predators. *Biological control. 3*: 207-214.

Symondson, W.O.C. and Liddell, J.E. (1993c). Differential antigen decay rates during digestion of molluscan prey by carabid predators. *Entomologia Experimentalis et Applicata. 69*: 277-287.

Thiele, H.U. (1977). *Carabid beetles in their environments. A study on habitat selection by adaptions in physiology and behaviour.* Springer-verlag, Berlin.

Thieling, K.M. and Croft, B.A. (1989). Toxicity, selectivity and sublethal effects of pesticides on arthropod natural enemies: A data-base summary. *Pesticides and Non-target invertebrates*. Ed. Jepson, P.C. Intercept, Dorset.

Thomas, M.B., Sotherton, N.W., Coombes, D.S. and Wratten, S.D. (1992). Habitat factors influencing the distribution of polyphagous predatory insects between field boundaries. *Annals of Applied Biology.* 120: 198-202.

Tomlin, J.R. (1935). Additions to the Coleoptera of Glamorgan. *Transactions of the Cardiff Nature Society*. 66: 87-102.

Tod, M.E. (1973). Notes on beetle predators of molluscs. *The Etomologist. 106*: 196-201.

Trelka, D.G. and Berg, C.O. (1977). Behavioral studies of the slug-killing larvae of two species of *Tetanocera* (Diptera: Sciomyzidae). *Proceedings of the Entomological Society of Washington. 79*: 475-489.

Van Emden, H.F. (1989). *Pest control.* Edward Arnold.

Verhoef, H.A., Nagelkerke, C.J. and Joosse, E.N.G. (1977). Aggregation pheromones in Collembola. *Journal of Insect Physiology. 23*: 1009-1013.

Vickerman, G.P and Sunderland, K.D. (1975). Arthropods in cereal crops: nocturnal activity, vertical distribution and aphid predation. *Journal of Applied Ecology. 12*, 755-765.

Vickerman, G.P and Sunderland, K.D. (1977). Some effects of dimethoate on arthropods in winter wheat. *Journal of Applied Ecology. 14*: 767-777.

Vickerman, G.P. (1992). The effects of different pesticide regimes on the invertebrate fauna of winter wheat. *Pesticides, cereal farming and the environment.* Ed; P.W. Greg-Smith, G.K. Frampton & A.R. Hardy, pp 110-131. HMSO, London.

Vinson, S.B. and Lewis, W.J. (1965). A method of host selection by *Cardiochiles nigriceps. Journal of Economic Entomology. 58*: 869-871.

Vinson, S.B. (1976). Host selection by insect parasitoids. *Annual Review of Entomology. 21*: 109-133.

Vinson, S.B., Barfield, C.S. and Henson, R.D. (1977). Oviposition behaviour of *Bracon mellitor*, a parasitoid of the boll weevil (Anthonomus grandis). II Associative learning. *Physiological Entomology. 2*: 157-164.

Waage, J.K. (1977). Behavioural aspects of foraging in the parasitoid *Nemeritis canescans* (Grav.). Unpublished Ph.D. thesis, *Imperial College of Science and Technology, London.*

Waage, J.K. (1978). Arrestment responses of the parasitoid, *Nemeritis canescans,* to a contact chemical produced by its host *Plodia interpunctella. Physiological Entomology. 3*: 135-146.

Waage, J.K. (1979). Foraging for patchily-distributed hosts by the parasitoid, *Nemeritis canescans. Journal of Animal Ecology. 48*: 353-371.

Wallin, H. (1985). Spatial and temporal distribution of some abundant carabid beetles (Coloeoptera; Carabidae) in cereal fields and adjacent habitats. *Pedobiologia. 28*: 19-34.

Wallin, H. (1986). Habitat choice of some field-inhabiting carabid beetles (Coleoptera; Carabidae) studied by recapture of marked individuals. *Ecological Entomology. 11*: 457-466.

Wallin, H. (1987). Distribution, movements and reproduction of carabid beetles (Coleoptera: Carabidae) inhabiting cereal fields. Plant Protection Reports, Dissertations 15, Uppsala, 25.

Wallin, H. and Ekbom. B.S. (1988). Movement of carabid beetles (Coleoptera; Carabidae) inhabiting cereal fields. A field tracing study. *Oecologia. 77*: 39-43.

Wallin, H., Chiverton, P.A., Ekbom, B.S. and Borg, A. (1992). Diet, fecundity and egg size in some polyphagous predatory carabid beetles. *Entomologia Experimentalis et Applicata. 65*: 129-140.

Webley, D. (1964). Slug activity in relation to weather. *Annals of Applied Biology. 53*: 407-414.

Weitz, B. (1956). Identification of blood meals of blood-sucking arthropods. *Bulletin of the World Health Organisation. 15:* 473-490.

Wheater, C.P. (1988). Predator-prey size relationships in some Pterostichini (Coleoptera: Carabidae). *The Coleopterist's Bulletin. 42*: 237-240.

Wheater, C.P. (1989). Prey detection by some predatory Coleoptera (Carabidae and Staphylinidae). *Journal of Zoology (A). 218*: 171-185.

Whitford, F. and Showers, W.B. (1987). Impact of insecticides on composition and abundance of ground-dwelling insect fauna in adult European Corn Borer (Lepidoptera: Pyralidae) action sites in Iowa. *Environmental Entomology. 16*: 231-236.

Williams, G. (1959). Seasonal and diurnal activity of Carabidae, with particular reference to *Nebria*, *Notiophilus* and *Feronia. Journal of Animal Ecology. 28*: 309-330.

Wratten, S.D. (1982). Proceedings. Natural enemies and insect pest dynamics. *Annals of Applied Biology. 101*: pp 143.

Wratten, S.D., Bryan, K., Coombes, D. and Sopp, P. (1984). Evaluation of polyphagous predators of aphids in arable crops. *Proceedings 1984 British Crop Protection Conference - Pests and Diseases*. pp 271-276. British Crop Protection Council, Thornton heath.

Young, A.G. and Port, G.R. (1989). The effect of microclimate on slug activity in the field. *1989 BCPC Mono. No. 41 Slugs and Snails in World Agriculture*. pp 263-269. Ed; Henderson, I.F. British Crop Protection Council, Thornton heath.

Appendix 2.1

Slug mortality when exposed to various beetle species at five temperatures. One day old *D.reticulatum* slugs were used in the experiment. Data for *L.pilicornis*, *P.madidus, H.rufipes*, *N.brevicollis* and *H.aeneus* are presented in section 2.2. The control column indicates the mortality of one day old *D.reticulatum* when slugs were confined individually in petri dishes overnight in the absence of predators.

Species	Temp. (°C)	No. reps	Slug eaten	Slug dead	Slugs alive	Preds. dead
A.apricaria	4	1	0	0	1	0
	12	1	1	0	0	0
	20	1	1	0	0	0
A.aulica	4	5	0	0	5	0
	12	4	2	1	2	0
	20	4	0	4	0	0
A.dorsale	12	3	1	2	0	0
	20	5	0	0	5	0
A.flavipes	20	1	0	0	1	0
A.fuliginosum	20	4	1	0	3	0
A.obscurum	20	13	0	0	13	0
A.plebeja	12	7	0	0	7	0
	20	9	0	0	9	2
A.similata	20	6	0	1	5	0
B.lampros	20	1	1	0	0	0
B.tetracolum	20	5	0	0	5	0
C.fossor	16	1	0	0	1	0
	20	10	0	0	10	0
C.melanocephalus	20	6	0	0	6	0
N.biguttatus	20	10	0	0	10	0
P.atrorufus	20	4	0	0	4	0
P.melanarius	20	9	6	0	3	0
P.nigrita	20	7	4	2	1	0
P.strenuus	20	11	3	1	7	0
S.nivalis	20	1	1	0	0	0
T.quadristriatus	8	8	0	0	8	0
	12	14	0	0	14	0
	20	11	0	0	11	0
Controls	4	30	-	0	30	-
	8	30	-	0	30	-
	12	30	-	0	30	-
	16	30	-	0	30	-
	20	30	-	0	30	-

Appendix 2.2

Run number and beetles used in section 2.3 of chapter two. *Pterostichus cristatus* and *Silpha atrata* were not used in the analysis.

Run Number	Species	Run Number	Species
01	*C.nemoralis*	27	*C.violaceus*
02	*C.nemoralis*	28	*C.caraboides*
03	*P.niger*	29	*C.caraboides*
04	*A.parallelepipedus*	30	*C.violaceus*
05	*A.parallelepipedus*	31	*C.violaceus*
06	*C.nemoralis*	32	*C.caraboides*
07	*C.nemoralis*	33	*C.violaceus*
08	*A.parallelepipedus*	34	*C.violaceus*
09	*A.parallelepipedus*	35	*C.violaceus*
10	*C.violaceus*	36	*C.nemoralis*
11	*C.caraboides*	37	*C.nemoralis*
12	*A.parallelepipedus*	38	*C.violaceus*
13	*A.parallelepipedus*	39	*C.violaceus*
14	*C.violaceus*	40	*C.caraboides*
15	*C.caraboides*	41	*C.violaceus*
16	*A.parallelepipedus*	42	*A.parallelepipedus*
17	*A.parallelepipedus*	43	*C.violaceus*
18	*A.parallelepipedus*	44	*A.parallelepipedus*
19	*A.parallelepipedus*	45	*A.parallelepipedus*
20	*P.niger*	46	*C.violaceus*
21	*P.niger*	47	*C.violaceus*
22	*C.nemoralis*	48	*C.nemoralis*
23	*S.atrata*	49	*S.atrata*
24	*P.cristatus*	50	*A.parallelepipedus*
25	*P.niger*	51	*A.parallelepipedus*
26	*C.violaceus*		

Appendix 2.3

Beetles used in section 2.3 of chapter two. The number in the second column indicates the number of beetles of each species used in the study. The third column indicates the number of beetles which did not contact a slug. The fourth column indicates the number of beetles which contacted a slug but did not make a kill. The fifth column indicates the number of beetles which contacted a slug and made a kill. The two species indicated by an asterisk, were not used in the analysis as they did not kill slugs in the study.

Species	No. of runs	Made no contacts	Made Contact /no kill	Contacts and kill
C.caraboides	6	1	1	4
C.nemoralis	8	3	1	4
C.violaceus	15	0	7	8
A.parallelepipedus	15	4	3	8
P.niger	4	0	1	3
* *P.cristatus*	1	0	1	0
* *S.atrata*	2	2	0	0

Appendix 2.4

Data concerning five parameters (time spent moving, time spent stationary, net speed of movement, degree of turn / second and distance covered) for five beetle species. The data were collected every time a beetle entered the inner slug zone during the night period. See text for details.

	INNER SLUG ZONE				
	Time moving	Time stationary	Net Speed	Deg/ sec	Dist.
C.violaceus	3.981	0	0.0189	1.599	0.075
	4.021	0	0.0172	5.694	0.069
	2.286	0	0.0185	3.125	0.042
	5.558	1.734	0.0134	8.878	0.074
C.caraboides	8.717	3.968	0.0107	16.32	0.093
	6.793	0.721	0.0010	12.98	0.075
	7.755	4.749	0.0138	17.95	0.107
	4.449	0	0.0165	0.60	0.073
	6.132	7.454	0.0144	14.29	0.088
	23.567	13.226	0.0105	25.25	0.249
	2.885	0	0.0212	3.66	0.061
	25.251	17.615	0.0113	20.99	0.286
	2.645	5.711	0.0225	10.29	0.059
	9.499	9.258	0.0158	31.24	0.150
	12.806	10.942	0.0138	22.63	0.177
C.problematicus					
	12.03	2.190	0.0220	49.59	0.262
	6.53	1.434	0.0200	50.81	0.130
	12.11	13.466	0.0230	44.82	0.278
	2.98	7.529	0.0279	41.05	0.083
	2.39	0	0.0263	15.14	0.062
	20.00	10.005	0.0086	13.71	0.172
	20.71	9.349	0.0102	11.74	0.212
	6.15	0	0.0114	5.01	0.070
	7.69	0	0.0075	0	0.058
	4.61	0	0.0112	5.13	0.051
	16.68	8.339	0.0035	2.66	0.059
	19.87	40.040	0.0262	15.39	0.522
	6.16	2.464	0.0197	33.98	0.121
	4.38	1.984	0.0165	21.02	0.072
	2.94	2.404	0.0205	26.70	0.060
	2.28	2.495	0.0212	17.81	0.048

Appendix 2.4 (continued)

INNER SLUG ZONE

	Time moving	Time stationary	Net Speed	Deg/ sec	Dist.
H.rufipes	2.385	12.167	0.0144	4.30	0.034
	10.378	11.212	0.0050	3.88	0.052
	10.616	35.661	0.0063	4.16	0.067
	15.507	111.053	0.0064	4.44	0.100
	2.862	10.854	0.0060	5.46	0.017
	12.763	5.845	0.0060	3.84	0.077
	8.708	0	0.0078	0.23	0.068
	6.322	3.220	0.0045	8.34	0.029
	10.020	14.791	0.0066	1.97	0.066
	10.020	138.608	0.0071	3.72	0.071
	10.855	6.322	0.0070	4.03	0.076
	9.185	2.505	0.0057	4.31	0.052
C.nemoralis	0.801	0	0.2166	8.133	0.017
	2.952	7.283	0.0235	14.095	0.069
	16.063	28.740	0.0173	29.186	0.278
	3.819	5.630	0.0185	14.384	0.070
	10.945	20.708	0.0203	24.966	0.222
	4.449	11.850	0.0241	30.693	0.107
	2.047	11.889	0.0301	34.314	0.061
	4.291	6.811	0.0152	28.436	0.065
	14.527	25.315	0.0201	33.946	0.293
	7.283	13.149	0.0199	23.943	0.145
	2.874	0	0.0128	16.414	0.036
	17.283	25.078	0.0169	23.314	0.292
	4.567	2.755	0.0171	25.240	0.078
	4.330	4.330	0.0189	17.544	0.081
	4.724	4.055	0.0185	22.082	0.087
	5.157	3.504	0.0185	32.152	0.095
	10.831	12.559	0.0207	27.440	0.224
	10.708	21.023	0.0182	27.148	0.195
	6.850	4.763	0.0748	39.336	0.119
	4.842	6.889	0.0479	19.203	0.087
	8.464	5.630	0.0178	26.819	0.151
	1.653	1.732	0.0163	8.977	0.026
	12.717	42.658	0.0194	35.448	0.247
	8.372	22.126	0.0215	34.486	0.180
	12.678	28.584	0.0174	35.898	0.220
	24.718	20.093	0.0177	33.260	0.438
	10.644	4.106	0.0159	26.134	0.169

Appendix 2.4 (continued)

INNER SLUG ZONE

	Time moving	Time stationary	Net Speed	Deg/ sec	Dist.
C.nemoralis	20.651	76.505	0.0216	39.038	0.446
	4.784	10.086	0.0188	33.673	0.089
	9.488	19.256	0.0201	43.164	0.191
	15.588	23.003	0.0180	38.862	0.281
	3.747	8.770	0.0224	19.790	0.083
	8.850	15.827	0.0202	34.476	0.179
	5.422	8.731	0.0176	22.957	0.095
	6.339	9.767	0.0194	34.382	0.122
	2.750	11.601	0.0198	27.196	0.054
	9.202	37.555	0.0246	41.857	0.226
	4.026	3.667	0.0147	10.118	0.059
	6.937	5.142	0.0157	39.918	0.109
P.niger	0.295	0	0.2494	9.773	0.073
	0.826	0	0.0690	42.933	0.057
	1.150	0.137	0.0646	82.107	0.074
	0.501	0	0.0857	5.299	0.043
	0.786	0.944	0.0844	66.460	0.066
	0.599	1.209	0.0773	137.380	0.046
	1.062	0.108	0.0632	43.284	0.067
	0.845	0	0.0752	26.096	0.063
	3.893	0.653	0.0208	31.356	0.081
	2.258	0.323	0.0320	21.736	0.072
	1.159	0	0.0416	22.342	0.048
	1.486	0	0.0445	10.119	0.066
	1.872	0	0.0333	17.294	0.062
	2.615	0	0.0233	19.878	0.061
	2.436	0.445	0.0317	18.628	0.077
	2.258	0.475	0.0208	19.594	0.056
	1.753	2.407	0.0443	51.357	0.077
	2.615	0	0.0276	23.406	0.072
	2.258	0	0.0297	43.768	0.067
	2.020	0.743	0.0387	15.773	0.078
	2.674	1.218	0.0294	4.001	0.078
	1.367	0.080	0.0359	15.804	0.049
	2.971	0.713	0.0265	9.718	0.078
	1.961	0.326	0.0374	51.664	0.073
	1.485	0.713	0.0393	1.281	0.058

Appendix 2.4 (continued)

INNER SLUG ZONE

	Time moving	Time stationary	Net Speed	Deg/ sec	Dist.
P.madidus	2.953	22.729	0.0291	31.410	0.086
	3.405	8.173	0.0250	36.384	0.085
	3.040	3.639	0.0254	40.535	0.077
	2.117	9.634	0.0272	18.730	0.057
	3.340	13.721	0.0315	23.081	0.105
	1.879	3.460	0.0270	8.046	0.050
	1.610	0	0.0365	18.935	0.058
	1.759	3.311	0.0317	11.339	0.055
	2.774	1.282	0.0228	23.016	0.063
	1.670	0	0.0167	19.056	0.028
	3.907	10.469	0.0216	19.360	0.084
	3.370	2.237	0.0265	26.631	0.089
	1.640	55.123	0.0563	17.927	0.092
	1.402	11.961	0.0365	30.261	0.051
	1.819	16.614	0.0497	19.341	0.090
	2.147	8.560	0.0256	26.986	0.055
	1.730	8.262	0.0379	49.196	0.065
	3.340	0.357	0.0176	8.119	0.059
	1.938	7.546	0.0336	15.975	0.065
	1.312	4.683	0.0329	11.184	0.043
	2.296	1.610	0.0207	11.700	0.047
	2.744	47.129	0.0582	24.115	0.159
	2.982	4.772	0.0262	17.184	0.078
	2.058	3.370	0.0304	9.660	0.062
	2.684	0.686	0.0235	25.148	0.063
	2.237	4.176	0.0237	19.097	0.053
	3.609	2.953	0.0239	31.561	0.086
	2.744	3.251	0.0296	26.070	0.081
	2.207	0.835	0.0289	15.088	0.063
	3.937	2.565	0.0213	14.796	0.084
	2.147	0.417	0.0285	11.515	0.061
	2.535	0.328	0.0247	6.066	0.062
	1.849	0.984	0.0217	21.807	0.040
	2.237	0	0.0320	14.440	0.071
	5.130	2.207	0.0236	33.095	0.121
	3.340	0	0.0247	8.842	0.082
	3.758	9.306	0.0258	24.080	0.097
	2.296	10.111	0.0360	27.495	0.082

Appendix 2.4 (continued)

INNER SLUG ZONE

	Time moving	Time stationary	Net Speed	Deg/ sec	Dist.
P.madidus	1.610	2.058	0.0250	16.697	0.040
	2.684	2.893	0.0214	14.805	0.057
	3.340	2.058	0.0229	28.732	0.076
	2.028	5.309	0.0363	50.835	0.073
	1.849	0	0.0294	13.613	0.054

Appendix 2.5

Data concerning five parameters (time spent moving, time spent stationary, net speed of movement, degree of turn / second and distance covered) for five beetle species. The data were collected every time a beetle entered the inner control zone during the night period. See text for details.

INNER CONTROL ZONE

	Time moving	Time stationary	Net Speed	Deg/ sec	Dist.
C.violaceus	3.272	1.892	0.0174	8.690	0.057
	3.429	0	0.0118	12.544	0.040
	4.139	0	0.0168	7.990	0.069
	3.390	2.247	0.0191	0.248	0.064
	3.587	0	0.0179	3.365	0.064
C.caraboides	4.268	0	0.0164	11.53	0.070
	1.262	0	0.0363	0	0.045
	1.803	0	0.0383	0	0.069
	2.705	0	0.0264	17.63	0.071
	1.984	0	0.0308	11.33	0.061
	4.629	0	0.0105	19.44	0.049
	3.547	0	0.0214	27.99	0.075
	1.923	0	0.0228	0	0.044
C.problematicus					
	0.757	0	0.043	0	0.032
	1.430	0	0.033	73.42	0.047
	1.354	0	0.042	25.48	0.056
	1.753	0	0.028	11.96	0.049
	1.314	0	0.052	0	0.068
	3.195	0	0.0167	0	0.053
	1.538	0	0.0234	3.23	0.036
	1.538	0	0.0184	0	0.028
	1.538	0	0.0129	0	0.019
	2.483	0	0.0238	0	0.059
	8.803	0	0.0077	0	0.068
	8.340	0	0.0074	0	0.061
	4.170	0	0.0133	0	0.055
	1.833	0	0.0246	1.77	0.045
	1.623	0	0.2826	2.71	0.458

Appendix 2.5 (continued)

INNER CONTROL ZONE

	Time moving	Time stationary	Net Speed	Deg/ sec	Dist.
C.problematicus					
	2.495	0	0.0283	12.57	0.070
	2.915	0	0.0252	2.47	0.073
	2.224	0	0.0196	17.63	0.043
H.rufipes	2.071	0	0.0235	26.02	0.050
	1.716	0	0.0377	10.41	0.064
	11.332	0	0.0060	7.76	0.068
	4.294	0	0.0154	4.50	0.066
	3.578	9.9	0.0006	11.09	0.022
	3.817	35.546	0.0125	4.60	0.047
	7.157	1.192	0.0070	4.25	0.050
	9.423	8.35	0.0074	4.77	0.070
	14.195	12.524	0.0057	6.14	0.080
	11.690	11.332	0.0068	6.54	0.080
	10.974	1.550	0.0065	1.00	0.072
C.nemoralis	1.632	0	0.0413	3.066	0.067
	1.839	0	0.0370	5.232	0.068
	1.454	0	0.0356	1.814	0.051
	2.519	0	0.0170	29.828	0.042
	1.929	0.433	0.0191	16.915	0.036
	1.968	0	0.0268	0.491	0.052
	1.338	0	0.0276	29.052	0.036
	1.850	0	0.0366	8.918	0.067
	1.913	0	0.0253	0.651	0.048
P.niger	1.077	0	0.0447	0	0.051
	0.629	0	0.0399	83.981	0.029
	0.973	0	0.0777	39.311	0.075
	1.111	0	0.0652	22.828	0.072
	0.708	0	0.0799	18.029	0.056
	0.683	0	0.0449	26.916	0.030
	0.445	0	0.0608	0	0.027
	0.802	0	0.0500	26.443	0.040
	0.505	0	0.0482	40.441	0.024

Appendix 2.5 (continued)

INNER CONTROL ZONE

	Time moving	Time stationary	Net Speed	Deg/ sec	Dist.
P.madidus	2.028	0	0.0317	11.838	0.064
	2.714	0	0.0253	18.581	0.068
	1.879	0	0.0207	2.473	0.038
	2.893	1.133	0.0200	6.556	0.057
	2.058	0	0.0313	31.000	0.064
	1.551	0	0.0392	1.194	0.060
	2.654	0.035	0.0230	1.890	0.061
	1.998	0	0.0233	18.797	0.046
	1.640	0	0.0409	10.399	0.067
	2.416	0	0.0267	11.390	0.064
	2.416	0	0.0222	1.316	0.053
	1.402	0	0.0338	2.007	0.057
	2.654	0.626	0.0246	25.311	0.065
	2.505	1.819	0.0263	21.586	0.065
	4.653	10.917	0.0237	33.153	0.110
	1.998	0	0.0306	6.315	0.061
	2.654	9.157	0.0285	21.338	0.075
	2.028	0	0.0343	32.243	0.069
	1.700	0.293	0.0267	16.540	0.045
	1.849	0.835	0.0303	11.616	0.056
	2.386	1.849	0.0274	4.893	0.065
	1.461	2.684	0.0406	7.039	0.059
	2.058	0	0.0284	24.463	0.058
	1.909	0	0.0342	57.715	0.065
	1.909	0	0.0335	15.633	0.064
	1.789	0	0.0363	11.073	0.064
	1.700	0	0.0372	4.172	0.063
	1.581	0	0.0290	9.784	0.045
	2.237	0.328	0.0251	18.981	0.056

Appendix 3.1 Decay rate of Deroceras/Arion antigens

The number of beetles used in each treatment is indicated by the (No) column. The number of positive ELISA signals is indicated in the corresponding (+) column. *N.brevicollis* abbreviated to *N.brev*. *A.parallelepipedus* abbreviated to *A.paral*.

Species		1 hr		1		3		5		10		15	
Temp. (°C)		No	+	No	+	No	+	No	+	No	+	No	+
P.madidus	4	6	6	6	5	6	6	6	4	6	4	6	2
	8	6	6	6	6	6	4	6	2	6	3	6	4
	12	6	6	6	4	6	1	6	0	6	0	6	1
	16	6	6	6	4								
	20	6	6	6	0	6	2	6	1	6	0	6	2
H.rufipes	4	6	6	6	2	6	0	6	0	6	0	6	0
	8	6	6	6	0	6	3	6	0	6	1	6	1
	12	6	6	6	1	6	2	6	0	6	1	6	2
	16	6	6	6	2	6	2	6	3	6	0	6	0
	20	6	6	6	2	6	0	4	0				
A.paral.	4	6	6	6	6	-	-	4	3	-	-	-	-
	12	6	6	6	6	6	5	6	2	6	5	6	4
	20	6	6	6	3	6	2	6	2	6	0	6	1
N.brev.	4	6	6	-	-	-	-	-	-	6	5	-	-
	12	6	2	6	6	6	3	6	4	6	5	6	4
	16	6	6	-	-	-	-	6	5	-	-	-	-
H.aeneus	12	6	5	-	-	4	0	4	0	-	-	-	-
	20	6	6	6	3	6	3	4	1	-	-	-	-
Carabus	8	4	4	-	-	-	-	3	3	-	-	-	-
	12	3	3	2	2	-	-	-	-	-	-	3	3
	20	4	4	-	-	-	-	1	1	3	3	1	1
Arion													
P.madidus	12	6	6	6	5	6	2	4	2	-	-	-	-
	20	6	6	6	6	6	5	6	6	6	6	6	6

Appendix 3.2 Antigen recovery and meal size

The number of beetles used in each treatment is indicated by the (No) column. The number of positive ELISA signals is indicated in the corresponding (+) column. *A.parallelepipedus* abbreviated to A.pa., *H.rufipes* abbreviated to H.ru., *P.madidus* abbreviated to P.ma., *H.aeneus* abbreviated to H.ae., *N.brevicollis* abbreviated to N.brev., *P.strenuus* abbreviated to P.st., *P.nigrita* abbreviated to P.ni. *A.fuliginosum* abbrevaited to A.fu. *N.brevicollis* larvae abbreivaited to N.br. lar.

Spp.	Tp °C	Number of replicates											
		one		two		three		four		five		ten	
		No	+	No	+	No	+	No	+	No	+	No	+
A.pa.	12									2	2	14	14
	20	7	7							6	6	6	6
H.ru.	04					1	1						
	12	2	0	1	0	2	0						
	20	4	0	2	0			1	0				
P.ma.	08	2	1	1	0	1	1						
	12									5	2		
	20	7	1			3	0			6	0		
H.ae.	08			2	0								
	20	1	0										
N.br	12	1	0	3	2								
P.st.	20	3	0										
P.ni.	20					2	1						
A.fu.	20	1	0										
N.br.	04	6	6					1	1				
lar.	08	7	7										
	12	2	2										
	16	6	2										
	20	6	1										

Appendix 3.3 Mode of predator death on Antigen recovery

The number of beetles used in each treatment is indicated by the (No) column. The number of positive ELISA signals is indicated in the corresponding (+) column.

	Treatment							
	fed slug/ drown		fed slug/ froze		starve/ drown		starve/ froze	
	No	+	No	+	No	+	No	+
H.rufipes	9	9	8	6	7	5	3	0
P.madidus	3	3	4	4	3	1	3	1

Appendix 4.1

The number of beetles found with / without slug remains in their gut from the three study sites (assessed by ELISA).

		1992	1993
All sites	No. positive	293	26
	No. negative	831	161
Clayton field	No. positive	230	20
	No. negative	600	126
Square field	No. positive	61	6
	No. negative	182	34
Bog field	No. positive	2	0
	No. negative	49	1

Appendix 4.2

The number of each common beetle species found with/without mollusc remains from the three study sites (assessed by ELISA). The data from an entires years catch are presented.

			1992	1993
P.melanarius	Clayton field	No. positive	0	2
		No. negative	25	12
	Square field	No. positive	5	4
		No. negative	42	25
	Bog field	No. positive	0	0
		No. negative	4	0
P.madidus	Clayton field	No. positive	3	5
		No. negative	44	29
	Square field	No. positive	0	2
		No. negative	4	5
	Bog field	No. positive	0	0
		No. negative	5	1
H.rufipes	Clayton field	No. positive	70	12
		No. negative	168	73
	Square field	No. positive	1	0
		No. negative	2	1
	Bog field	No. positive	0	0
		No.negative	2	0
N.brevicollis	Clayton field	No. positive	4	0
		No. negative	5	0
	Square field	No. positive	38	0
		No. negative	57	0
	Bog field	No. positive	1	0
		No. negative	10	0
A.similata	Clayton field	No. positive	143	0
		No. negative	322	0
	Square field	No. positive	15	0
		No. negative	65	0
	Bog field	No. positive	0	0
		No. negative	26	0

Appendix 4.3

The number of beetles found with/without mollusc remains in the two summer periods. Data from all beetles presented in 'All sites' section. Data from all beetles also presented at each site. Data for three species also presented as a combined total from all three sites.

		Summer 1992	Summer 1993
All sites	No. positive	293	15
	No. negative	830	88
Clayton field	No. positive	230	13
	No. negative	599	70
Square field	No. positive	61	2
	No. negative	182	18
Bog field	No. positive	2	0
	No. negative	49	0
P.melanarius	No. positive	5	2
	No. negative	71	22
P.madidus	No. positive	3	5
	No. negative	53	20
H.rufipes	No. positive	71	8
	No. negative	172	41

Appendix 4.4

The Mollusc fauna of the three study sites in the summer seasons of 1992 and 1993. The values represent the total number of molluscs of each species found over the summer seasons. *Arion ater* has been divided into the two forms: *Arion ater ater* and *Arion ater rufus*.

	1992	1993
Clayton field		
Deroceras reticulatum	38	45
Arion ater ater	1	0
Arion ater rufus	0	1
Arion hortensis	1	0
Square field		
Deroceras reticulatum	166	11
Arion ater ater	0	1
Arion hortensis	1	0
Bog field		
Deroceras reticulatum	37	50
Arion ater ater	2	0
Arion ater rufus	0	1
Arion hortensis	2	0

Appendix 4.5

Deroceras reticulatum activity density in the three field sites in the summer periods of 1992 and 1993. Data presented as the number of slugs per tile trap. Clayton field abbreviated to CF. Square field abbreviated to SQ. Bog field abbreviated to BG.

	Summer 1992						Summer 1993				
	22/6	29/6	6/7	13/7	20/7	27/7	28/6	5/7	12/7	19/7	26/7
CF	0.10	0.15	0.35	0.20	0.55	0.55	0.6	0.3	0.2	3.0	0.1
SQ	0.65	0.75	1.95	1.65	0.45	2.85	0.3	0.0	0.3	0.5	0.0
BG	0.15	0.05	0.85	0.40	0.05	0.35	0.9	0.8	0.8	2.2	0.3

Appendix 4.6

Weight (g) of individual *D.reticulatum* slugs, collected from the three study sites on June 30th and July 08th 1992 when the fields were under oilseed rape.

Square		Clayton		Bog	
June 30	July 08	June 30	July 08	June 30	July 08
0.057	0.710	0.176	0.575	0.244	0.479
0.206	0.717	0.315	0.625	0.599	
0.344	0.662	0.315	0.263	0.512	
0.154	0.753		0.744	0.523	
0.102	0.715		0.747	0.314	
0.137	0.058		0.727	0.236	
0.469	0.071		0.165	0.207	
0.481	0.459			0.611	
0.721	0.328			1.097	
0.130	0.336			0.660	
0.871	0.040			1.496	
1.494	0.722			0.878	
0.883	0.797			1.298	
0.898	0.786			0.119	
0.526	0.475			0.672	
	0.785			1.158	
	0.363			0.529	
	0.423				
	0.611				
	0.236				
	0.282				
	0.341				
	0.304				
	0.086				
	0.279				
	0.507				
	0.678				
	0.436				
	0.154				
	0.295				
	0.458				
	0.224				
	0.452				
	0.630				
	0.778				
	0.471				
	0.864				
	0.389				
	0.157				

Appendix 4.7

Weekly maximum and minimum field temperatures (°C) at Clayton, Square and Bog field in June and July, 1992.

		Week ending on the following dates				
		29/06	06/07	13/07	20/07	27/07
Clayton	Max.	-	26	22	23.5	21
	Min.	-	17	11.5	10	11
Square	Max.	18	19	20	22.3	22
	Min.	10.5	11.5	5	5.2	5.7
Bog	Max.	20.5	13	23.5	27	22.5
	Min.	6	8.5	8.2	8.5	8.7

Appendix 4.8

Laboratory measured consumption rates of one day old *D.reticulatum* by six species of carabids at five temperatures. An asterisk indicates an upper predation limit was not determined in this treatment.

	Temperature (°C)				
	04	08	12	16	20
A.parallelepipedus			10*	10*	10*
H.rufipes	3		4		4
P.madidus		3	5		10*
H.aeneus		1		2	
N.brevicollis			2		
A.similata			1		1

Appendix 5.1

Weekly carabid catch in Clayton field

	Summer 1992						Summer
Date of sample	22/06	29/06	06/07	13/07	20/07	27/07	totals
Pterostichus madidus		6	9	16	11	9	51
Pterostichus melanarius		3	11	7	2	2	25
Pterostichus cristatus					3		3
Pterostichus niger			1	2	3	2	8
Pterostichus strenuus			1	1		1	3
Abax parallelepipedus			1				1
Nebria brevicollis		5	5		1		11
Harpalus rufipes	3	34	49	62	62	37	247
Harpalus latus						2	2
Harpalus aeneus		2	7	5	4	2	20
Cychrus caraboides			1				1
Carabus violaceus		1		1	1	1	4
Calathus melanocephalus				2	4		6
Calathus fuscipes						4	4
Loricera pilicornis			4	6	8	7	25
Agonum dorsale	2	7	27	30	13	1	80
Amara similata	8	32	163	214	35	24	476
Amara aenea		1	2	1		3	7
Amara familiaris		1	3				4
Amara aulica		1	2		2	2	7
Amara apricaria		2	1	1			4
Amara plebeja				1			1
Amara lunicollis		3	1				4
Amara spp			6		4		10
Bembidion spp							
Badister bipustulatus				1			1
Patrobus atrorufus				1			1
Notiophilus biguttatus					1		1
Trechus quadristriatus						1	1
Totals	13	98	294	351	154	98	1008

	Autumn 1992							Autumn	02/11-21/12
	02/11	16/11	23/07	30/11	07/12	14/12	21/12	totals	totals
P.madidus	1								1
P.melanarius									
P.cristatus									
P.niger									
P.strenuus									
A.parallelepipedus									
N.brevicollis									
H.rufipes									
H.latus									
H.aeneus									
C.caraboides									
C.violaceus									
C.melanocephalus									
C.fuscipes									
L.pilicornis								1	1
A.dorsale		1							
A.similata									
A.aenea									
A.familiaris									
A.aulica									
A.apricaria									
A.plebeja									
A.lunicollis								3	4
Amara spp	1	3						4	4
Bembidion spp		1	2		1				
B.bipustulatus									
P.atrorufus									
N.biguttatus						4		4	5
T.quadristriatus	1								
Totals	3	5	2	0	1	4	0	12	15

Appendix 5.1 (continued)

Weekly carabid catch in Clayton field

Spring 1993

Date of sample	12/04	26/04	03/05	10/05	17/05	24/05	01/06	Spring totals	12/04-01/06 totals
Pterostichus madidus			1		1	2	1	5	5
Pterostichus melanarius		1						1	1
Pterostichus niger									
Pterostichus strenuus				4				4	4
Pterostichus nigrita				1	1			2	2
Nebria brevicollis									
Harpalus rufipes				6	1	2	18	27	27
Harpalus latus									
Harpalus aeneus	1	1		3	3	2	1	11	11
Calathus melanocephalus									
Loricera pilicornis			2	4	2	10	3	21	21
Agonum dorsale				4		1	3	8	8
Agonum assimile				1				1	1
Amara similata			1			3	3	7	7
Amara aenea									
Amara aulica									
Amara plebeja			1	2		3		6	6
Amara spp		1		6				7	7
Notiophilus biguttatus	2			2	1			3	5
Clivina fossor									
Bembidion tetracolum					1			1	1
Bembidion unicolor				2				2	2
Bembidion lampros	1			1				1	2
Bembidion obtusum				1				1	1
Synuchus nivalis									
Totals	4	3	5	37	10	23	29	108	111

Summer 1993

	14/06	21/06	28/06	05/07	12/07	19/07	26/07	01/08	11/08	Summer totals	14/06-11/08 totals
P.madidus	2	5	3	7	5	4	7	3	5	31	41
P.melanarius	1	2	4	3	5		2		1	16	18
P.niger							1	1		1	2
P.strenuus											
P.nigrita											
N.brevicollis		1								1	1
H.rufipes	7	24	6	11	15	5	4	3		65	75
H.latus		1								1	1
H.aeneus		1	2							3	3
C.melanocephalus						1				1	1
L.pilicornis	6	2	1	1		3		2	5	7	20
A.dorsale			1							1	1
A.assimile											
A.similata		1	1							2	2
A.aenea		1								1	1
A.aulica		1		2						3	3
A.plebeja						1				1	1
Amara spp.											
N.biguttatus											
C.fossor	1										1
B.tetracolum	1										1
B.unicolor											
B.lampros											1
B.obtusum	1										
S.nivalis						1				1	1
Totals	19	39	18	24	25	15	14	9	11	135	174

Appendix 5.1 (continued)

Weekly carabid catch in Square field

Date of sample	Summer 1992 23/06	29/06	06/07	13/07	20/07	27/07	Summer totals
Pterostichus madidus	1	1		1		1	4
Pterostichus melanarius		5	5	13	15	10	48
Pterostichus cristatus		1		1	1		3
Pterostichus niger				1	3	1	5
Pterostichus strenuus	1	1				1	3
Nebria brevicollis	34	43	20	7	1	2	107
Harpalus rufipes				1	2		3
Harpalus aeneus					4		4
Calathus melanocephalus		1		2	1	1	5
Calathus fuscipes							
Loricera pilicornis	1	17	20	39	31	14	122
Agonum assimile		1	1		1		3
Amara similata	2	5	20	41	9	7	84
Amara plebeja				1			1
Amara lunicollis			1	1	4		6
Amara spp.				2	3		5
Patrobus atrorufus				1	1		2
Notiophilus biguttatus		2			1		3
Trechus quadristriatus							
Patrobus assimilis		1					1
Clivina fossor					1		1
Bembidion tetracolum			2	2			4
Bembidion obtusum			1				1
Totals	39	78	70	113	78	37	415

	Autumn 1992 02/11	16/11	23/11	30/11	07/12	14/12	21/12	Autumn totals	02/11-21/12 totals
P.madidus									
P.melanarius									
P.cristatus									
P.niger									
P.strenuus						1	1	2	2
N.brevicollis									
H.rufipes									
H.aeneus									
C.melanocephalus									
C.fuscipes									
L.pilicornis									
A.assimile									
A.similata									
A.plebeja									
A.lunicollis									
Amara spp.									
P.atrorufus								1	1
N.biguttatus		1				3		3	3
T.quadristriatus									
P.assimilis									
C.fossor								2	2
B.tetracolum		1		1		1		1	1
B.obtusum									
Totals	0	2	0	1	0	5	1	9	9

Appendix 5.1 (continued)

Weekly carabid catch at Square field

Spring 1993

Date of sampling	12/04	26/04	03/05	10/05	17/05	24/05	01/06	Spring totals	12/04-01/06 totals
Pterostichus madidus							1	1	1
Pterostichus melanarius				1				1	1
Pterostichus cristatus									
Pterostichus niger									
Pterostichus strenuus									
Pterostichus nigrita									
Nebria brevicollis							5	5	5
Harpalus rufipes									
Carabus problematicus							1	1	1
Calathus melanocephalus									
Calathus fuscipes							1	1	1
Loricera pilicornis			2	2	6	3	3	16	16
Agonum dorsale						1		1	1
Agonum mulleri							1	1	1
Agonum fuliginosum							1	1	1
Amara similata			1		1	1		3	3
Amara aenea					2			2	2
Amara aulica							1	1	1
Amara plebeja				2		1	1	4	4
Notiophilus biguttatus		1			1	1		3	3
Bembidion tetracolum				4	4	2		10	10
Bembidion lampros					1	1		2	2
Bembidion obtusum						1	1	2	2
Totals	0	1	3	9	15	11	16	55	55

Summer 1993

	14/06	21/06	28/06	05/07	12/07	19/07	26/07	01/08	11/08	Summer totals	14/06-11/08 totals
P.madidus	1	1	1		1	2	1		1	6	7
P.melanarius	1	5	1	3	3	3	3	3	11	18	22
P.cristatus							1			1	1
P.niger							1			1	1
P.strenuus		1	1							2	2
P.nigrita								1			1
N.brevicollis	9	9								9	18
H.rufipes						1				1	1
C.problematicus											
C.melanocephalus		1	2							3	3
C.fuscipes								1			1
L.pilicornis	5	1		3	1	2	1	2		8	15
A.dorsale	1		1							1	2
A.mulleri											
A.fuliginosum											
A.similata		1								1	1
A.aenea											
A.aulica											
A.plebeja											
N.biguttatus											
B.tetracolum											
B.lampros											
B.obtusum						1				1	1
Totals	17	19	6	6	5	9	7	7	0	52	76

Appendix 5.1 (continued)

Weekly carabid catch in Bog field

Date of sample	Summer 1992 22/06	29/06	06/07	13/07	20/07	27/07	Summer totals
Pterostichus madidus		1		2	1	1	5
Pterostichus melanarius				1	1	2	4
Pterostichus strenuus	1	1	2		1		5
Nebria brevicollis	2	7	2	2			13
Harpalus rufipes				1	3		4
Harpalus aeneus		1	1		7		9
Cychrus caraboides						1	1
Calathus melanocephalus		1		6	4	4	15
Calathus fuscipes						4	4
Loricera pilicornis	3	12	12	9	7	13	56
Agonum dorsale	1		1				2
Agonum assimile			1				1
Amara similata	1	13	16	31	11	5	77
Amara familiaris	1	1		1			3
Amara aulica						2	2
Amara aenea	1						1
Other Amara spp	1		2				3
Notiophilus biguttatus							0
Trechus quadristriatus							0
Bembidion tetracolum				1	3		4
							0
Totals	11	37	37	54	38	32	209

Date of sample	Autumn 1992 02/11	16/11	23/07	30/11	07/12	14/12	21/12	Autumn totals	02/11-21/12 totals
Pterostichus madidus									
Pterostichus melanarius									
Pterostichus strenuus									
N.brevicollis		1	1					2	2
Harpalus rufipes									
Harpalus aeneus									
Cychrus caraboides									
Calathus melanocephalus									
Calathus fuscipes									
Loricera pilicornis									
Agonum dorsale									
Agonum assimile									
Amara similata									
Amara familiaris									
Amara aulica									
Amara aenea									
Other Amara spp		1						1	1
N.biguttatus							2	2	2
T.quadristriatus						7	8	15	15
Bembidion species		7	2		2			11	11
Totals	0	9	3	0	2	7	10	31	31

Appendix 5.1 (continued)

Weekly carabid catch in Bog field

Spring 1993

Date of sample	12/04	26/04	03/05	10/05	17/05	24/05	01/06	Spring totals	12/04-01/06 totals
Pterostichus madidus						1		1	1
Pterostichus nigrita									
Nebria brevicollis					1		7	8	8
Calathus melanocephalus				1			1	2	2
Calathus fuscipes									
Loricera pilicornis				1	1	3		5	5
Agonum dorsale				1		1	9	11	11
Amara similata						1		1	1
Amara plebeja						1		1	1
Notiophilus biguttatus						1		1	1
Trechus quadristratus	1								1
Bembidion tetracolum					2	2		4	4
Bembidion lampros						1		1	1
Bembidion spp.						1		1	1
Totals	1	0	0	3	4	12	17	36	37

Summer 1993

	14/06	21/06	28/06	05/07	12/07	19/07	26/07	01/08	11/08	Summer totals	14/06-11/08 totals
P.madidus											
P.nigrita							1			1	1
N.brevicollis	5	3				1				4	9
C.melanocephalus		1	1	2	2		1	1		7	8
C.fuscipes					1					1	1
L.pilicornis	1				1			1		1	3
A.dorsale	5	2								2	7
A.similata				1						1	1
Amara plebeja											
N.biguttatus											
T.quadristriatus											
B.tetracolum	2										2
Totals	13	6	1	3	4	1	2	2	0	17	32

Appendix 6.1

The number and species of carabid beetles found in the twenty eight miniplots, prior to experimentation.

Species	29/06	01/07	06/07
A.similata	1	2	2
A.plebeja	1		
A.familiaris			2
L.pilicornis	1	1	7
H.aeneus	5	6	1
H.rufipes	1	4	1
N.biguttatus			1
Bembidion spp.	1		

Appendix 6.2

The number of *D.reticulatum* slugs recovered from the twenty eight miniplots, prior to experimentation.

	23/06	29/06	01/07	06/07
D.reticulatum	1	0	4	5

Appendix 6.3

The mean amount of slug damage to the chinese cabbage plants in each of the beetle, unprotected and blank treatments. The Blank and unprotected miniplots were used to investigate the relationship between slug density and slug damage. When miniplot number 21 (second assessment) is left out of the calculation, a correlation exists between slug density and slug damage (see text).

		First Assessment	Second Assessment
H.rufipes	1/plot	138.25	109.96
H.rufipes	5/plot	93.72	154.50
H.rufipes	10/plot	81.92	279.52
A.parallelepipedus	1/plot	96.66	101.44
A.parallelepipedus	5/plot	2.19	2.73
A.parallelepipedus	10/plot	16.65	9.73
P.melanarius	1/plot	57.37	27.94
P.melanarius	5/plot	72.19	32.85
P.melanarius	10/plot	104.16	56.99
P.niger	1/plot	85.23	88.38
P.niger	3/plot	98.41	107.66
Unprotected miniplots		154.74	159.85
Blank miniplots		50.92	223.74
Breakdown of the control miniplots (figure in brackets = miniplot number)			
Unprotected miniplots	(12)	270.59	235.78
	(16)	38.89	83.93
Blank miniplots	(08)	96.43	114.98
	(21)	5.41	332.50

Appendix 7.1

Table of Authorities. Where possible, species have been given their full name, author and date.

Annelidae:
Allolobophora longa (Ude)
Elisenia fuetida (Savigny)
Lumbricus terrertris (Linnaeus)
Oetolasion lacteum (Oerley)

Coleoptera; Carabidae:
Abax ater (de Villers C.J. 1789)
Abax parallelepipedus (Piller and Mitterpacher 1783)
Agonum assimile (Paykull)
Agonum dorsale (Pontoppidan 1763)
Agonum fuliginosum (Panzer 1809)
Agonum obscurum (Herbst 1784)
Agonum mulleri (Herbst 1785)
Agonum viduum (Panzer 1797)
Agonum thoreyi Dejean 1828
Agonum fuliginosum (Panzer 1809)
Amara aenea (Degeer 1774)
Amara aulica (Panzer 1797)
Amara apricaria (Paykull 1790)
Amara convexior Stephens 1828
Amara familiaris (Duftschmid 1812)
Amara lunicollis Schioedte 1837
Amara plebeja (Gyllenhal 1810)
Amara similata (Gyllenhal 1810)
Asaphidion flavipes (Linnaeus 1761)
Badister bipustulatus (Fabricius 1792)
Bembidion lampros (Herbst 1784)
Bembidion tetracolum (Say)
Bembidion unicolor Chaudoir 1850
Bembidion obtusum Serville 1821
Calathus fuscipes (Goezze 1777)
Calathus piceus (Marsham 1802)
Calathus melanocephalus (Linnaeus 1758)
Calathus micropterus (Duftschmid 1812)
Calosoma frigidum Kirby
Carabus catenulatus Fabricius 1801
Carabus nemoralis (Mueller, O. F. 1764)
Carabus violaceus Linnaeus 1758
Carabus arvensis Herbst 1784
Carabus problematicus (=*catenulatus*) Herbst 1786
Clivina fossor (Linnaeus 1758)
Cychrus attenuatus Fabricius
Cychrus caraboides (Linnaeus 1758)
Demetrias atricapillus (Linnaeus 1758)

Coleoptera; Carabidae (continued):
Harpalus aeneus (Fabricius 1775)
Harpalus rufipes (Degeer 1774)
Harpalus latus (Linnaeus 1758)
Harpalus pensylvanicus Degeer
Harpalus erraticus Say
Harpalus compar Lec.
Loricera pilicornis (Fabricius 1775)
Nebria brevicollis (Fabricius 1792)
Nebria gyllenhalli (Schoenherr 1806)
Notiophilus biguttatus (Fabricius 1779)
Patrobus atrorufus (Stroem, H. 1768)
Patrobus assimilis Chaudoir 1844
Pterostichus anthracinus (Illiger 1798)
Pterostichus chalcites
Pterostichus cupreus (Linnaeus 1758)
Pterostichus cristatus (Dufour, l. 1820)
Pterostichus diligens Sturm 1824
Pterostichus madidus (Fabricius 1775)
Pterostichus melanarius (Illiger 1798)
Pterostichus minor (Gyllenhal 1827)
Pterostichus niger (Schaller 1783)
Pterostichus nigrita (Fabricius 1792)
Pterostichus strenuus (Panzer 1798)
Pterostichus vernalis (Panzer 1796)
Pterostichus versicolor Sturm 1824
Scaphinotus interruptus (Menetries)
Scaphinotus striatopunctatus (Chandoir)
Synuchus nivalis (Panzer 1797)
Trechus quadristriatus (Schrank 1781)

Coleoptera; Stapylinidae:
Oxypoda vittata Maerkel 1842

Coleoptera:
Coccinella 7-punctata L.
Psyllobora 22-punctata (L.)
Rodolia cardinalis (Mulsant)
Silpha atrata (Linnaeus 1758)

Collembola:
Podura minor (Lubb)

Chilopoda:
Lithobius forficatus (L.)

Dermaptera:
Forficula auricularia L.

Diplopoda:
Glomeris marginata (Villers)
Oxidus gracilis Koch
Polydesmus angustus Latzel
Polymicrodon polydesmoides (Leach)
Tachypodoiulus niger (Leach)

Diptera:
Coboldia fuscipes Meigen 1830
Megaselia aequalis (Wood, J. H. 1909)
Tetanocera elate (Meigen)
Tetanocera plebeia Loew

Hemiptera:
Brevicoryne brassicae (L.)
Icerya purchasi Maskell 1878
Nezara viridula (L.)
Notonecta glauca Linnaeus 1758
Podisus maculiventris Say
Rhopalosiphum padi (Linnaeus 1758)
Sitobion fragariae (Walker 1848)
Sitobian avenae (F.)

Homoptera:
Conomelus anceps (Germar)

Hymenoptera:
Bracon mellitor Say
Cardiochiles nigriceps Viereck
Encarsia formosa Gahan 1924
Goniozus natalensis Gordh
Ibalia leucospoides (Hochenwarth 1785)
Megarhyssa nortoni nortoni (Cresson)
Nemeritis canescens (Gravenhorst 1829)
Pracon volucre (Haliday 1833)
Rhyssa persuasoria (Linnaeus 1758)
Sirex noctilio Fabricius 1793
Trichogramma evanescens Westwood 1833

Isopoda:
Armadillidium vulgare (Latreille)
Oniscus asellus Linnaeus
Philiscia muscorum (Scopoli)
Porcellio scaber Letrielle
Porcellio spinicornis Say
Trichoniscus pusillus (Brandt)

Lepidoptera:
Eldana saccharina Walker
Heliothis virescens (F.)
Pieris brassicae Linnaeus
Pieris rapae (linnaeus 1758)

Mollusca:
Arion ater (Linne 1758)
Arion rufus (Linne 1758)
Arion circumscriptus Johnston 1828
Arion hortensis Ferussac 1819
Arion subfuscus (Draparnaud 1805)
Arion intermedius Normand 1852
Arion fasciatus (Nilsson 1823)
Cochlicopa lubricella (=*minima*) (Porro 1838)
Deroceras caruanae (Pollonera 1891)
Deroceras laeve (Muller 1774)
Deroceras reticulatum (Muller 1774)
Helix aspersa (Muller 1774)
Limax maximus Linne 1758
Limax tenellus Muller 1774
Limnaea truncatula (Muller)
Milax budapestensis (Hazay 1881)
Milax gagates (Draparnaud 1801)
Zonitoides nitidus (Muller 1774)