The Biology, Behaviour and Control of the Field Slug *Deroceras reticulatum* (Müller)

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For my Nan
Peggy Kathleen Smith
(1923-2002)

and

For my Parents

With Love and Thanks

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Abstract

Deroceras reticulatum (Müller) is the most destructive slug pest of arable crops in Britain. Control on a field scale relies heavily on molluscicide pellets. These are often only partially effective; considerable economic loss and collateral damage still occur. More effective targeting of pellets will depend on accurate predictions of damage severity through a better understanding of population dynamics, reliable estimates of surface activity and behavioural studies to evaluate current control strategies.

Lifecycle parameters of *D. reticulatum* were studied. There was considerable variation in growth even under identical conditions and growth rate was inversely related to survival. For field collected slugs, the association between growth rate and temperature was low but negative for spring hatching individuals; however, this was relatively trivial compared to the high and positive association exhibited by those hatching in autumn. For self-fertilised slugs, egg development took longer and the hatching rate was lower than for slugs laid by field collected adults, but the growth rate was faster. It is suggested that field populations may be composed of fast and slow growers, and that this might be determined by whether eggs are fertilised with auto- or allosperm. An equation was derived to predict female-phase maturity from body weight.

Refuge traps sampled approximately one third of the surface active population over a 24 hour period. The timing and number of trap entries and exits did not differ between small and large *D. reticulatum.*

Behavioural studies of *D. reticulatum* demonstrated that the time elapsed and distance travelled before feeding on bait pellets was shorter when they were broadcast compared to drilled. Soil splash did not reduce pellet efficacy. Trails were more sinuous on fine than coarse seedbeds and were reduced by pellets.

The practical implications of these results for assessing and controlling *D. reticulatum* in arable crops are discussed.

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Chapter 1
General Introduction

The aim of this chapter is to present an overview of the nature and scope of the problems posed to British agriculture, in particular to arable crops, by the limacid slug *Deroceras reticulatum* (Müller). *Section 1.1* will outline general features of the biology of this species as a basis for understanding how it has attained pest status in agricultural environments. The pest status of *D. reticulatum* will be expanded upon in *section 1.2* which will also review the difficulties concerning current methods of control and the associated costs incurred by the farming industry. Finally, *section 1.3* summarises the resultant motivations and objectives of this study and describes the structure of the thesis.

1.1 General Biology

1.1.1 Taxonomy

All British terrestrial slugs belong to the Phylum Mollusca, Class Gastropoda, Subclass Pulmonata, Order Stylommatophora. There are five families comprising the Arionidae; Boettgerillidae; Milacidae; Testacellidae and Limacidae, the last to which *D. reticulatum* belongs (South, 1992). Keys for their identification include those of Quick (1960) and Cameron *et al.* (1983). They may generally be characterised as possessing a broad flattened foot which extends the length of the body and sometimes a short distance up the side, forming a 'foot fringe'. The visceral mass is contained within the body cavity (head-foot) and exhibits torsion to some degree. Situated dorsally is the distinctive mantle area which is drawn in anteriorly to form a flap that covers the head and neck when contracted. To the right of the mantle is a structure called the pneumostome, which is used in respiration and beneath the mantle is the mantle cavity; the rudimentary shell, if present, is usually internal and is also found beneath the mantle. The distinct head region possesses two

pairs of retractile tentacles, the posterior pair of which bear eyes. A comprehensive account of the internal anatomy of slugs is provided by Runham and Hunter (1970) and South (1992).

1.1.2 *Deroceras reticulatum*

D. reticulatum is also known by the synonym *Agriolimax reticulatus* in the older literature and is one of the most common British slugs (South, 1992). It measures 3.5-5 cm when fully extended and the body colour can vary from bluish-black to pale white, but is typically a greyish cream as in *Fig. 1.1.* The dorsal surface is usually covered in a mottled pattern of darker spots and is ridged with tubercles, giving the integument a slightly rough appearance. A raised ridge on the midline of the body, known as the keel, extends up to two-thirds the distance to the mantle from the tail and the mantle surface itself is textured with a 'fingerprint-like' pattern of concentric ridges (Cameron *et al.*, 1983). The foot is a pale cream colour becoming darker towards the centre and the mucus produced is a sticky, milky white when the slug is irritated, but otherwise is colourless (Runham & Hunter, 1970).

Figure 1.1: Deroceras reticulatum *(Müller), the field slug, one of the most serious arable crop pests in Britain.*

D. reticulatum is indigenous to Europe, but has been introduced through trade to many other temperate regions, e.g. parts of the USA (Godan, 1983) and New Zealand (Barker, 1999). It is widely dispersed throughout Britain and thrives in unstable habitats, for example, agricultural land (South, 1974), but is also found in gardens, grassland and hedgerows (Runham & Hunter, 1970). The local spatial distribution tends to be rather patchy (Barnes & Weil, 1944) and is determined by factors such as the provision of food (Hunter, 1966), availability of moisture and shelter (Stephenson, 1967; Duval, 1970). Relatively non-aggressive species such as *D. reticulatum* are known to 'huddle' in groups to conserve water in adverse conditions, e.g. very dry, cold or frosty weather (Waite, 1988) and, in common with other species, are often aggregated following hatching (South, 1965).

Although omnivorous (Pallant, 1969), this species tends to feed primarily on live and decaying plant material, showing a preference for green leafy vegetation (Duthoit, 1964; Airey, 1986). The basis of food choice is likely to involve a number of factors including the physical properties of the substrate and the presence of feeding stimulants or inhibitors (Stephenson, 1979). The activity of *D. reticulatum* is not restricted by cold conditions to the same extent as other species (Crawford-Sidebotham, 1972) and it is known to feed normally at temperatures as low as 0°C (Mellanby, 1961).

D. reticulatum is a simultaneous hermaphrodite. Development is protandric, i.e. the male-phase precedes the female-phase although there is considerable overlap between the two (Runham, 1978). The reproductive system is described in detail in *Chapter 5.* Courtship, which precedes sperm transfer, is often complex and protracted, involving elaborate sequences of behaviour such as trail following, pairing and circling (Nicholas, 1984). The details vary widely between species (Taylor, 1902-1907; Quick, 1960), but in *D. reticulatum* it usually takes place on damp ground on mild evenings or during the night (South, 1992). The period between mating and oviposition ranges from 8-10 days (Runham & Hunter, 1970).

The lifecycle of *D. reticulatum* is strongly influenced by temperature (South, 1982) and hence it varies with location and the time of year (Port & Port, 1986). In cold

climates the population is observed to consist of a single generation each year (Dmitrieva, 1969) whereas in more temperate regions, such as Britain, there are two peak breeding seasons per annum, in spring and autumn (Bett, 1960; Hunter, 1968b). These were initially thought to correspond to two separate generations (Hunter, 1968b), however, the lifespan was later shown to be nine months or more in the field (Hunter & Symonds, 1971) and since *D. reticulatum* is capable of breeding throughout the year whenever conditions are favourable the pattern is likely to be more complex. Indeed there may be several overlapping generations present at any one time (Hunter & Symonds, 1971; Hunter, 1978; South, 1989a).

Eggs are commonly laid under objects such as stones and leaves or in soil crevices (South, 1992). *D. reticulatum* is one of the most fecund species with up to 500 eggs recorded per individual (Port & Port, 1986) in batches of approximately 30 separate eggs (Carrick, 1938) (*Fig. 1.2*). Oviposition on soil is influenced by the water content; maximum numbers of eggs are laid when the soil is 75% saturated (Arias & Crowell, 1963) and the drier it becomes, the deeper they are found (Carrick, 1942). Eggs are very sensitive to extremes of temperature early in development and failure to hatch is often the result of desiccation (South, 1989b). The incubation period is temperature-dependent, ranging from a minimum of 175 days at 4.4°C (Judge, 1972) to 15 days at 20°C (Carrick, 1942). At higher temperatures the incubation period increases again and the numbers remaining viable declines (Pakhorukova & Matekin, 1977). The young slugs hatch fully formed and break their way out of the egg by rasping at its membrane with the radula (Carrick, 1938).

Stages in post-embryonic development are traditionally distinguished by the growth rate. Abeloos (1944) defined three phases based on a study of *Arion* species: infantile (rapid growth), juvenile (slow growth) and mature (minimal growth as slugs commence egg laying). *D. reticulatum* exhibits only two of these; the infantile phase is highly suppressed to the extent that it merges with the juvenile phase (South, 1982). Mortality is highest in slugs in very early stages post-hatching and then remains more stable until the end of the life span (South, 1989b). Signs of senescence are indistinct, but may include a darkening of the body wall and a loss of weight (Szabó, 1935 in South, 1992).

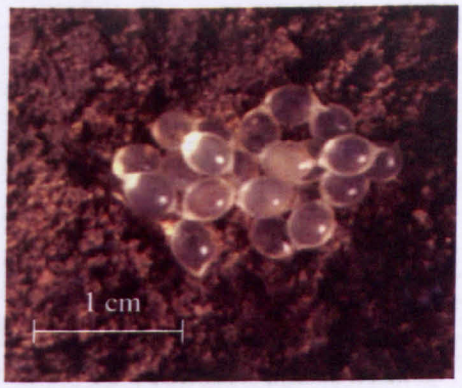


Figure 1.2: A batch of slug eggs laid in a soil crevice.

1.1.3 Regulation of activity

A prerequisite for activity in terrestrial slugs is the ability to withstand wide fluctuations in water content (Howes & Wells, 1934). Reserves are continually depleted by evaporation from the body surface even in constant conditions and this is exacerbated by the production of mucus for locomotion and vital processes such as excretion and respiration (Dainton, 1954b; South, 1992). This would rapidly lead to dehydration were it not replenished from the food intake by 'drinking' and by absorption through the skin in a process known as contact-rehydration (Prior, 1989). The capacity of slugs to regulate their body water content in this way, in addition to behavioural adaptations such as crawling downwind (Dainton, 1954b), enables them to exploit environments normally denied to animals whose integuments must remain moist, yet have no impermeable cuticle (Runham & Hunter, 1970).

D. reticulatum is predominantly active at night (Newell, 1966) exhibiting, in common with all slugs, an endogenous circadian rhythm of activity (e.g. Rollo, 1991; Hommay *et al.*, 1998). This is thought to be an adaptation to avoid unfavourable weather conditions (Rollo, 1982) since it is entrained by exogenous, environmental factors (zeitgebers). There is some disagreement in the literature as to whether light or temperature is the most important of these; earlier studies suggested that changes in temperature were mainly responsible for promoting activity (e.g. Dainton, 1954a; Karlin, 1961), but have been criticised for using low light intensities to which some species, such as *D. reticulatum*, are insensitive (Rollo, 1982) and for focussing on the

initiation rather than maintenance of entrained rhythms (Rollo, 1991). More recent work indicates that light plays a greater role in preserving activity cycles (e.g. Newell, 1965; Sokolove *et al.*, 1977; Morton, 1979). The majority of these studies were, however, based on laboratory experiments and in the field it is likely that other weather variables modify patterns of expression, e.g. soil moisture, relative humidity, air movement and fluctuating air temperatures (Lewis, 1969a; Wareing & Bailey, 1985; Cook & Ford, 1989; Young & Port, 1989).

Homing behaviour has been described for a number of slug species, including *D. reticulatum* (e.g. Taylor, 1902-1907; Newell, 1965; Duval, 1972). When individuals are released into a novel environment, after an initial dispersal phase they establish a home range containing a home site or sites to which they return after nocturnal foraging (South, 1965). The home site may be occupied by a single individual, or may be shared between a number of conspecifics (Rollo & Wellington, 1981). Slugs are capable of homing without following a mucus trail or using visual cues (Gelperin, 1974) although failure to relocate a home site often results in a period of trail following (Cook, 1979). These observations were explained by postulating a dual pheromone mechanism whereby a volatile pheromone acts as an 'olfactory beacon' in distance chemoreception of the home site and a non-volatile pheromone, detectable only in contact with mucus, is responsible for trail following (Cook, 1980). The pheromones are probably detected by the tentacles; the posterior pair are known to be sensitive to smell and are implicated in olfaction of air-borne chemicals (Gelperin, 1974; Chase & Croll, 1981) while the anterior pair are involved in taste reception and are likely to mediate trail following (Kittel, 1956 in South, 1992). Weather conditions influence the approach to home sites; in calm dry weather the paths are direct, whereas in rain and wind they are spiralled (Rollo & Wellington, 1981).

1.1.4 Mortality factors

Density-independent climatic factors have been shown to influence slug populations through their effects on the life cycle suggesting that population numbers are not stable, but are limited most of the time by the short period in which they can increase

before harsh conditions intervene (South, 1992). This has been shown for *D. reticulatum* in a number of long-term studies that have monitored population numbers in relation to weather conditions over a period of several years (e.g. Dmitrieva, 1969; South, 1989b; South, 1989a). It was generally found that within a particular area exceptionally dry or cold conditions led to local extinctions, but that these were redressed by rapid re-colonisation when the weather became more favourable again (Waldén, 1981 in South, 1992).

Competition, a density-dependent regulatory factor, is generally influential where population densities or species diversity are high (Rollo, 1983b; Rollo, 1983a). In natural slug populations, however, it is unlikely to have any significant effects on numbers as a result of niche differentiation and the relative distribution of aggressive and non-aggressive species. The former serves to separate competing species that co-exist in a stable environment such that they are not vying for similar resources, e.g. food and resting sites. There are numerous examples of this in the literature, (e.g. Hunter, 1966; Jennings & Barkham, 1979; Cook & Radford, 1988). In unstable environments such as arable land the dominant species, e.g. *D. reticulatum* and *Arion hortensis*, tend to be relatively unaggressive (Rollo & Wellington, 1979) and well-adapted to such conditions (South, 1982).

Imperfectly density-dependent factors that act to regulate slug populations include predators, parasites and disease. Early records of predation on slugs are mostly anecdotal (Taylor, 1902-1907). There are a number of inherent difficulties in compiling a comprehensive list of predators. Direct evidence from field observations is almost impossible to obtain in a systematic fashion. Laboratory choice-experiments of slug-predator interactions are informative in identifying species that will accept slugs as a food source, but cannot indicate the extent to which the slugs consumed form a 'normal' part of the predator's diet under natural conditions (South, 1992). Until recently, it was difficult to identify slug remains in gut contents of field collected slugs since digestion is rapid and the only remaining parts are the jaw, radula and occasionally the internal shell (South, 1980), but the application of immunological techniques is now helping to overcome this problem (e.g. Symondson & Liddell, 1993; Ayre & Port, 1996; Dodd *et al.*, 2003).

Representatives of all vertebrate groups have been reported to consume slugs, although none in sufficient quantities to make an appreciable difference to population numbers (Port & Port, 1986) and many of these instances may have been incidental (South, 1992). Birds are often hindered by the copious production of sticky mucus, especially by larger slug species, which therefore do not form a major part of their diet (Boycott, 1934; South, 1980). Amphibians, particularly frogs and toads, have long been regarded as key predators of slugs (Taylor, 1902-1907) along with small mammals such as hedgehogs, which prey especially on surface dwelling *D. reticulatum* and do not appear to find the mucus a deterrent (South, 1980).

Invertebrates are more important natural enemies of slugs than vertebrates, in particular carabid beetles (Cornic, 1973; Ayre & Port, 1996; Mair & Port, 2001; Armsworth *et al.*, 2003) (*Fig. 1.3*). Of these the larger species are more likely to eat slugs as their greater strength permits them to hold the slug away from the head region so they are less hindered by the mucus produced and their strongly developed mouth parts allow them to dispatch their prey efficiently and quickly (Tod, 1973). Other invertebrates have been reported to scavenge moribund slugs including fly larvae (Reidenbach *et al.*, 1989) and species of the carnivorous slug genus, *Testacella* (Stubbs, 1934).

A number of parasites may infest slugs, most notably nematodes (e.g. Arias & Crowell, 1963; Grewal *et al.*, 2003), but also platyhelminths (e.g. Cragg, 1957 and Foster 1958 in South, 1992) and protozoa (e.g. Jones & Selman, 1984). The nematode, *Phasmarhabditis hermaphrodita* (Schneider) has been extensively studied and exploited in recent times as a biological control agent for *D. reticulatum* (Wilson *et al.*, 1994; Speiser & Andermatt, 1996; Iglesias *et al.*, 2001; Ester *et al.*, 2003) (*Fig. 1.4*). The effect of parasitism on the slug and the site of infestation depend on the particular species involved. Smaller slugs are probably more important intermediate hosts of vertebrate parasites as they are preyed on more readily than the larger species (South, 1992).

Figure 1.3: Carabid beetle, Pterostichus madidus *(Fabricius), an invertebrate predator of* Deroceras reticulatum.

Little is known of the diseases that affect slugs (Godan, 1983); few viruses have been reported and knowledge of mollusc immunity is limited (South, 1992). Infections with fungi e.g. *Arthrobotrys* and *Fusarium* species are, however, commonly observed in laboratory cultures and often develop on eggs or dead adults (Arias & Crowell, 1963).

Figure 1.4: Deroceras reticulatum *infested with the nematode* Phasmarhabditis hermaphrodita *(front). Note the swollen mantle in comparison to that of a healthy slug (behind).*

1.2 *D. reticulatum* and British Arable Farming

1.2.1 Crops at risk and damage caused

D. reticulatum has long been a dominant pest species on arable land in Britain (e.g. Miles *et al.*, 1931; Martin & Kelly, 1986; Willis *et al.*, 2003) and is responsible for an estimated 70% damage due to slugs (Schley & Bees, 2003) (*Fig. 1.5*). It is a particular pest of oilseed rape and cereals, e.g. winter wheat (Martin & Kelly, 1986; Glen, 1989; Glen *et al.*, 1996b).

Figure 1.5: Slug damage on arable land (Heddon Banks Farm, Northumberland). Note the patchy areas of crop growth.

Crops are most vulnerable at the time of establishment when the main types of damage caused are grain hollowing (Gould, 1961; Duthoit, 1964) and grazing of young shoots (South, 1992). Grain hollowing (*Fig. 1.6*) is generally considered the most serious of these and results from slugs eating the germ and endosperm of seeds soon after drilling, preventing germination. Grazing of emerging shoots retards growth and can lead to a thin crop stand; in severe cases, this too may lead to the

complete loss of plants (Port & Port, 1986). This is a particular risk during periods where the temperature at night is warm enough for slug activity, but too low in the daytime for plant growth (Runham & Hunter, 1970).

Leaf shredding, where fleshy parts of the leaf are eaten away, is a common sign of slug attack in older plants (*Fig. 1.7).* This is generally less critical than earlier damage as plants are usually able to grow fast enough to compensate (Anon., 1979); indeed, some leaf damage can encourage tillering in wheat, ultimately increasing yield (Jessop, 1969).

Figure 1.6: Grain hollowing damage to wheat seeds. Seeds on the left have been eaten; the endosperm has been eaten from those on the right.

Figure 1.7: Leaf shredding damage to an oilseed rape plant by Deroceras reticulatum *(Photograph: Louise Simms, with permission).*

1.2.2 Methods of slug control

1.2.2.1 Chemical control

Molluscicides are the most frequently used method of slug control on an agricultural scale (Glen & Moens, 2002) and were applied to 17% of all arable crops in 2002. The two most commonly used active ingredients were metaldehyde and methiocarb, which respectively accounted for 79% and 14% total area treated; thiodicarb was used on a further 5% of treated crops (all statistics from Garthwaite & Thomas, 2003).

Metaldehyde and methiocarb can be applied as sprays or dusts (Howling, 1990), but are most commonly formulated as pelleted baits (Glen & Wilson, 1995). Commercially produced pellets generally have a cereal or bran base which acts as a feeding stimulant and attractant, along with a stabiliser, binder, fungicide and coloured dye to discourage consumption by other animals (Bailey, 2002) (*Fig. 1.8*). As the concentration of metaldehyde and methiocarb in pellets increases, they become repellent to slugs and the amount eaten declines (Wright & Williams, 1980; Wedgwood & Bailey, 1986; Bourne *et al.*, 1988), which may result in sub-lethal poisoning (Wedgwood & Bailey, 1988). Consequently, the percentage of active ingredient included in pellets is determined by the balance between efficacy and palatability, and is typically within the range of 2-8% (Howling, 1990).

Figure 1.8: Deroceras reticulatum *approaching a molluscicide pellet.*

Both metaldehyde and methiocarb act by paralysing slugs, although the course of action of the two chemicals differs. Metaldehyde poisoning is characterised by rapid immobilisation, punctuated with brief outbursts of uncoordinated muscular activity and excessive mucus production whereas methiocarb poisoning tends to be manifested by a brief period of hyperactivity, followed by flaccidity of muscles with ensuing paralysis, but no increase in mucus production (Cragg & Vincent, 1952; Frain & Newell, 1983; Young, 1986; Mills *et al.*, 1989). Slugs suffering from

metaldehyde poisoning often look shrivelled while methiocarb results in a bloated appearance (Bourne *et al.*, 1988); in both cases the ultimate cause of death is frequently dehydration since the slugs cannot move to find shelter.

The toxic effects of molluscicides to non-target organisms have been investigated in a number of studies. Occasional cases of poisoning in domestic pets (Studdert, 1985) and farm stock (Longbottom & Gordon, 1979) have been reported, but these are generally the unfortunate outcome of poor containment of pellets. As regards wildlife, metaldehyde appears to be one of the less toxic pesticides to soil fauna, with no reported mortality of earthworms (Bieri *et al.*, 1989) or carabid beetles (Büchs *et al.*, 1989) exposed to pellets under laboratory conditions. Methiocarb, in contrast, results in considerable collateral damage; it has been observed to cause up to 5% mortality in earthworm populations (Martin & Forrest, 1969) and is also damaging to both carabid and staphylinid beetles (Runham & Hunter, 1970; Purvis, 1996). There is little published on molluscicide poisoning in small wild mammals, e.g. shrews and hedgehogs, largely due to difficulties in obtaining data since they tend to seek cover when affected (Barnett *et al.*, 2002). Field trials have, however, shown that populations of wood mice, *Apodemus sylvaticus* (L.), which are common small mammal species on arable land, are reduced in fields where methiocarb has been applied (Tarrant & Westlake, 1988; Shore *et al.*, 1997).

1.2.2.2 Biological control

Biological methods to control pests involve the introduction and management of their natural enemies (Runham & Hunter, 1970). Although this has been used with some success in isolated environments, such as greenhouses (e.g. Symondson, 1989), the only biocontrol agent shown to have potential on an agricultural scale to date is the parasitic nematode, *P. hermaphrodita* (e.g. Wilson *et al.*, 1994; Glen & Wilson, 1997; Hass *et al.*, 1999), as mentioned in *section 1.1.4.* The use of this nematode is feasible on technical grounds, however, cost prohibits its use in arable crops at present (Glen *et al.*, 1996a) and it is not a realistic option for oilseed rape or cereal farmers.

1.2.2.3 Cultural control

Cultural control entails modifications to the husbandry of a crop to reduce slug damage. This may include repeat cultivations of land followed by compaction to alter the soil structure (Gould, 1961; Hunter, 1967; Stephenson, 1975; Glen *et al.*, 1989), appropriate crop rotations, adjustments to sowing time (Port, 1989) and removing the residue of the previous crop from the soil surface to reduce the amount of shelter, food and oviposition sites available (Port & Port, 1986; Howling, 1990). The use of less susceptible cultivars may also help, although this is more relevant to potatoes, where slugs are known to have distinct preferences for certain varieties (Gould, 1965; Winfield *et al.*, 1967). They are less discerning as far as cereals are concerned. Recent studies have, however, shown that some modern oilseed rape cultivars are more prone to attack than older varieties (Glen *et al.*, 1990; Glen *et al.*, 1996b). In practice, the extent to which each of these modifications is viable in a given situation depends largely on the weather, soil type, market requirements and competing demands on the farmer's time.

1.2.2.4 Integrated control

Integrated control (also referred to as integrated pest management, IPM) has been defined as 'a pest management system that, in the context of the associated environment and population dynamics of the pest species, utilises all suitable techniques and methods in as compatible manner as possible and maintains the pest populations at levels below those causing economic injury' (Smith, 1967). The implication is that control measures should be used when they will have the optimum effect on the slugs present, in such a way that they do not have detrimental effects on beneficial organisms in the environment and only when absolutely necessary, i.e. when slug densities exceed a critical threshold for damage and not prophylactically (Runham & Hunter, 1970). Such a strategy is inherently specific to a particular species, location and time. The prediction of slug damage is central to this approach, but is a complex task as slugs are localised and damage depends intimately on weather and cultural conditions.

1.2.3 Problems past and present

1.2.3.1 1970s to 1980s

The pest status of *D. reticulatum* increased throughout the 1970s and 1980s largely as a result of changes in agricultural practice involving arable crop production (Martin & Kelly, 1986), along with a succession of damp springs and mild autumns.

The area devoted to oilseed rape rose from 39 thousand hectares in 1975 to 296 thousand hectares in 1985 (Bunting, 1984; Martin & Kelly, 1986). The total crop area of cereals also increased; in particular the area of winter wheat grown rose by 54.3% (calculated from MAFF Agricultural statistics in Martin & Kelly, 1986). The dense foliage of oilseed rape and the associated large volume of post-harvest debris affords shelter from adverse conditions and predators, in addition to supplying ample food (Stephenson & Bardner, 1976), while winter cereals extend the duration of the growing season providing favourable habitats for slugs over a longer period of time, aiding survival and prolonging the traditional breeding cycle. Both of these factors individually promoted rapid population expansion, and this was compounded by crop rotations where oilseed rape was followed by wheat (Port & Port, 1986).

Financial pressures and increased emphasis on production costs in the early 1980s, along with environmental concerns, were the impetus for a move towards reduced cultivation techniques. Traditional methods such as ploughing require almost three times the energy input of minimum tillage cultivations using tines and discs, and over ten times the energy consumed by direct drilling (MAFF leaflet 'Cultivations for Cereals' 1982 in Martin & Kelly, 1986). Any economic gain from less intensive soil management was, however, offset by a resultant increase in slug populations. Minimum cultivation techniques led to increased crop residues (Port & Port, 1986) and did not have the adverse effects on slug numbers that are associated with traditional methods, such as mortality due to physical injury (Hunter, 1967) and reducing shelter sites by creating a fine, firm seedbed with fewer air spaces between soil particles (Stephenson, 1975). Direct drilling into straw or stubble (zero-tillage) resulted in much increased and more severe crop damage (Edwards, 1975; Stephenson & Bardner, 1976). This is likely to be because seeds are easily

accessible in the drill slit unless conditions favour its closure (Hughes & Gaynor, 1984) and, as mentioned previously, crop residues support population expansion by providing food and shelter. The practice of stubble burning alleviated some of the these problems to a certain extent (Glen *et al.*, 1984; Smith *et al.*, 1985) although it is not clear whether this was due to the heat, removal of food and shelter or the resulting ash deposits; it is probably a combination of these factors, but slug numbers were observed to decrease as a consequence.

1.2.3.2 1990s to the present

Despite advances in understanding the factors that contribute to high levels of damage, slugs continued to be major pests in British arable crops throughout the 1990s and remain so at present (Glen *et al.*, 2003; Willis *et al.*, 2003). Reasons for this are varied. Agricultural policy and market demands mean that what is 'ideal' from the perspective of control is not necessarily practical for economic or legislative reasons. For example, stubble burning was banned in 1993 on environmental grounds and is therefore no longer a viable intervention for reducing populations. Current alternative means of straw disposal include incorporation into the soil by shallow cultivation or chopping and spreading on the surface (Glen & Wilson, 1995), both of which aggravate slug problems by providing plentiful cover and food.

Avoiding crop rotations that are known to increase the risk of serious damage is not always feasible because they are also designed to achieve other aims, such as profitability and promoting soil fertility (Jordon and Hutcheon 1996 in Glen *et al.*, 1996b); these may take precedence. The influence of set-aside to damage levels in crop rotations has yet to be fully assessed. Conditions are certainly ideal for slugs (Glen *et al.*, 1996b), but subsequent preparations prior to sowing crops may reduce numbers below thresholds of economic injury.

There is currently a demand for modern 'double-low' cultivars of oilseed rape which have low glucosinolate and erucic acid concentrations. These are considerably more vulnerable to slug attack than older 'single-low' varieties (Glen *et al.*, 1990) and exacerbate the risk of damage to a following wheat crop. Where slug populations build to very high levels even the preparation of fine seedbeds or deep drilling of

seeds in coarse seedbeds, which usually offer some protection (Glen *et al.*, 1992), can fail to prevent attack; this is simply delayed until after emergence and severe loss of plant stand may occur.

As a consequence of agronomic practices continuing to encourage slug population growth, the use of molluscicides has increased. The Department for the Environment, Food and Rural Affairs (DEFRA) conducts biennial surveys of pesticide usage in arable crops and the latest data available is for 2002 (Garthwaite & Thomas, 2003). This indicates that, although the area of arable crops grown (excluding set-aside) fell by 9% between 1992 and 2002 to 4.8 million hectares, molluscicide usage steadily increased by four times over that 10 year period. For wheat, 32% of the area grown was treated with molluscicides and for oilseed rape this figure was 56%. In 2002, a total of 718 tonnes of active ingredient were applied to 1.1 million hectares of land.

In spite of the increased use of molluscicides, control of *D. reticulatum* is not always effective and losses are still incurred. Reasons for this are unclear. It has been suggested, however, that molluscicides may be rendered ineffective when splashed by soil as a result of heavy rainfall or when they are washed into crevices becoming concealed from slugs (Hass *et al.*, 1999; Simms *et al.*, 2002). It may also be that, following disruption caused by seedbed preparation, slugs continue to hatch from eggs or resume normal surface activity patterns whilst crops are still susceptible and after molluscicides have lost their potency. Indeed, Glen *et al.* (1991) showed that, at best, molluscicides kill approximately 50% of the slug population and speculated that this is due to a large proportion of slugs not being surface active during the period of time that molluscicides are effective which may explain why dense populations are not reduced sufficiently to prevent economic levels of damage.

The optimum time to apply pellets is at the weakest point in the slug lifecycle. This is when initial numbers are low and before egg laying commences so that the subsequent increase in numbers is kept in check. In practice this is not always possible because the initial low densities of slugs are very hard to detect. Furthermore, as described in *section 1.1.2*, the breeding season is moderated by annual weather patterns. There is consequently a narrow window of time when

pellets have maximum benefit. This is itself highly variable and is not always compatible with an appropriate stage in the growth cycle of the crop. Local factors also need to be taken into consideration; slugs must be active on the soil surface in order for pellets to have any effect and therefore the moisture and temperature on the day of application must be appropriate (South, 1992).

1.2.3.3 Costs of damage

Accurate estimates of the economic impact of slug damage can be difficult to obtain since signs of attack are often confused with those of other pests (South, 1992) and thus the basis of the estimate is not always reliable. Those assessments of damage that have been made are based on restricted factors and are largely concerned with a particular crop. For example, in wheat, estimates variously concern equivalent grain loss (Strickland, 1965), area redrilled (Hunter, 1969) and area treated with molluscicides (Sly, 1986).

Solely for wheat, Stephenson and Bardner (1976) suggested that economic loss due to slugs amounted to 0.22% of the value of the crop based on the combined costs of redrilling, yield loss and application of control measures. This gave a value of £2.69 million in 1985 (Port & Port, 1986) which was updated to £4 million in 2001 (Shirley *et al.*, 2001). These figures are, however, likely to be conservative since costs of factors associated with Stephenson and Bardner's estimate are expected to have increased since 1976.

For arable crops in total, the cost of molluscicide pellets alone was estimated at £18.5 million at 2000 values (Schüder, 2004); the additional costs associated with crop loss where molluscicides are ineffective and the costs of application itself would make this figure higher still.

1.2.4 The future

It is probable that arable farmers will continue to be restricted in the extent to which they can manipulate cultivations in order to reduce slug damage, unless agricultural policy changes significantly (McDonald, 1995), and the use of *P. hermaphrodita* as a

biological control agent in arable crops is, as described earlier, not feasible on economic grounds at present. Genetic modification of crop plants to produce cultivars that are resistant to feeding by slugs has shown promise in laboratory trials (e.g. Walker *et al.*, 1999; Mulligan *et al.*, 2003), but the future potential of this on a field scale is uncertain; there is much public opposition to such technology. Molluscicide pellets are, therefore, likely to remain the main method of slug control in the immediate future. Since molluscicides are a restricted market globally, it is doubtful that products based on new chemistry will become available for some time and, therefore, there is a need to improve the efficiency and targeting of current products in order to reduce costs to farmers and minimise collateral damage. Improved targeting of molluscicides will rely on accurate predictions of damage severity through a better understanding of why current control has variable success. Key to this are reliable estimates of slug activity, an increased knowledge of population dynamics and behavioural studies to assess the risk associated with control strategies presently used by farmers. These needs are reflected in the objectives of the project presented in this thesis.

1.3 Project Objectives

The work presented in this thesis forms part of a Sustainable Arable LINK (SAL) Project which is funded by the government (Department for the Environment, Food and Rural Affairs), the Home Grown Cereals Authority and industry (Bayer, Lonza, De Sangosse, and a number of farmers and consultants). The project commenced in September 2001 and is due to be completed in September 2005.

The overall aim of the SAL project is to devise a rational risk assessment system for the integrated control of slugs in arable crops, in particular winter wheat and oilseed rape (see factsheet, *Appendix A*). This was broken down into a number of broad primary objectives (*Table 1.1*).

Table 1.1: Primary Objectives of Sustainable Arable LINK Project.

Objective	*Description*
1	To assess and quantify the impact of key factors such as soil, weather and agronomic conditions, including the timing and method of pellet application, on control efficacy.
2	Quantify relationships between slug populations and conditions in the previous crop to evaluate their use as a damage indicator in the succeeding crop
3	Develop a trapping system that is a reliable predictor of crop damage
4	Predict the need for, and timing of, slug pellets as part of an integrated control strategy

A list of different study areas was compiled for each primary objective and these were divided between the three collaborating research institutions: ADAS, Rothamsted Research and the University of Newcastle upon Tyne. This list is extensive and only those areas assigned to the University of Newcastle upon Tyne are shown in *Table 1.2.* These are addressed in the studies comprising this Ph.D. thesis.

Table 1.2: Aspects of SAL Project assigned to the University of Newcastle upon Tyne.

To investigate egg hatch, growth and survival of *Deroceras reticulatum*
To investigate the relationship between weight and sexual maturity
To critically assess the effects of pellet application method and pellet condition upon slug activity
To characterise surface activity patterns of individual slugs on different seedbeds in the presence and absence of pellets
To assess the efficacy of refuge traps in estimating surface active slug populations

1.4 Thesis Outline

Following this general introduction (*Chapter 1*), data chapters in the thesis are presented in three sections based on the different aspects of slug biology and control that were studied. *Section A* is centred on the basic lifecycle parameters of *D. reticulatum*, including hatching, growth, development and maturity and incorporates *Chapters 2-5*. *Section B* focuses on control efficacy in relation to pellet application (*Chapters 6* and *7*) and *Section C* concerns population assessment using refuge traps (*Chapter 8*). Finally, the general discussion (*Chapter 9)* draws together the findings of the thesis as a whole and evaluates the contribution these can make to improving slug control strategies.

The individual chapters have been written with a view to publication and are laid out in paper format with standard headings (Abstract, Materials and Methods, Results, Discussion and Conclusions). The references for all chapters, however, appear in a single section at the end of the thesis.

Section A

Lifecycle of *Deroceras reticulatum* (Müller)

The chapters presented in this section investigate selected aspects of the hatching, growth, survival and sexual maturation of *D. reticulatum*. They are comprised of a continuum of laboratory based studies that follow the lifecycle of a cohort of slugs over three generations.

Generation one slugs hatched from eggs laid by field-collected adults and were therefore assumed to be cross-fertilised. They were reared in isolation and laid self-fertilised eggs that resulted in generation two. A small number of these second generation slugs, also reared in isolation, laid eggs which constituted generation three. These generation three eggs, however, did not hatch.

Chapters 2 and *3* focus on generation one. The former investigates various factors which may influence batch size, development time and hatching rate of eggs, while the latter follows up the subsequent growth and survival of these individuals.

Chapter 4 concerns generation two. The hatching, growth and survival of these self-fertilised slugs are reported and compared with the cross-fertilised generation one slugs.

Chapter 5 investigates the relationship between sexual maturity, age and weight. It is based on material from preserved generation one slugs which were dissected to ascertain the developmental state of the reproductive system.

Chapter 2

Factors Influencing the Batch Size, Development and Hatching Rate of *Deroceras reticulatum* (Müller) Eggs

Abstract

The effects of laying season (spring or autumn) and incubation temperature on early stages in the lifecycle of *Deroceras reticulatum* (Müller) were investigated. Eggs laid by wild-caught parents were kept at ambient temperature, constant 12°C or constant 15°C. Egg batch size was positively associated with parental weight in spring, but not in autumn. Batch size, but not laying season or incubation temperature, was a significant predictor of whether any of the eggs in a batch hatched. Development time was inversely related to incubation temperature and this was modified by the laying season. Hatching rate, however, did not vary with temperature or laying season, and the overall mean value (± S.E.) was 65.9 ± 5.8 %.

2.1 Introduction

Underpinning effective slug control in arable crops is the need to predict when and where particular increases in populations are likely to occur so that steps can be taken in advance to minimise the economic loss that would otherwise result (Schley & Bees, 2003).

Various models have been proposed in recent years to forecast changes in slug population dynamics as a consequence of environmental factors and their impact on growth and mortality (e.g. Bohan *et al.*, 1997; Shirley *et al.*, 2001; Choi *et al.*, 2004). Due to the complexity of slug behaviour and limited data on some aspects of their fundamental biology, however, these are often based on a number of assumptions about lifecycle parameters and have, to a greater or lesser extent, only been able to

predict confidently part of the overall picture. There is a need, therefore, for studies to bridge these gaps in the knowledge of *Deroceras reticulatum* biology so that models can give more accurate predictions.

The potential of a slug population to attain pest proportions is largely determined by its initial size and the speed with which it can complete its lifecycle whilst favourable conditions prevail. The initial population size in short-lived, annual species is effectively dependent upon the number of eggs laid, and how many of them develop and hatch to give rise to the following generation. A number of early studies have shown that temperature affects hatching, and that under natural conditions, hatching takes longer in autumn as eggs over-winter (e.g. Carrick, 1942; Arias & Crowell, 1963; Pinder, 1969). There is nothing published, however, that assesses the effect on development time and hatching rate of rearing eggs laid in different seasons at the same temperature; this may be an important first step in beginning to refine predictions to take into account the impact of unseasonable weather conditions on the next cohort, such as the exceptionally mild autumns or winters experienced in recent years. Furthermore, there is little that addresses the question of whether parental slug weight influences batch size. These aspects are investigated in the studies presented in this chapter.

Whilst this chapter is concerned with early stages in the lifecycle of *D. reticulatum*, focusing on the period from egg laying to hatching, the work forms part of an extensive study into the development of *D. reticulatum*. After hatching, individuals entered into the next stage of the study which assessed growth and survival rates (*Chapter 3*).

2.2 Materials and Methods

2.2.1 Materials

2.2.1.1 Eggs

The eggs used in each of the spring and autumn experiments were laid by 50 adult *D. reticulatum*. The slugs were collected from under refuge traps at Close House Field Station, Heddon-on-the-Wall, Northumberland (Grid reference NZ 127659). After collection slugs were weighed, using a Mettler MT5 balance, and placed in individual Petri dishes lined with moist laboratory tissue. They were fed with Chinese cabbage and carrot *ad libitum*. Cuttle fish bone was provided as a source of calcium. The slugs were maintained at a constant temperature of 20 ± 2°C (mean ± S.E.) in a Sanyo MIR-253 incubator with a constant photoperiod of 16L:8D and were cleaned weekly by transferring them to a clean dish with fresh food. Over a two week period a total of 60 egg batches were collected. Each batch was placed on fine grade netting and rinsed with distilled water to remove any soiling before being transferred into another Petri dish lined with moist laboratory tissue. The number of eggs per batch was recorded.

2.2.2 Methods

2.2.2.1 Experimental treatments

In each season the 60 egg batches were allocated equally and at random to three temperature treatments; two constant (12 ± 2°C and 15 ± 2°C) (mean ± S.E.) and one fluctuating (ambient). The mean ambient temperature (± S.E.) during the hatching period was 13.3 ± 0.1°C (range 4.3-24.1°C) in spring and 5.7 ± 0.1°C (range -3.0-21.8°C) in autumn. Constant temperatures were maintained in Sanyo MIR-235 incubators with a photoperiod of 16L:8D, provided by two fluorescent tubes (1 x 15 Watt and 1 x 13 Watt). The temperatures were monitored using Tinytalk® data loggers (Gemini Data Loggers, UK). For the ambient treatment Petri dishes containing egg batches were placed in a plastic tank and housed outside the Ridley Building, University of Newcastle upon Tyne. The temperature inside and outside

the tank was also recorded using a Tinytalk® data logger and there was no additional light (i.e. natural photoperiod).

2.2.2.2 Egg maintenance and hatching

Egg batches were prevented from drying out by remoistening with distilled water as required. Hatching was checked weekly. Regular monitoring continued until two full weeks had elapsed since the last slug hatched. On each occasion any offspring were removed from the Petri dishes and entered into the next stage of the study (*Chapter 3*). The development time and number of slugs hatching per batch were recorded.

2.2.3 Statistical analyses

Regression was used to analyse variables between which a cause-effect relationship was postulated; for binomial data binary logistic regression was applied, otherwise linear regression was used. Comparisons between regression lines were made according to the procedure detailed in Zar (1999).

Continuous data were tested for normality and transformed if necessary. Percentages were arcsine transformed. In all cases this resulted in parametric data which were then analysed using analysis of variance (ANOVA) followed by Tukey (equal variances assumed) or Dunnett (equal variances not assumed) post-hoc tests as appropriate.

2.3 Results

2.3.1 Relationship between parental weight and batch size

Parents laying eggs in spring were significantly heavier than those laying eggs in autumn (ANOVA: $F_{1,67} = 6.266$, $P < 0.001$). The mean parental weight (± S.E.) in spring was 585.43 ± 22.73 mg and in autumn it was 469.10 ± 18.31 mg. The mean

batch size did not differ between treatments either between or within seasons (*Table 2.1*).

Table 2.1: Results of two-way analysis of variance (ANOVA) to compare mean batch size of Deroceras reticulatum *eggs between incubation treatments within and between seasons.*

Factor	*df*	*SS*	*MS*	*F*	*P-value*
Temperature	2	61.22	30.61	0.43	*n.s*
Season	1	6.08	6.08	0.09	*n.s*
Interaction	2	33.95	16.98	0.24	*n.s*
Error	114	8155.75	71.54		

For eggs laid in spring there was a significant relationship between parental weight and both the size of the first batch laid (linear regression: $N = 31$, $R^2 = 0.280$, $P < 0.01$) and the mean batch size during the collection period (linear regression: $N = 31$, $R^2 = 0.142$, $P < 0.05$) (*Fig. 2.1 (a) & (b)*). Although the P-values are both below the critical 5% level, however, the R^2 value which indicates the amount of variation in one variable that is explained by the other, is relatively small. There was no significant relationship between parental weight and either the size of the first batch laid (linear regression: $N = 38$, $R^2 = 0.094$, *n.s.*) or the mean batch size during the collection period (linear regression: $N = 38$, $R^2 = 0.011$, *n.s.*) for the eggs laid in autumn (*Fig. 2.1 (c) & (d)*). In *Fig. 2.1*, the regression lines are shown for eggs laid in spring and were set to intercept at the origin since the number of eggs must equal zero when parental weight equals zero.

2.3.2 Relationship between hatching and batch size

The analyses presented in this section were confined to constant temperature treatments only since heavy rain in spring 2002 resulted in flooding of a number of ambient treatment batches and the loss of some eggs.

Batch size, laying season and incubation temperature were assessed as potential predictors of whether any of the eggs in a batch hatched. Batch size had a significant

influence; the larger the batch size the more likely it was that at least one of the eggs would hatch (Binary logistic regression: N = 76, Z = 2.65, $P < 0.01$, percentage concordant pairs = 78.5%) (*Fig. 2.2*). Specifically, the odds ratio of 1.18 indicated that the chances of at least one egg in a batch hatching increased 1.18 times with each successive single egg increase in batch size (i.e. approximately 20%). Incubation temperature and laying season had no effect.

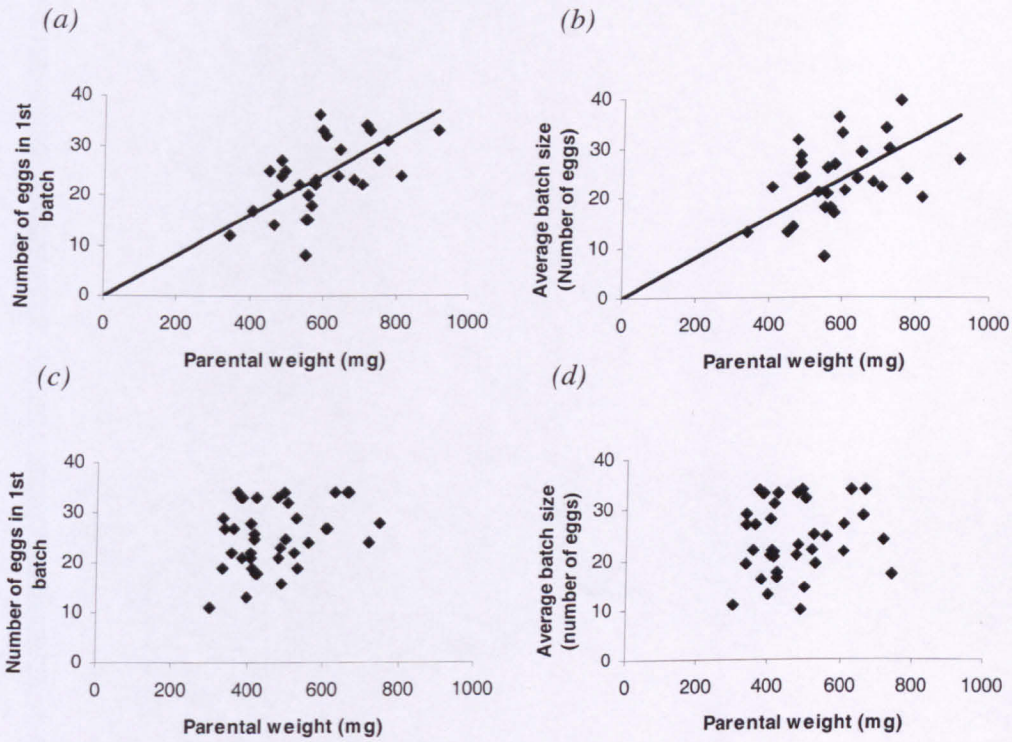


Figure 2.1: Scatterplots of the number of Deroceras reticulatum *eggs in the first batch and the mean batch size of eggs laid in spring (a) & (b) and autumn (c) & (d) against parental weight. Regression equation for (a): y = 0.040x; (b) y = 0.040x.*

The total number of eggs hatching was related to batch size in a linear fashion (*Fig. 2.3*). For every combination of laying season and incubation temperature the number of eggs hatching increased with batch size (linear regression: Spring: 15°C: N = 20, $R^2 = 0.61$, $P < 0.001$; 12°C: N = 20, $R^2 = 0.52$, $P < 0.001$; Autumn: 15°C: N = 20, $R^2 = 0.64$, $P < 0.001$; 12°C: N = 16, $R^2 = 0.36$, $P < 0.01$). Comparisons between the four regressions using analysis of covariance showed that there were no significant

differences in their slopes or elevations, i.e. the four regressions are coincident and, therefore, at least at the constant temperatures studied here, the number of eggs hatching per batch is unaffected by laying season or incubation temperature (ANCOVA: $F_{6,68} = 0.41$, *n.s.*). The regression lines were set to intercept at the origin since the number hatching must equal zero when batch size equals zero.

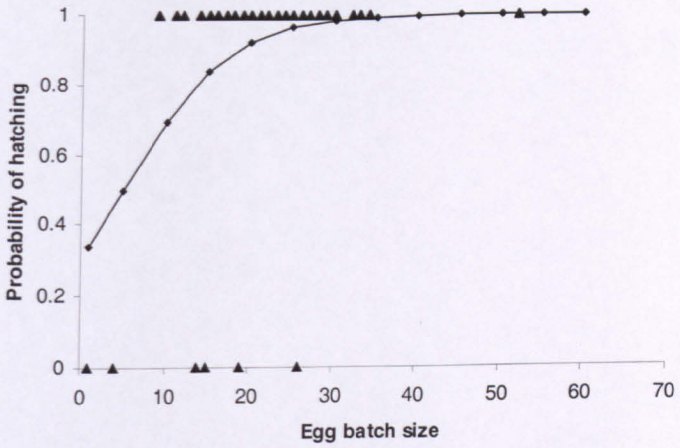


Figure 2.2: Results of binary logistic regression analysis to investigate the relationship between batch size and whether any eggs hatched for Deroceras reticulatum. *Binary logistic regression equation: Probability of hatching = (exp (-0.842 + 0.1659 x batch size))/(1 + exp (-0.842 + 0.1659 x batch size)). (Binary response indicated by black triangles; probability of hatching predicted by binary logistic regression indicated by solid line).*

For the purposes of comparison with eggs incubated at field temperatures, regression analysis was performed on the data from autumn laid eggs incubated at ambient temperature, which were not affected by flooding. The trend observed at constant temperatures was not confirmed; there was no significant relationship between batch size and the number of eggs hatching for autumn laid eggs reared at ambient temperature (linear regression: Autumn: ambient: $N = 20$, $R^2 = 0.08$, *n.s.*).

2.3.3 Influence of incubation temperature and laying season on development time

This analysis is based on data from all three incubation temperatures. Evidence suggests that *D. reticulatum* eggs can withstand complete immersion in water for a number of days with no effect on development (Arias & Crowell, 1963; Rollo & Shibata, 1991). It is therefore assumed that the brief period of flooding described in *section 2.3.2* did not have any detrimental effect on remaining eggs. Incubation temperature, laying season and an interaction between these two factors all influenced egg development time (*Table 2.2*).

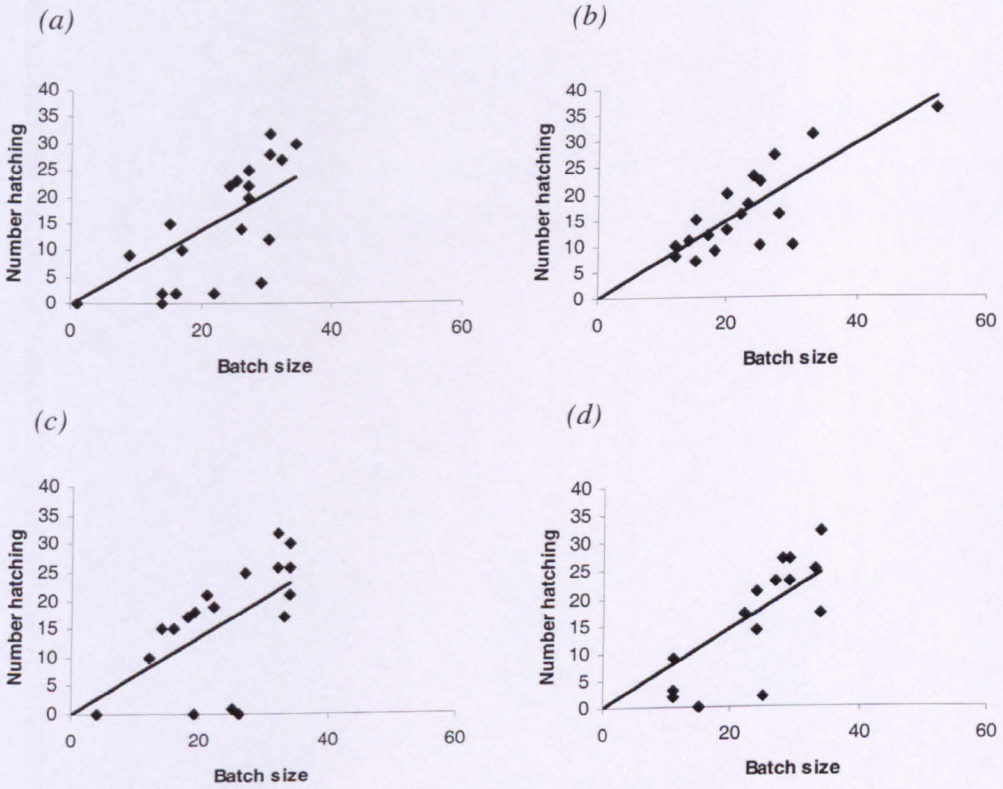


Figure 2.3: Scatterplots of the number of Deroceras reticulatum *eggs hatching and the batch size for eggs laid in spring at (a) 12°C & (b) 15°C and in autumn at (c)12°C & (d) 15°C. Regression equation for (a) y = 0.700x; (b) y = 0.738x; (c) y = 0.680x; (d) y = 0.723x.*

Table 2.2: Results of two-way analysis of variance (ANOVA) to compare the development time of Deroceras reticulatum *eggs at three incubation temperatures (ambient, 12°C and 15°C) in each of two seasons (spring and autumn).*

Factor	*df*	*SS*	*MS*	*F*	*P-value*
Temperature	2	1.35	0.67	148.47	*< 0.001*
Season	1	0.34	0.34	75.12	*< 0.001*
Interaction	2	0.43	0.21	47.10	*< 0.001*
Error	101	0.46	4.53×10^{-3}		

Inspection of the mean values for each combination of incubation temperature and laying season (*Fig. 2.4*) indicated that for eggs laid in spring those incubated at 15°C developed sooner than those at 12°C or ambient temperature. There was little difference between these latter two temperatures (mean spring ambient temperature during hatching (± S.E.) = 13.3 ± 0.1°C). The mean development time of eggs laid in autumn differed between all three incubation temperatures with hatching occurring sooner the higher the temperature (mean autumn ambient temperature during hatching (± S.E.) = 5.7 ± 0.1°C).

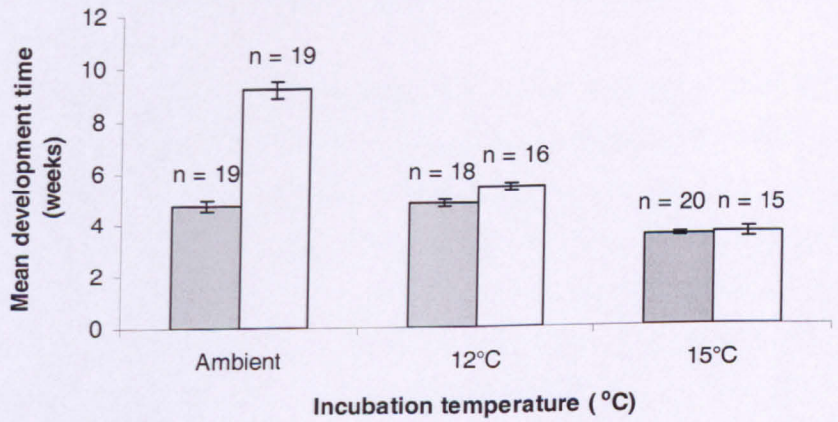


Figure 2.4: Mean development time for Deroceras reticulatum *eggs laid in spring and autumn, incubated at ambient temperature, 12°C and 15°C (± S.E.) (grey bars = spring; white bars = autumn).*

Since there is a much greater seasonal difference in development time at ambient temperature than at either constant temperature, the analysis was repeated for constant temperatures only, to see whether the interaction was still apparent *(Table 2.3)*. Although the significance level for season and the interaction term were reduced in this second analysis the overall results were unaltered. As there is virtually no seasonal difference in the development time at 15°C it can be inferred that the interaction is due mainly to the 12°C treatment for the constant incubation temperatures.

Table 2.3: Results of two-way analysis of variance (ANOVA) to compare the development time of Deroceras reticulatum *eggs between two constant incubation temperatures (12°C and 15°C) in each of two seasons (spring and autumn).*

Factor	*df*	*SS*	*MS*	*F*	*P-value*
Temperature	1	42.22	42.22	131.53	*< 0.001*
Season	1	2.03	2.03	6.32	*< 0.05*
Interaction	1	1.40	1.40	4.35	*< 0.05*
Error	65	20.86	0.32		

2.3.4 Influence of incubation temperature and laying season on hatching rate

Hatching rate is expressed as the percentage of the total egg batch that hatched. Since the fate of eggs washed away by flooding in the spring 2002 ambient treatment was unknown (*section 2.3.2*), these results pertain only to the constant incubation temperatures. *Table 2.4* shows the mean hatching rate (± S.E.) for each laying season and incubation temperature. There were no significant differences between treatments (*Table 2.5*). For the purposes of comparison the hatching rate of slugs laid in autumn and reared at ambient conditions, which were not affected by flooding, was 66.0 ± 7.11%. The results from constant incubation temperatures in the laboratory therefore give a good indication of what is observed at field temperatures.

Table 2.4: Mean hatching rate (± S.E.) of Deroceras reticulatum *eggs at each of two laying seasons and incubation temperatures.*

Laying Season	*Incubation Temperature (°C)*	*n*	*Mean Hatching Rate ± SE (%)*
Spring	12	20	60.0 ± 8.55
	15	20	74.5 ± 4.72
Autumn	12	20	65.1 ± 8.94
	15	16	63.6 ± 8.17

Table 2.5: Results of two-way analysis of variance (ANOVA) to compare the hatching rate of Deroceras reticulatum *eggs between two constant incubation temperatures (12°C and 15°C) in each of two seasons (spring and autumn).*

Factor	*df*	*SS*	*MS*	*F*	*P-value*
Temperature	1	462.08	462.08	0.65	*n.s*
Season	1	301.55	301.55	0.42	*n.s*
Interaction	1	698.06	698.06	0.98	*n.s*
Error	72	51153.12	710.46		

2.4 Discussion

2.4.1 Parental weight and batch size

It is difficult to study the relationship between parental weight and batch size in wild-caught slugs because we do not know their laying history prior to being brought into the laboratory. There is evidence from work on the snail, *Helix aspersa* (Müller), that the number of batches previously laid affects batch size (Madec *et al.*, 2000). These authors showed that for wild-caught individuals brought into the laboratory towards the end of hibernation there were significantly more eggs in the first batch laid than in subsequent batches. Since slugs do not hibernate we do not have a similar 'start-point' to indicate whether a batch is the first that an individual has laid, or another in a long sequence of batches. Furthermore, it has been reported in 'higher' molluscs that the nutritional status of the parent affects batch size (Steer *et al.*, 2004). This may also be of importance in slugs, but again, it is an unknown

factor in field collected specimens. The results presented here, therefore, need to be interpreted with caution particularly in view of the low, albeit significant, R^2 values. They are offered as a preliminary exploration of this relationship that could be investigated more comprehensively in future studies.

That there is a link between parental weight and number of eggs produced has been reported in a number of species, for example in the molluscs *Loligo vulgaris* (Lamarck) (Coelho *et al.*, 1994) and *Helix aspersa* (Madec & Daguzan, 1993) as well as crustaceans (Runge, 1984; Austin, 1998) and fish (Coward & Bromage, 1999). In the studies detailed here both the mean batch size for a given parent during the collection period and the size of the first batch laid in the laboratory were positively associated with parental weight of slugs collected in spring, but not in autumn. There are a number of potential explanations for this observation. Firstly, slugs laying eggs in spring have over-wintered as eggs or juveniles themselves and consequently have developed slowly over a longer period of time than those laying eggs in autumn which probably hatched in spring of the same year and reached maturity more rapidly. This difference in development time may result in spring laying parents being a more homogeneous group than the autumn parents, with a more stable reproductive physiology. A study using *Helix aspersa* found that parents with slower growth contributed more to offspring production than faster growing parents and suggest that this may be due to differences in reproductive ability (Dupont-Nivet *et al.*, 2000). This may also be the case for *D. reticulatum.*

A second explanation could be that this seasonal difference is related to temperature conditions experienced by slugs prior to being brought into the laboratory. Under natural conditions slugs laying eggs in spring have been adapting to gradually rising temperatures following winter. Although the incubation temperature used in these experiments is, at 20°C, higher than ambient conditions for spring in the North East of England, it is in the same direction that the slugs have been acclimatising to, i.e. warmer than in winter. Slugs laying eggs in autumn would, conversely, have been acclimatising to gradually cooler temperatures towards winter and the incubation temperature is, therefore, in the opposite direction to natural conditions for the time of year. Perhaps this resulted in 'thermal shock' which disrupted egg laying. It has

been shown that the mean supercooling point of *D. reticulatum* rises in the winter, i.e. slugs freeze at higher temperatures in the winter, although they are able to survive this freezing for almost twice as long compared to the summer (Cook, 2004). Evidently there is a shift in thermal tolerance during autumn and slugs may be more sensitive to unseasonably high temperatures at this time of transition.

A third possibility is that the lack of an association between batch size and parental weight in autumn is due to egg laying becoming less coordinated towards the end of the reproductive period. By the time the parents were collected from the field in autumn they may have been near the end of their peak in egg production whereas those collected in spring were more likely to be at the start of this process. This may explain why the association was stronger earlier in the year.

2.4.2 Hatching and batch size

There is a scarcity of studies in the literature that investigate the relationship between hatching and batch size. In the experiments presented here, the larger the batch, the greater was the chance that at least one of the eggs would hatch. The incremental rise in the odds of hatching with each additional egg in the batch seemed, initially, to be relatively high at just under 20%. There is nothing in the literature, however, that details the baseline chance of hatching, i.e. the absolute probability that a single egg will hatch. This increase in the odds of hatching may actually be 20% of a very small number. Unfortunately, it was not possible to estimate this from the current study since there was only one batch where a single egg was laid.

The relationship between number of eggs hatching and batch size was more or less linear at constant incubation temperatures, which follows from the previous result. The comparison of regression lines for this relationship indicated that, at least at 12°C and 15°C, the number of eggs hatching was unaffected by the laying season or rearing temperature. This supports the previous analysis of factors influencing whether or not at least one egg in a batch hatches; laying season and incubation temperature were found to have no effect here either.

Interestingly, however, for the eggs laid in autumn and incubated at ambient temperature, there was no relationship between batch size and the number of eggs hatching. There were no significant differences in batch size between temperature treatments and since egg batches were allocated to the treatments randomly any differences in egg quality would be expected to affect hatching equally. It seems improbable that any insulation effect, with eggs in the middle of batches being more protected from very cold temperatures by eggs on the edges of the batches, would be strong enough to alter hatching dynamics either; temperature differences would not be great enough. A more likely explanation of this difference between constant and field temperatures is that the assessment period favoured the former. Assessment ceased when two full weeks had elapsed since the last egg hatched. This period was chosen for reasons of practicality as the next stage of the experiment was underway and was very labour intensive (*Chapter 3*). The results from the study of development times, however, show that this is faster and there is less variation at constant incubation temperatures, i.e. the standard errors are smaller (*section 2.3.3*). If hatching had been monitored for longer than two weeks after the last slug hatched then perhaps continued sporadic hatching would have been observed at ambient conditions and a pattern between number of eggs hatching and batch size similar to that observed at the constant temperatures may have been apparent.

2.4.3 Development time

It was observed that egg development time was generally inversely related to temperature. This is in agreement with the findings from other studies (e.g. Carrick, 1942; Judge, 1972; South, 1989b; Rollo & Shibata, 1991). It was shown by Rollo and Shibata (1991) that the development time for *Deroceras laeve* (Müller) eggs was determined by the mean of fluctuating temperature regimes rather than the magnitude of the temperature range. This could explain why the development times for *D. reticulatum* in the spring experiments presented here did not differ significantly between the ambient and the constant 12°C treatments. Although the mean ambient spring temperature of 13.3 ± 0.1°C was slightly higher than the constant 12°C this was not sufficient to result in a significant difference in development time between these treatments. Development times at both of these temperatures, however, were

longer than at 15°C. In autumn, mean ambient temperature during the development period was considerably lower than either constant temperature, at 5.7 ± 0.1°C and the inverse relationship between temperature and development time was clear (*Fig. 2.4*).

The effect of season on development time was very interesting. The ambient treatments effectively acted as a control to confirm that eggs were developing normally for the given time of year. The development time here was, as expected, more prolonged in cooler autumn conditions compared to spring. The contrast between seasonal development times at each of the constant temperatures was, however, surprising. If temperature was the main factor influencing development time, given uniform moisture, humidity and day light regime, then we would expect that it would take the same length of time at a given constant temperature, regardless of the laying season, i.e. we would expect day-degrees to determine the development time. There was, however, a significant interaction between temperature and season for constant temperature treatments, meaning that the development times at a given temperature are not the same in both seasons, i.e. day-degrees alone do not fully account for differences in development times. The results indicate that at 15°C the development times were almost the same in spring and autumn (*Fig. 2.4*) implying that the interaction is due mainly to the 12°C treatment. This suggests that there may be a temperature threshold below which laying season influences development time, but above which the increased temperature 'over-rides' the effect of season. These findings are supported by a study of the freshwater snail, *Lymnaea auricularia* (L.), where differences were observed in the development time of spring and summer laid eggs incubated at the same constant temperatures, although a threshold was not postulated (Salih *et al.*, 1981).

In the North East, 12°C corresponds to a warm spring/cool autumn whereas 15°C would be an above-average autumn temperature. Perhaps autumn laid eggs have something inherently different about them that means development is delayed unless temperatures are consistently above the threshold. The adaptive significance of this is that development of autumn laid eggs would not be accelerated as a result of brief periods of mild weather following which a return to progressively cooler conditions

might cause considerable mortality among hatchlings. To explore this idea further development times of *D. reticulatum* eggs at temperatures in between 12°C and 15°C, along with switches between temperatures, could be studied to see whether it is possible to pinpoint more accurately a putative 'threshold' value. It would also be necessary to repeat the experiment at temperatures above 15°C to confirm that season continues to have no effect on development time.

2.4.4 Hatching rate

Temperature and laying season had no effect on hatching rate under the conditions tested in these experiments. The overall mean hatching rate was 65.9 ± 5.8 %. Although Judge (1972) found similar hatching rates for *D. reticulatum* at temperatures within the range examined here, he found that at considerably higher incubation temperatures the hatching rate decreased, for example, to 20-37% at 27°C. Such high temperatures would not normally be reached during the peak period of egg laying in the UK. Furthermore, *D. reticulatum* usually lays eggs in crevices in the soil or under organic matter on the soil surface such as leaves or logs and they are therefore likely to be buffered from extremes of temperature to a certain extent. It would seem, therefore, that under natural temperature conditions experienced around peak egg-laying, hatching rate is reasonably uniform. For the 'background' level of egg laying that continues throughout the year in this species, however, particularly in summer when short-term temperatures can approach Judge's experimental conditions, this may have a significant effect on percentage hatching. That temperature in lower ranges has no effect on hatching rate is supported by work on a related slug species, *Deroceras panormitanum* (Lessona and Pollenera), a pest of hardy nursery stock (Schüder, 2004). Schüder found that slug eggs incubated at 12°C, 15°C and 20°C hatched at similar rates, although the percentage hatching, at 84-89%, was higher than observed in *D. reticulatum*. This may be because *D. panormitanum*, which was originally a Mediterranean species (Kerney & Cameron, 1979), is well adapted to the warmer and less variable ambient conditions found in horticultural greenhouses.

2.4.5 Implications for control

In addition to providing useful insights into the basic biology of *D. reticulatum*, the results of these experiments also have a practical application in informing aspects of its control. In an ideal control strategy, a solid understanding of the pest's biology underpins confident predictions of how it will respond to environmental changes such as annual weather fluctuations leading to effective prevention of economically important levels of damage.

In a highly adaptable generalist herbivore, such as *D. reticulatum*, this ideal is not easily attained. By an improved appreciation of how factors like season and temperature, acting independently or in combination, impact on the early stages of its lifecycle, population dynamics models can be refined. The more reliable the input of such models, the more confidence we can have in their later predictions. Although the results in this chapter require further testing before they can usefully help to parameterise models they indicate areas where adjustments could be valuable.

2.5 Conclusions and Future Work

The data presented in this chapter were collected as part of an extensive study of the developmental biology of *D. reticulatum* under different conditions. The investigations described were necessarily restricted in scope by the need to continue later stages of the study. It is, therefore, acknowledged that data collection may have been carried out in different ways had the work been done in isolation; for example, hatching may have been monitored daily and a wider range of temperatures could have been investigated. Nevertheless, the results provide support for other studies in this area of slug biology and indicate some avenues for future research.

In conclusion it was found that:

1. Laying season influenced the association between parental weight and batch size and also modified the relationship between incubation temperature and development time.

2. Incubation temperature was inversely related to development time.
3. Batch size was a significant predictor of whether any of the eggs in a batch hatched.
4. Hatching rate did not vary with temperature or laying season.

As noted in the discussion, there are a number of ways in which this work could be expanded in future studies. Repeating the experiments at a wider range of temperatures would help to provide a more complete picture of early stages in the lifecycle by showing whether trends at the temperatures tested here are maintained over a wider range. Whilst temperature effects on development and hatching of slug species are relatively widely reported in the literature there is a marked scarcity of studies into seasonal effects on these processes, or indeed combined effects of temperature and season. This would be an obvious area to develop. It would allow further investigation of the idea that there may be a threshold above which laying season fails to influence development time. In addition, it would be valuable to study in more detail the influence of parental weight on batch size. If this were to be a strong relationship when tested more stringently, in combination with the results on batch size, hatching and development times it could be a very useful 'rough and ready' guide as to the potential population size in the following year.

Chapter 3
Seasonal and Temperature Effects on the Growth Rate and Survival of *Deroceras reticulatum* (Müller)

Abstract

The growth and survival of *Deroceras reticulatum* (Müller) hatching in different seasons (spring and autumn), but reared at the same temperatures (ambient, 12°C and 15°C) were investigated. There was considerable variation in growth under identical conditions. Growth was influenced by hatching season at all rearing temperatures; at ambient temperature it was faster in spring, but at the two constant temperatures it was faster in autumn. The association between temperature and growth was negative in spring and positive in autumn. Survival was influenced by hatching season at ambient temperature and 15°C, but not 12°C; at ambient temperature slugs survived longer in autumn than spring whereas at 15°C they survived longer in spring than autumn. Within a season survival differed between ambient and each constant rearing temperature, but was similar between the two constant temperatures; in spring ambient survival was lower than at 12°C and 15°C, but in autumn it was higher.

3.1 Introduction

It is well-established that temperature influences many aspects of the biology of terrestrial slugs, including growth rate (e.g. Runham & Hunter, 1970; Godan, 1983; South, 1992). For *Deroceras reticulatum* most studies have shown that the relationship between growth rate and temperature is approximately hyperbolic; there is a positive association up to an optimum of 17-19°C after which higher temperatures have a detrimental effect on development (e.g. Dainton, 1954a; Arias & Crowell, 1963; Dmitrieva, 1969; South, 1982). An exception to this is the work of Judge (1972) whose data indicate that growth is faster at cooler temperatures. All of

these studies are based on slugs hatching at one particular point in time, i.e. a single season, although none actually state which season this is.

D. reticulatum is capable of breeding throughout the year if conditions are favourable, but there are peaks in spring and autumn (Bett, 1960; Hunter, 1968b; Runham & Laryea, 1968). The autumn population have to over-winter either as eggs or recently hatched juveniles and it is postulated that there may be something inherently different in the physiology of these slugs compared to the spring population that adapts them for development and survival at lower seasonal temperatures. If so, it might be expected that the growth trajectories of spring and autumn hatching slugs will differ when reared under identical conditions. This may explain the discrepancy between the work of Judge (1972) and other authors.

There are no published data that directly assess the effect of hatching season on slug growth. The experiments presented in this chapter were, therefore, designed to investigate this. The growth trajectories of slugs hatching in spring and autumn were compared at three rearing temperatures, ambient, 12°C and 15°C, along with their survival. It was also possible to contrast growth and survival between temperatures within a given season to see how trends compare with published studies.

These experiments continue the work described in *Chapter 2* on factors affecting development and hatching rate. Similarly, in addition to finding out more about the biology of *D. reticulatum*, the results of this chapter may be of practical application in refining population dynamics models used in risk assessments for the control of slugs in arable crops.

3.2 Materials and Methods

3.2.1 Materials

3.2.1.1 Slugs

Newly hatched *D. reticulatum* from the eggs studied and described in *Chapter 2* were used in these experiments. The eggs had been laid in the laboratory by field collected adults in spring or autumn 2002 and were incubated at one of three temperatures (ambient, 12°C or 15°C).

3.2.1.2 Food

Slugs were fed on a mixed diet of Chinese cabbage and carrot. Cuttlefish bone was provided as a source of calcium.

3.2.2 Methods

3.2.2.1 Experimental treatments

The experimental treatments described in *Chapter 2* (*section 2.2.2.1*) were maintained in this second phase of the study, i.e. in each of two hatching seasons (spring or autumn) slugs were reared at one of three temperatures, ambient (fluctuating), 12°C or 15°C (both constant), corresponding to the temperature at which they hatched. Methods of temperature control and photoperiod remained unchanged. At each of the three rearing temperatures for a given season a total of 200 individuals were initially monitored. Due to very low mortality rates in all treatments, this number was subsequently reduced to 100 individuals per treatment in order to make the experiment more manageable. Throughout the experiment slugs were handled using a square-ended paintbrush.

3.2.2.2 Culturing procedure

Hatchlings were gently removed from the Petri dishes in which egg batches had been incubated. Each individual was placed into a separate 9 cm diameter Petri dish lined with blue laboratory tissue moistened with distilled water. Food was provided *ad libitum*. The slug was then returned to the temperature treatment at which it hatched.

Dishes were cleaned weekly when food was replaced. During cleaning the slug was transferred to the lid of the Petri dish. The moist laboratory tissue was then replaced and any soiling was wiped from the surfaces of the dish. Fresh food was added and the slug was transferred back into the dish. Mortality was recorded weekly.

3.2.2.3 Weighing regime

Slugs were weighed at hatching (week 0) and fortnightly thereafter using a Mettler MT 5 balance to an accuracy of 0.01 mg, allowing a brief settling period for the reading to stabilise. A small plastic dish (3.5 cm diameter) was used as a weighing receptacle and the balance was tared to zero before the slug was added. Slugs were transferred from the Petri dish to the weighing receptacle as quickly as possible in order to minimise the amount of mucus lost in weighing.

3.2.2.4 Monitoring period

Slugs hatching from eggs laid in spring were monitored for a total of 20 weeks, by which time autumn laid eggs began hatching and it was not practicable to maintain both sets of slugs simultaneously. It was, however, possible to monitor the autumn hatching slugs for a longer period as there were no further individuals entering the experiment. This set was monitored for a total of 34 weeks during which time some began to lay eggs. If batches of five or more were laid they were collected for the next stage of the experiment (*Chapter 4*) and the monitoring of the individual that laid them ceased.

3.2.2.5 Preservation of slugs

When monitoring ceased, slugs were kept for future dissection in the final part of the experiment (*Chapter 5*). They were individually preserved in 70% ethanol in glass tubes measuring 5 x 1.2 cm. The ethanol was changed two weeks after slugs were first preserved to ensure that it did not become diluted by diffusion of body fluids.

3.2.3 Statistical analyses

All comparisons of growth and survival between spring and autumn hatching slugs are based on weeks 0-20, since this is the period of time observed that is common to

slugs from both hatching seasons (*sections 3.3.1* and *3.3.2*). Additional analyses on the longer observation period for autumn alone concern data from weeks 0-34 (*section 3.3.3*).

All analyses exclude data from individuals removed from the experiment when numbers were reduced to 100 slugs per treatment. For those analyses concerning growth, results relate to the subset of slugs that were alive for the full monitoring period only.

Continuous weight data were analysed using a repeated measures analysis of variance (ANOVA). To account for the non-parametric nature of the data, as confirmed by Mauchley's test of sphericity and Box's M-test, the ANOVA was adjusted by applying the lower-bound epsilon correction. Tukey post-hoc tests were carried out as appropriate.

Survival data were analysed with the Kaplan-Meier procedure, using the Breslow test to compare between treatments. Hazard ratios were calculated.

3.3 Results

3.3.1 Growth: spring and autumn comparisons (weeks 0-20)

Table 3.1 shows the numbers of slugs alive for the full 20 week monitoring period in each treatment. All analyses in *section 3.3.1* are based on these slugs.

Table 3.1: Numbers of Deroceras reticulatum *alive for the full 20 week monitoring period in each experimental treatment.*

Hatching	*Rearing Temperature (°C)*			
Season	*Ambient*	*12*	*15*	*Total*
Spring	60	97	94	251
Autumn	94	97	96	287
Total	154	194	190	538

The mean (± S.E.) ambient temperature during the 0-20 week growth period was 12.7 ± 0.1°C (absolute range: 1.2-24.1°C; mean daily range: ± 4.9°C) in spring and 5.6 ± 0.1°C (absolute range:-3.0-14.0°C; mean daily range: 2.8°C) in autumn.

3.3.1.1 Growth rate variation

Considerable variation in weight was observed in all treatments, even within groups of slugs reared under identical conditions. An example of this is illustrated in *Fig. 3.1* which shows two spring hatching slugs of the same age reared under identical conditions (15°C). Taking into account this within treatment variation, there were still significant differences in growth between treatments. These are described in *sections 3.3.1.2* and *3.3.1.3.*

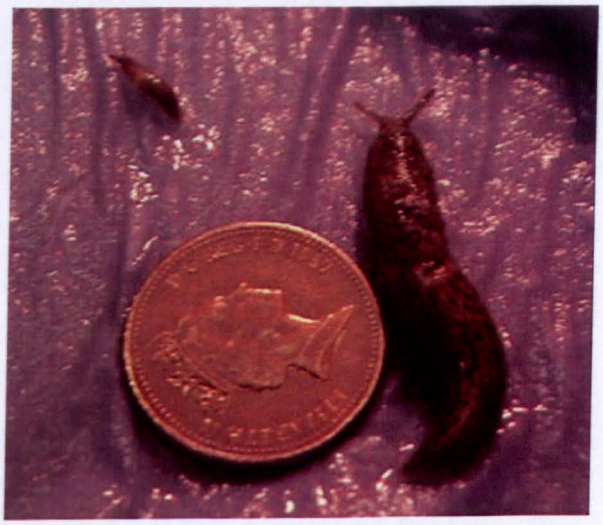


Figure 3.1: Weight variation in Deroceras reticulatum *hatching in spring and reared at 15°C. Both slugs are 20 weeks of age. Scale is indicated by a 1 pence piece.*

3.3.1.2 Seasonal effects on growth

At each of the three rearing temperatures the hatching season had a significant effect on growth rate (*Table 3.2*).

Table 3.2: Results of repeated measures analysis of variance (ANOVA) to compare the growth rate of Deroceras reticulatum *hatching in spring and autumn, reared at ambient temperature, 12°C or 15°C (weeks 0-20).*

Rearing Temperature (°C)	*N*	*df*	*F*	*P-value*
Ambient	154	1	12.964	*< 0.001*
12	194	1	316.249	*< 0.001*
15	190	1	419.232	*< 0.001*

At all rearing temperatures the growth rate from hatching until week 4-5 was similar in both seasons, but then began to diverge, and this became more marked with time. At ambient temperature slugs hatching in spring grew faster than those hatching in autumn for most of the 20 week monitoring period, although those hatching in autumn began to catch up and overtake in weeks 19-20. At the two constant temperatures, however, the reverse was observed; growth was considerably faster for autumn hatching slugs throughout the monitoring period. By week 20, autumn hatching slugs were on average four times larger than spring hatching slugs at 12°C, rising to eight times larger at 15°C (*Fig. 3.2 (a)-(c)*).

The absolute differences in mean weight between hatching seasons at week 20 were greatest for slugs reared at 15°C and smallest for those reared under ambient conditions (*Table 3.3*).

Table 3.3: Mean weight (± S.E.) (mg) at week 20 of Deroceras reticulatum *hatching in spring and autumn, reared at ambient temperature, 12°C or 15°C.*

Hatching Season	*Rearing Temperature (°C)*		
	Ambient	*12*	*15*
Spring	170.05 ± 13.62	163.04 ± 16.64	121.55 ± 12.53
Autumn	180.37 ± 7.41	671.56 ± 25.52	1002.58 ± 45.00

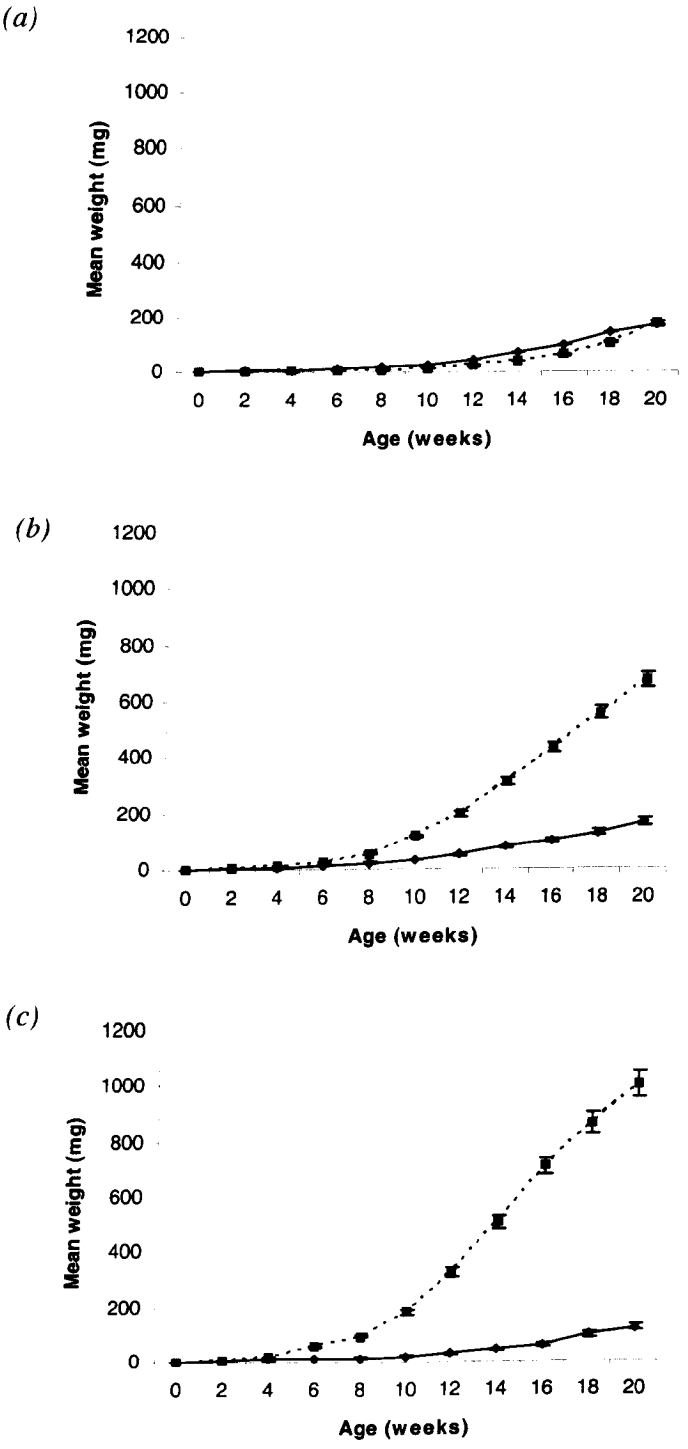


Figure 3.2: Mean weight (± S.E.) (mg) of Deroceras reticulatum *reared at (a) ambient temperature, (b) 12°C and (c) 15°C in spring (solid line) and autumn (dotted line).*

3.3.1.3 Temperature effects on growth

Further inspection of *Table 3.3* shows that the marked differences in growth between seasons at a given rearing temperature are also apparent between some rearing temperatures in a single season, most notably in autumn. There are opposite trends between the seasons in the rearing temperatures at which maximum and minimum mean growth is reached; in spring the highest mean weight is reached in ambient conditions and the lowest at 15°C whereas in autumn the reverse is observed.

Within a given season growth rate was significantly influenced by rearing temperature (ANOVA: spring: $F_{2, 248} = 7.333$, $P < 0.01$; autumn: $F_{2, 284} = 241.343$, $P < 0.001$). Tukey post-hoc tests showed that in spring growth at 15°C was significantly slower than at 12°C ($P < 0.01$) and ambient conditions ($P < 0.01$), but the differences between these latter two temperatures did not reach statistical significance. Indeed, it can be seen that there is a very close correspondence in growth at 12°C and ambient temperature throughout the monitoring period (*Fig. 3.3*). In contrast, autumn hatching slugs reared at 15°C grew considerably faster than at both of the other two rearing temperatures; those at ambient conditions grew slowest. The differences between all three rearing temperatures were significant at $P < 0.001$ (*Fig. 3.4*).

Note: Figs. 3.3 and 3.4 are shown on the same scale for the purposes of comparison. To view Fig. 3.3 on an expanded scale refer to Appendix B.

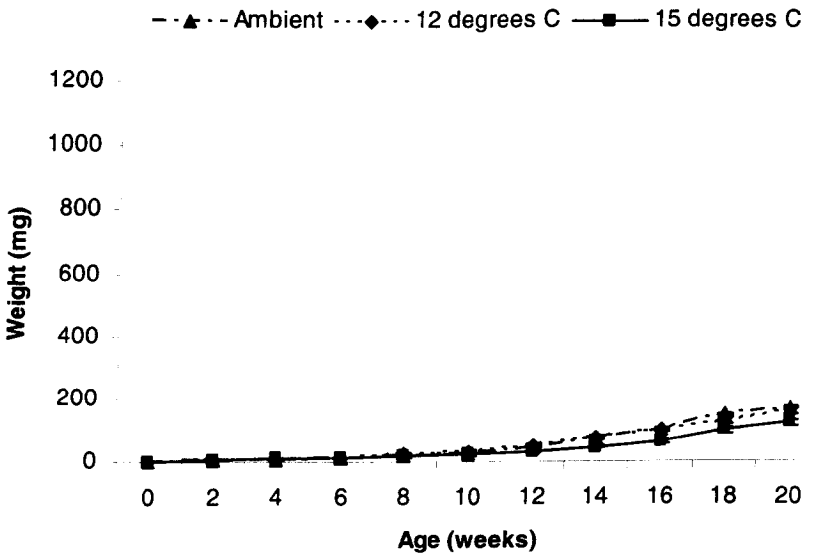


Figure 3.3: Mean weight (± S.E.) (mg) of Deroceras reticulatum *hatching in spring and reared at ambient temperature, 12°C or 15°C (weeks 0-20).*

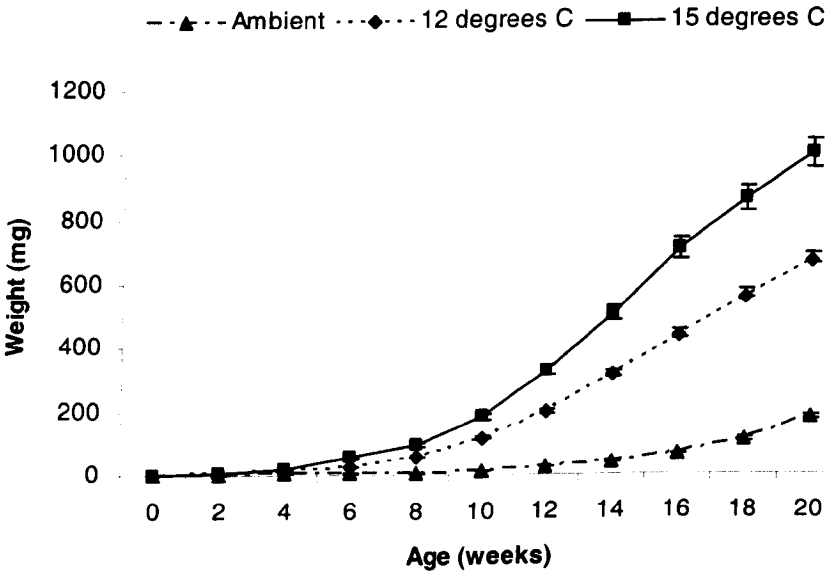


Figure 3.4: Mean weight (± S.E.) (mg) of Deroceras reticulatum *hatching in autumn and reared at ambient temperature, 12°C or 15°C (weeks 0-20).*

3.3.2 Survival: spring and autumn comparisons (weeks 0-20)

Table 3.4 shows the numbers of slugs in the experiment up to week 20, excluding those that were removed when numbers were reduced to 100 per treatment. All analyses in *section 3.3.2* are based on these slugs.

Table 3.4: Numbers of Deroceras reticulatum *in the experiment up to week 20, excluding those that were removed when numbers were reduced to 100 per treatment.*

Hatching	*Rearing Temperature (°C)*			
Season	*Ambient*	*12*	*15*	*Total*
Spring	141	116	114	371
Autumn	105	121	135	361
Total	246	237	249	732

The Kaplan-Meier procedure was used to analyse survival. In this procedure the cumulative chance of survival between each time interval, *S(t)*, is calculated. This value is called the Kaplan-Meier estimator and it is plotted against survival time to give a classic survival curve known as the Kaplan-Meier estimator plot such as in *Figs. 3.5 & 3.6.* Treatments are compared using the Breslow test.

3.3.2.1 Seasonal differences in survival

Fig. 3.5 (a)-(c) compares seasonal differences in survival at each of the three rearing temperatures. The survival rate differed significantly between spring and autumn hatching slugs reared at ambient temperature and 15°C, but not for those reared at 12°C (Kaplan-Meier (Breslow test): Ambient: N = 246, df = 1, $\chi^2 = 49.33$, $P < 0.001$; 12°C: N = 237, df = 1, $\chi^2 = 0.63$, *n.s.*; 15°C: N = 249, df = 1, $\chi^2 = 5.06$, $P < 0.05$). *Table 3.5* shows the mean survival times of slugs at each combination of hatching season and rearing temperature.

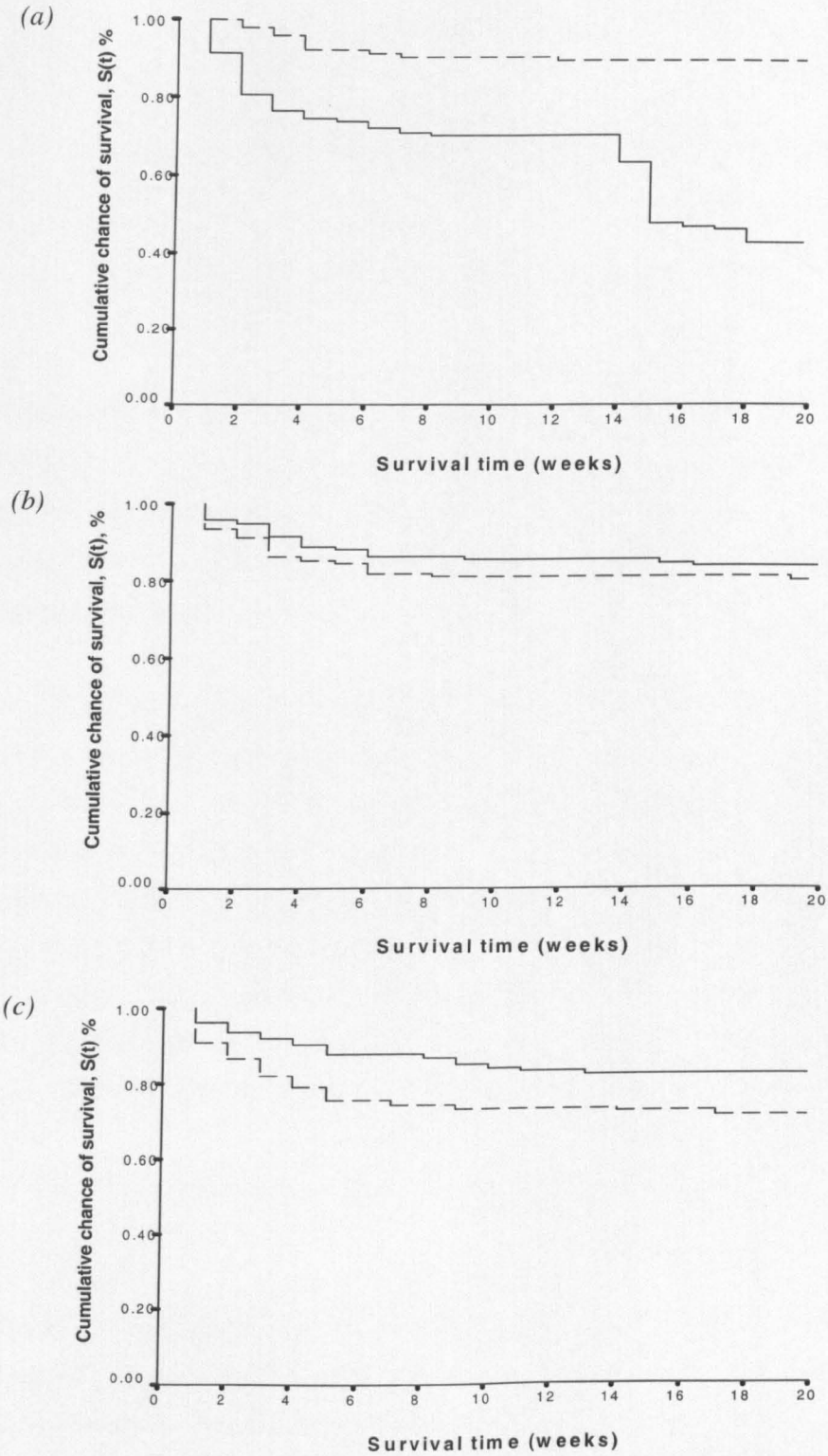


Figure 3.5:Kaplan-Meier estimators for Deroceras reticulatum *reared at (a) ambient temperature, (b) 12°C and (c) 15°C in spring (solid line) and autumn (dashed line).*

Table 3.5: Mean survival times (± S.E.) (weeks) for Deroceras reticulatum *hatching in spring and autumn and reared at ambient temperature, 12°C or 15°C (weeks 0-20).*

Hatching Season	*Rearing Temperature (°C)*		
	Ambient	*12*	*15*
Spring	13.50 ± 0.63	17.48 ± 0.55	17.36 ± 0.55
Autumn	18.39 ± 0.47	16.74 ± 0.61	15.36 ± 0.65

Hazard ratios indicate how much the survival rate differs between two groups of individuals. At ambient conditions, slugs hatching in spring had lower survival rates than those hatching in autumn (hazard ratio = 0.13), whereas the converse was seen in slugs reared at 15°C; survival was greater for spring hatching than autumn hatching slugs (hazard ratio = 1.83).

3.3.2.2 Temperature differences in survival

Within a given hatching season survival rates between the two constant rearing temperatures did not differ significantly. There were, however, significant differences in survival of slugs reared at each of these temperatures and ambient conditions (*Table 3.6*). For spring hatching slugs, those reared at ambient temperature had a lower survival rate than at 12°C (hazard ratio = 0.21) and 15°C (hazard ratio = 0.23) whereas for autumn hatching slugs the reverse was observed; those reared at ambient temperatures had a higher survival rate than at 12°C (hazard ratio = 2.07) and 15°C (hazard ratio = 3.20) (*Fig. 3.6 (a) & (b)*).

3.3.3 Growth and survival: autumn hatching slugs (weeks 0-34)

3.3.3.1 Growth

At the end of the longer monitoring period for autumn hatching slugs, the number of individuals per treatment that were alive for the entire 34 weeks was considerably less compared with those alive for the 20 week period which was contrasted with spring hatching slugs. This was particularly so in the constant temperature treatments (ambient: n = 67; 12°C: n = 18; 15°C: n = 13) and is due to the removal

from the experiment of slugs laying batches of five or more eggs, in addition to natural mortality; slugs began to lay eggs much sooner under constant conditions. Consequently, statistical analysis of this data needs to be interpreted with extreme caution as the test is highly unbalanced. The results in this section are therefore exploratory in nature and to confirm initial trends further experiments would be required.

Table 3.6: Results of Kaplan-Meier survival analysis (with Breslow test) to compare differences in survival rates between Deroceras reticulatum *hatching in the same season (spring or autumn) and reared at different temperatures (ambient, 12°C or 15°C).*

Laying season	*Pairwise comparison between rearing temperatures (°C)*	*N*	*df*	*Breslow statistic ($\chi 2$)*	*P-value*
Spring	Ambient vs 15°C	255	1	32.82	*< 0.001*
	Ambient vs 12°C	257	1	36.54	*< 0.001*
	12°C vs 15°C	230	1	0.04	*n.s.*
Autumn	Ambient vs 15°C	240	1	12.96	*< 0.001*
	Ambient vs 12°C	226	1	4.35	*< 0.05*
	12°C vs 15°C	256	1	2.59	*n.s.*

(a)

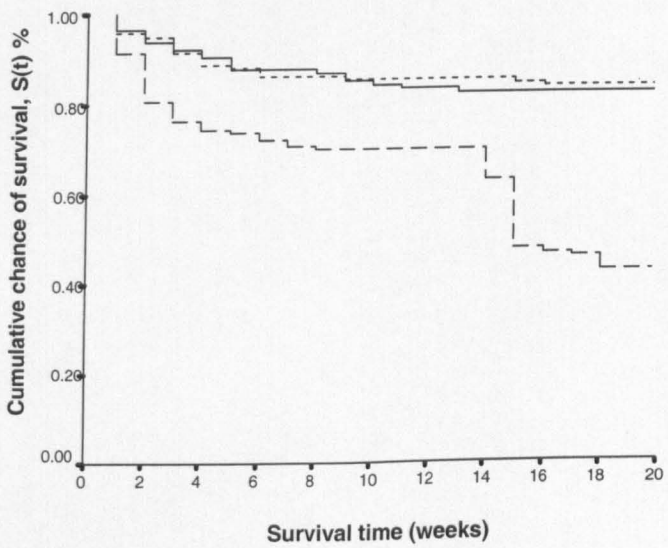


Figure 3.6: (a) Kaplan-Meier estimators for Deroceras reticulatum *hatching in spring and reared at ambient temperature (dashed line), 12°C (dotted line) or 15°C (solid line).*

(b)

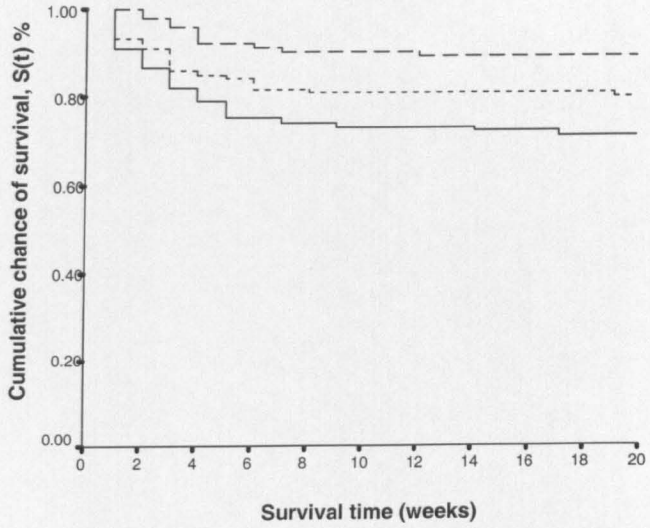


Figure 3.6 (cont../): (b) Kaplan-Meier estimators for Deroceras reticulatum *hatching in autumn and reared at ambient temperature (dashed line), 12°C (dotted line) or 15°C (solid line).*

Over the full 34 week observation period rearing temperature was found to have no effect on growth (ANOVA: $F_{2, 95} = 1.844$, *n.s.*). Contrasting the mean weights of these slugs (*Fig. 3.7*) with autumn hatching slugs alive for 20 weeks, as described previously (*Fig. 3.4*), indicates that the mean weights are lower at all rearing temperatures in the 34 week group. This is observed on a week by week basis, but becomes particularly marked with increasing time.

Fig. 3.7 shows that slugs reared at ambient conditions eventually 'caught-up' with and exceeded the growth rate of slugs reared at constant temperatures. This 'overtaking' by ambient reared slugs occurred at week 26 for slugs at 12°C and week 29 for those reared at 15°C.

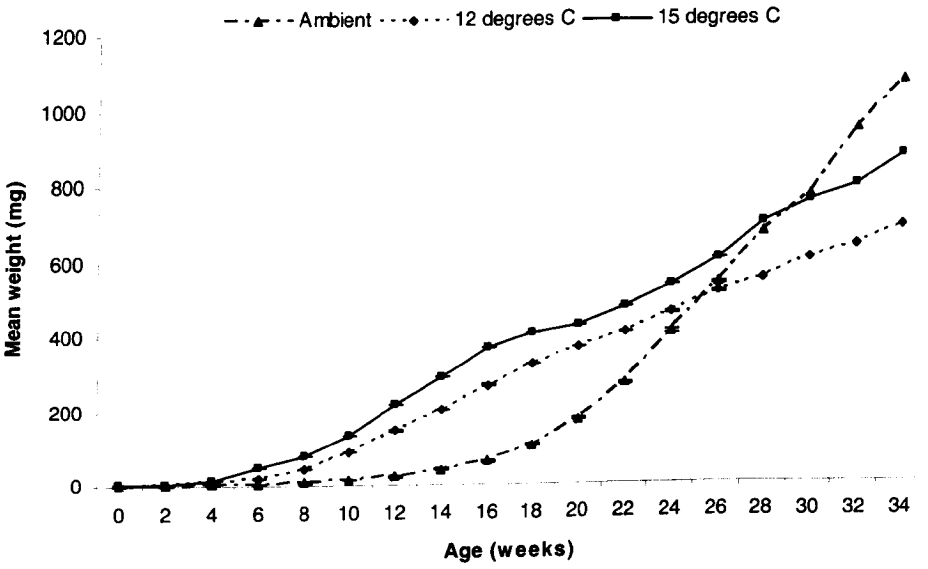


Figure 3.7: Mean weight (± S.E.) (mg) of Deroceras reticulatum *hatching in autumn and reared at ambient temperature, 12°C or 15°C (weeks 0-34).*

3.3.3.2 Survival

Slugs for which monitoring ceased after laying batches of five or more eggs are classified as 'censored' in survival analyses because their ultimate fate within the monitoring period is unknown, i.e. it is not known whether they would have stayed alive or died between the time monitoring stopped and week 34. This is taken into account during the analysis and such individuals are indicated by a cross on Kaplan-Meier estimator plots.

The mean survival time was highest for slugs reared at ambient conditions, followed by those at 12°C and finally 15°C which survived, approximately 6 weeks less than those at ambient conditions (*Table 3.7*).

Table 3.7: Mean survival times (± S.E.) (weeks) for autumn hatching Deroceras reticulatum *at each rearing temperature during the 34 week monitoring period.*

Rearing Temperature (°C)		
Ambient	*12*	*15*
30.31 ± 0.89	27.88 ± 1.11	24.67 ± 1.19

Although the mean survival times indicate that ambient conditions are generally more favourable for autumn hatching slugs, the patterns in survival rates over the entire 34 week monitoring period show that this becomes less so with increasing time (*Fig. 3.8*). The differences seen between rearing temperatures in weeks 0-20 are maintained up to week 26. After this time the survival rate of ambient reared slugs begins to decline until, at week 30, it equals that of slugs reared at 12°C and at week 32 it falls below this group. Slugs reared at 15°C consistently exhibit the lowest survival throughout this monitoring period.

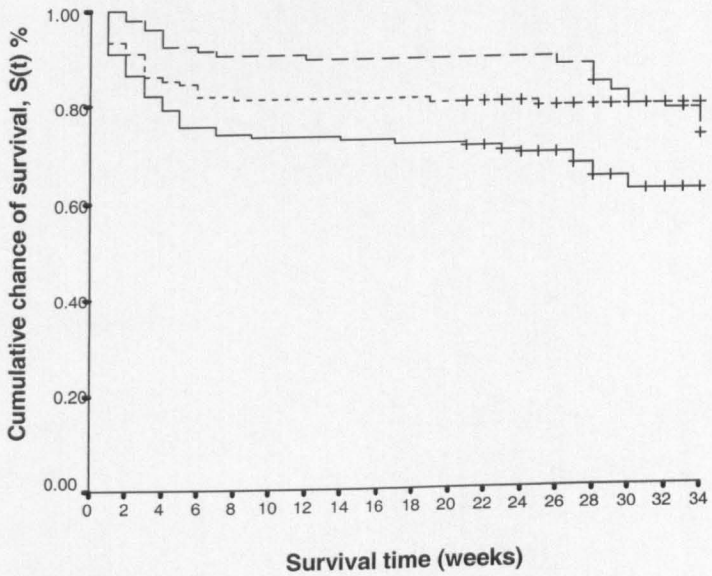


Figure 3.8: Kaplan-Meier estimators for Deroceras reticulatum *hatching in autumn and reared at ambient temperature (dashed line), 12°C (dotted line) or 15°C (solid line). Crosses indicate weeks when there were censored individuals (slugs that were removed from the experiment when they laid batches of 5 or more eggs).*

Overall, the differences in survival times were significant between slugs reared at 15°C and each of the other two temperatures, but there were no differences between those reared at ambient and 12°C (*Table 3.8).*

Table 3.8: Results of Kaplan-Meier survival analysis (with Breslow test) to compare differences in survival rates between Deroceras reticulatum *hatching in the autumn and reared at ambient temperature, 12°C or 15°C (weeks 0-34).*

Laying season	*Pairwise comparison between rearing temperatures (°C)*	*N*	*df*	*Breslow statistic (χ2)*	*P-value*
Autumn	Ambient vs 15°C	240	1	12.05	*< 0.001*
	Ambient vs 12°C	226	1	0.02	*n.s.*
	12°C vs 15°C	256	1	4.04	*< 0.05*

3.4 Discussion

This chapter comprises the second part of a study of *D. reticulatum* development, continuing the work described in *Chapter 2.* The experiments investigated the extent to which slugs hatching at different seasons of the year exhibit the same growth trajectories under identical rearing temperatures and whether this impacts on survival. It was also possible to contrast the growth and survival of slugs reared at different temperatures within a single season allowing a comparison with published studies.

3.4.1 Growth variation

That there is considerable variation in the size of slugs at hatching and during subsequent growth, even when reared under identical conditions, has been widely reported in the literature for a number of species (e.g. Hunter, 1978; Prior, 1983; Shibata & Rollo, 1988; South, 1992). Such variation is common in pulmonates generally (Peake, 1978) and is confirmed in the current study for *D. reticulatum.* Since individuals of the same age varied between approximately 5-100 fold in their weight and, by inference, slugs of a given weight vary considerably in their age, the results presented here support the conclusion of Prior (1983) for *Limax maximus* (L.)

that 'one cannot use body weight to estimate the absolute or relative age of animals accurately'. It is clear that the terms 'juvenile' and 'adult' should be used to describe the developmental state rather than the chronological age of slugs.

Little is known about the mechanisms underlying this exceptional divergence in size, although it is unlikely to be adaptively neutral since it is so consistently maintained over time (Rollo & Shibata, 1991). For example, it allows that there will be some mature individuals in the population capable of mating whenever conditions are favourable, maximising reproductive opportunities. Shibata and Rollo (1988) put forward a number of hypotheses to explain the basis of this phenomenon in *Deroceras laeve* (Müller), a largely self-fertilising species. These included maternal diet and egg quality, 'nutritional imprinting' (i.e. influence of early nutritional experience), density effects and egg size. Of these, they found that only egg size had a significant effect on growth with slugs hatching from smaller eggs growing faster than those hatching from larger ones. This was, however, only the case for slugs fed a high quality diet post-hatching and since size variation was still observed in experiments where egg size was controlled and diet was of a standard quality, it would suggest that there are likely to be multiple factors that influence growth trajectories either singly or in concert. This is supported by the experiments presented in this chapter; egg size was not measured, but since batches were allocated to treatments at random prior to incubation (*Chapter 2*) any variations in egg size would be expected to be distributed evenly amongst treatments. Whilst differences in egg size may, therefore, explain growth variation within treatments this would not account for the significant differences observed between them.

Size differences between slugs of the same age generally became more pronounced with time, agreeing with the findings of South (1982). Shibata and Rollo (1988) suggest that growth during early development is exponential and small differences in rates as a result of different weights at hatching translate into a 'rapid divergence in body sizes'. Certainly the growth rate increased with time in all the experimental treatments presented here and this explanation would seem feasible.

Since slugs were fed the same diet and were reared in isolation, nutritional imprinting and density effects can also be ruled out as explanations of growth variation in *D. reticulatum*. Indeed, it has been shown that interspecific competition in this non-aggressive species is minimal and has little effect on growth (Rollo, 1983b). Furthermore, size variation is comparable between isolated and communally reared individuals (South, 1982).

3.4.2 Seasonal and temperature effects on growth

Hatching season influenced growth at all temperatures assessed. Although seasonal differences in size between slugs from week 0-4 were small in all treatments, there was a rapid divergence after this time. The ambient reared slugs acted as controls and confirmed previous work that showed under field temperatures *D. reticulatum* grows faster in spring than autumn (Carrick, 1938). At constant temperatures of 12°C and 15°C, however, the converse was observed; growth of slugs hatching in autumn was faster than that of those hatching in spring.

The 20 week monitoring period encompassed the months of May–September for spring hatching slugs and November/December–March/April for the autumn hatching slugs, depending on the hatching date. Ambient autumn temperatures were considerably lower, albeit fluctuating, than the constant treatments assessed in this study during the same monitoring period whereas ambient spring temperatures were more similar (mean ambient temperature (± S.E.) for autumn hatching slugs = 5.6 ± 0.1°C; spring hatching slugs = 12.7 ± 0.1°C). It may be that the autumn hatching slugs exhibited a much greater growth response to the constant temperatures than those hatching in spring because they are more 'unseasonably high' for this group. If there is something inherently different about slugs hatching from autumn laid eggs that adapts them to withstand over-wintering then it might be expected that they would show a greater capacity to capitalise on consistently and markedly more favourable conditions than usually experienced through the winter months. In a species considered to be an r-strategist (South, 1982) the ability to respond to disturbed and changing environments is key. The finding that *D. reticulatum* is able to 'step up' its growth in response to prolonged and unexpectedly mild conditions for

the time of year concords with this. Whilst, therefore, these results support the importance of temperature on growth, it seems that season is, independently, also influential.

The influence of photoperiod on growth of *D. reticulatum* is not clear. Work on reproduction in this species indicates that the weight of individuals mirrors closely the development of the reproductive tract (Runham, 1978). Sokolove and McCrone (1978), working on *L. maximus,* showed that photoperiod affected reproductive development and it could, therefore, be inferred that it also has an effect on growth. South (1989a), however, argued that since *D. reticulatum* is capable of breeding throughout the year whenever temperature conditions are favourable it is unlikely that day-length is influential. The results of the current study cannot resolve this question. The photoperiod was appropriate to the time of year for the ambient reared slugs whereas it was maintained at 16L:8D throughout for the slugs reared at constant temperatures and therefore cannot be separated from the effects of season in either case and, additionally, temperature in the former. It could be that the longer day regime acted as an environmental cue to the autumn laid eggs reared at constant temperatures in tandem with the 'unseasonably good' temperatures conditions for growth as proposed above, but further experiments would be required to investigate this hypothesis.

Most published studies based on slugs hatching at a given point in time have shown a high positive association between growth and temperature up to an optimum, after which there is a decline in growth along with other physiological functions leading to rapid mortality (e.g. Abeloos, 1944; Arias & Crowell, 1963; Dmitrieva, 1969; Pinder, 1969; South, 1982). Judge (1972), however, found the opposite; growth was greater at lower temperatures. In the experiments presented here a positive association was confirmed for slugs hatching in autumn, but not for those hatching in spring where individuals reared at 15°C were observed to grow significantly more slowly than those at 12°C and ambient temperature, agreeing with Judge (1972). Studies in the literature rarely state the season in which slugs hatched. It could be that those indicating a positive association between growth and temperature were based on slugs hatching in autumn whereas those indicating a negative association used slugs

hatching in spring. The results of the current experiments, therefore, may not be contradictory, but rather help to explain this discrepancy.

Seasonal modification of the temperature-growth relationship brings into question the optimal temperature for growth of *D. reticulatum*. Reports suggest that this is between approximately 17-19°C (Dainton, 1954a; Dmitrieva, 1969; South, 1982). The autumn results of the current experiments support this range in that the maximum temperature assessed approaches these values and was indeed that at which growth was fastest. It would be advantageous for slugs to grow quickly in mild conditions during autumn so that they mature more rapidly and increase the likelihood of oviposition before the onset of winter. This would allow time for some embryonic development and a concomitant increase in tolerance of eggs to freezing conditions before the coldest months of the year arrive (Arias & Crowell, 1963). In spring, however, depressing growth at warmer temperatures, e.g. transient mild snaps, would serve to restrict maturation so that peak egg laying occurs, as observed, in autumn and not during the hotter months of summer when desiccation is more of a risk. There is evidence that slugs are able to make seasonal adjustments in their physiology that better adapt them to survive at the prevailing temperatures. It has, for example, been shown that the mean supercooling point (SCP) of *D. reticulatum* changes between summer and winter (Cook, 2004). Furthermore, it has been suggested that *L. maximus* and *Philomycus carolinianus* (Bosc) are 'cold adapted' in spring and 'warm adapted' in autumn (Rising & Armitage, 1969). It seems feasible that this seasonal adjustment could also include a change in the optimal temperature for growth.

Seasonal differences in activity may also be relevant to the results of the current experiments. Wareing and Bailey (1985) showed that slug activity is affected by constant temperatures and that some seasonal adjustment occurs. They suggest that this is controlled partly by endogenous rhythm and partly by day-length. Under long-days the optimum temperature for locomotion was 17°C, whilst under short-days it was 13°C. The optimum temperature for feeding remained at 14°C regardless of day-length. In the experiments presented here the long-days were maintained for the 15°C and 12°C treatments in both hatching seasons. The results of Wareing and

Bailey (1985) would imply that, as 15°C is nearer the optima for both locomotion under long-days and feeding, slugs reared at this temperature are likely to grow more than at 12°C in both seasons as they are likely to have eaten a larger quantity of food and been more active. Since this was only observed for the autumn hatching slugs it suggests that endogenous factors may have a greater influence than day-length.

Egg laying was initially sporadic with isolated eggs being produced, rather than batches. This is in accordance with the observations of Carrick (1938) who states that 'a few abnormal eggs are laid before the normal egg-masses begin to be produced by young slugs which have just reached maturity'. When five or more eggs were laid they were collected for the next stage of the experiment (*Chapter 4*). Although five is an arbitrary number to constitute a batch it was chosen because it was noted that once about five eggs were laid they tended to be in obvious groups, rather than spread at random individually around the Petri dish. It was, therefore, felt that this number reasonably represented a proper batch and would be likely have a greater chance of hatching than the earlier isolated eggs. After an egg batch was collected weight monitoring ceased as it was not practical to continue rearing all slugs until they died naturally and at this point they were considered to be mature. Rollo (1988) suggests that there is a trade-off between growth and reproduction such that once oviposition begins all reserves are switched to reproduction and growth stops, i.e. the two processes are mutually exclusive. Growth was therefore regarded as complete once the first batch of eggs was laid.

The results of prolonged monitoring of slugs hatching in autumn need to be interpreted with caution. As noted in *section 3.3.3.1* the number of autumn hatching slugs alive from weeks 0-34 was considerably less than from weeks 0-20, particularly in the constant temperature treatments, due to the gradual removal of egg-layers from the experiment. These two groups are not, therefore, composed of the same individuals; those in the 34 week analysis exclude any that laid batches of five or more eggs. The results are, nevertheless, informative. At constant rearing temperatures the mean weight of slugs in the 34 week group was lower at all time intervals than the 20 week group suggesting that the considerable variation in growth that is seen within a treatment may be partitioned into 'slow growers' and 'fast

growers'. Fast growers seem to be able to take advantage of the warmer than ambient, constant conditions, growing to large sizes and laying eggs rapidly. Slow growers, in contrast, gain weight at comparable rates to ambient reared slugs, regardless of the elevated constant temperatures (there were no significant differences in growth between rearing temperatures over the 34 week period). Despite within treatment variation in growth rate, the slugs reared at ambient temperature generally grew more slowly and began laying eggs later than slugs at constant temperatures and would, therefore, have consisted of a mixed group of fast and slow growers for longer. During weeks 20-34, however, their mean growth overtook that of slugs at 12°C and 15°C. These weeks correspond to May-August, i.e. the conditions experienced by spring hatching ambient reared slugs during weeks 0-20. The emerging pattern, with ambient slugs beginning to grow faster than those at constant temperatures reflects what was seen in the spring hatching group and could be explained by an increase in mean weight as faster growers reach maturity. It may also be that fluctuating temperatures affect growth in a different way to constant temperatures. These results require further investigation

Egg size may be an important determinant of whether a slug is a fast or slow grower (Shibata & Rollo, 1988), as described in *section 3.4.1*, but genetic differences may also play a role. Self-fertilisation is possible in species that normally cross-fertilise e.g. the genus *Philomycus* (McCracken & Selander, 1980), although it is not the norm. Furthermore, some species lay mixed batches of eggs, fertilised by both autosperm and allosperm, e.g. *Arion* (Duncan, 1975). Whilst it is reported in the literature that *D. reticulatum*, a normally cross-fertilising species, lays eggs when reared in isolation these are said to be infertile (South, 1982; Nicholas, 1984). In the current study, this was found not to be the case; some eggs laid by isolated slugs were fertile. It may be that *D. reticulatum* is also capable of both cross-fertilisation and self-fertilisation. If this were so then fast and slow growth may be determined by whether the egg is fertilised by autosperm or allosperm. These ideas are expanded upon in *Chapter 4*; additionally, genetic studies to investigate this hypothesis would be of great value.

3.4.3 Seasonal and temperature effects on survival

Whilst the mortality of *D. reticulatum* under different environmental conditions has been described in the literature in terms of absolute or relative numbers, there is little work that has formally assessed survival over time and, as for growth, none that compares this between seasons. In the experiments presented here it was found that season significantly affected the survival rate at ambient temperature and 15°C, but not at 12°C. Under ambient temperature slugs survived longer in autumn than in spring whereas at 15°C survival was greater in spring.

The ambient results indicate that under field temperatures *D. reticulatum* is better able to survive in cool than warm conditions. It is known that slugs possess physiological mechanisms to cope with low temperatures, for example, they can enter a state of chill coma (Mellanby, 1961), i.e. they cool to below the temperature at which freezing would normally occur (supercooling point) without becoming immobilised, and can survive freezing temperatures for longer in winter than other times of the year (Cook, 2004). They are more vulnerable to warm temperatures, however, having to rely to a greater extent on behavioural adaptations to withstand extremes. Although the mean ambient temperature in spring (± S.E.), at 12.7 ± 0.1°C, is not at the upper limit of their tolerance, this group were subject to a mean daily range in temperature that was almost double that of the autumn hatching ambient reared slugs (± 4.9°C c.f. ± 2.8°C). Furthermore the maximum recorded temperature in spring was 24.1°C compared to 14.0°C in autumn; hence spring hatching slugs were subject to larger extremes of temperature and this may also impact negatively on their survival.

Comparisons of survival between rearing temperatures within a season showed that, although there was no difference between the two constant temperatures in spring or in autumn, in both seasons these differed significantly from ambient. In spring survival at ambient temperature was lower than at both constant temperatures whereas in autumn it was higher. It is not clear why there was no difference between the constant temperatures; perhaps survival is simply not very sensitive to small

temperature changes. The ambient results are, however, consistent with previous suggestions that slugs are better adapted to lower temperatures.

South (1982) included an assessment of mortality in his experiments of growth at different controlled temperatures. In general it was found that the percentage of *D. reticulatum* surviving with time was inversely related to temperature, however no formal assessment of whether the differences between rearing temperatures were statistically significant was presented. South's results cannot be directly contrasted with the current experiment as the time of year that studies were carried out was not stated and nor were the same rearing temperatures assessed. A small subset of individually reared slugs was, nevertheless, compared with a larger group of communally reared individuals and it was found that their survival was similar, indicating that the results of the current experiment are unlikely to be inflated due to the rearing procedure.

Over the longer 34 week period survival patterns of autumn hatching slugs from weeks 0-20 were largely maintained. The chances of survival continued to decline with time for slugs reared at 15°C and this group had the lowest survival rate throughout. The survival of ambient reared slugs, though remaining high for most of the 34 week observation period, began to decline towards the end and eventually fell below that of the slugs reared at 12°C. At the time when ambient survival began to decline it was late May/early June and, as observed with growth patterns, the survival rates begin to revert to the trends observed for spring reared slugs from weeks 0-20. Had the slugs been monitored for longer it may be speculated that the ambient survival would also have dropped below that of the slugs reared at 15°C.

3.4.4 Implications for population dynamics

This study was laboratory based and as such provides information on the influence of different rearing temperatures and laying seasons on the potential capacity for growth and ultimately survival of *D. reticulatum*. Under field conditions many other factors will modify this response, e.g. predation, parasitic infestation (Glen *et al.*, 2000), food availability (Rollo, 1988) and climatic factors (Young & Port, 1989). It is,

therefore, necessary to be cautious in the extent to which the present findings can be extrapolated to such situations.

It was seen that growth accelerates under unseasonably mild conditions and that survival is greater at lower temperatures. It may be, therefore, that population booms after mild autumns are caused not by an increase in survival, but by a subset of fast growing slugs that mature rapidly. If, over the much longer term, mean temperatures were to rise as a result of global warming the decrease in survival at warmer temperatures may cause the distribution of *D. reticulatum* to move northwards; small changes in minimum temperatures have been shown to affect the range of other invertebrates (Crozier, 2004).

If the survival results are contrasted with those for growth (*section 3.4.2*) it is immediately obvious that, at least for slugs reared at ambient and 15°C, there is an inverse relationship between growth and survival; as slugs grow faster their chances of survival become progressively lower, agreeing with South (1982). At 12°C, however, no such relationship was apparent. At this temperature the growth rate increased significantly in autumn compared to spring with no change in survival rate yet at 15°C, a rise of just 3°C, the much greater increase in growth of autumn hatching slugs was accompanied by a significant decrease in survival. If there is a 'trade-off' between growth and longevity, it would seem that this operates within certain confines. Perhaps 12°C represents a transition temperature, not warm enough to reduce survival in autumn, but not cool enough to enhance it in spring. At temperatures where this inverse relationship holds, the implication is that harsh conditions may not affect an entire generation equally; smaller, slower growing slugs have an increased chance of surviving to replace larger faster growing individuals providing a high degree of flexibility in response to a changing environment at the population level.

Hunter and Symonds (1971) suggested that there are overlapping ('leapfrogging') generations of *D. reticulatum* in temperate regions such as the United Kingdom. Under this scheme the slug population consists of two generations separated by an interval of about nine months. In generation A, slugs hatch in autumn, over-winter

and lay eggs the following spring (equivalent to the autumn hatching slugs in the current study) whereas generation B hatch in late spring and then mature and lay eggs in late autumn (equivalent to the spring hatching slugs). This accounts for the two peaks in slugs numbers in spring and autumn whilst allowing that there cannot be two complete generations in a year due to a lifespan of nine or more months from egg to adult (Hunter, 1968b). South (1989a) points out that generations A and B are not necessarily distinct 'races' as some slugs may mature early/late and slip into the alternative generation. The results of the study presented here support South (1989a), suggesting that rather than being an exception, this intermixing of the two generations may be the norm. Perhaps the situation is more analogous to Aesop's 'hare and tortoise fable' (Gibbs, 2003); slow growing individuals may exist in the population simply growing steadily in small increments (the tortoises) while their fast growing contemporaries race ahead, but ultimately die early (the hares) and lose in the survivorship stakes. The 'tortoises' of one generation are to be found with the 'hares' of the following generation.

3.4.5 Implications for control

Any situation where the growth of a pest increases, but survival doesn't decrease poses a problem for control. For the constant rearing temperatures a difference of 3°C resulted in a significant change in growth; in spring growth was slower at 15°C than 12°C whereas in autumn this was reversed. Survival, however, remained unaltered at 12°C in both seasons. This could have appreciable short term effects on damage levels if a similar trend were confirmed for fluctuating temperatures. A cool spring where temperatures are nearer 12°C than 15°C could promote faster growth of *D. reticulatum* populations while a cool autumn would have the opposite effect with concomitant changes in crop damage. This would be particularly critical in winter crops sown in mild autumns, e.g. winter wheat. In the longer term, if the distribution of *D. reticulatum* were to move northwards due to a rise in mean temperature its status as a pest would be likely to increase in areas where it is not currently a significant problem.

3.5 Conclusions and Future Work

The experiments supported the hypothesis that autumn hatching slugs exhibit different growth trajectories to spring hatching slugs when reared under identical conditions suggesting that the temperature-growth relationship is more complex than previously thought. Not only does it vary with rearing temperature, but this is further modified by the hatching season. Studies of *D. reticulatum* growth, therefore, need to take into account the hatching season. A series of assessments may give misleading results unless they are all carried out at the same time of the year. Survival was inversely related to rearing temperature, but season modified this only at 15°C and ambient temperature.

In conclusion it was found that:

1. There was considerable variation in the growth of *D. reticulatum* reared under identical conditions.
2. Growth was influenced by hatching season at all rearing temperatures; at constant temperatures of 12°C and 15°C growth was faster in autumn than spring whereas at ambient temperature the reverse was observed.
3. Within a season the association between growth and temperature was low, but negative in spring; however, it was high and positive in autumn.
4. Survival was influenced by hatching season for slugs reared at ambient temperature and 15°C and was inversely related to growth rate, but had no effect at 12°C.
5. Within a season the chances of survival improved at lower rearing temperatures.

This series of experiments has suggested a number of avenues for future research. In particular, genetic studies would help to determine the influence of paternity on growth trajectories, i.e. fertilisation by allosperm or autosperm and would allow the detailed exploration of phenotypic plasticity. The ambient treatments acted as controls to confirm the growth and survival patterns of *D. reticulatum* under field temperatures. These temperatures were, of course, fluctuating whereas the other

treatments assessed constant temperatures. South (1982) found that a fluctuating temperature of 10/18°C did not have the same effect on the lifespan of *D. reticulatum* as the corresponding mean temperature of 12.7°C. This may also be the case for growth and survival and repeating the experiments at a wider range of constant and fluctuating temperatures would be informative.

Chapter 4
Hatching, Growth and Survival of Self-Fertilised *Deroceras reticulatum* (Müller)

Abstract

Eggs laid by *Deroceras reticulatum* (Müller) reared in isolation were incubated at ambient temperature, constant 12°C or constant 15°C. Appreciable numbers developed only at 15°C. These self-fertilised slugs developed more slowly and the hatching rate was lower than the parental population. There was considerable variation in the growth of those that hatched, despite identical rearing conditions. Their growth rate was significantly faster and their cumulative chance of survival was lower than the parental population. Eggs laid by this self-fertilised generation failed to hatch. The possible adaptive advantages of low level self-fertilisation in this predominantly cross-fertilising species are discussed.

4.1 Introduction

In the literature, *Deroceras reticulatum* is regarded as an obligate outcrosser (e.g. Runham & Hunter, 1970; McCracken & Selander, 1980; Niklas and Hoffmann 1981 in South, 1992). Whilst some authors have recorded instances of this species laying eggs when reared in isolation these are said to have been infertile (South, 1982; Nicholas, 1984).

Many other members of the genus *Deroceras*, however, can successfully self-fertilise. For example, this has been observed in *D. meridonale* (Reygrobellet) (Maury & Reygrobellet 1963 in South, 1982), and *D. agreste* (L.) produced five successive generations by self-fertilisation in the laboratory (Chen *et al.*, 1984). A study of genetic variation of different slug species in the eastern USA showed that *D. laeve* (Müller) reproduced by both cross- and self-fertilisation (McCracken & Selander,

1980), supporting the assertion of Duncan (1975) that different modes of fertilisation may even operate in the same species under different conditions.

Self-fertilisation may have adaptive benefits. Foltz *et al.* (1984), in a survey of genetic variation in terrestrial slugs, showed that self-fertilising species were considerably more successful at colonising new habitats than cross-fertilised species. They concluded that the type of fertilisation system was, therefore, related to colonisation ability, a conclusion that was supported by Anderson and McCracken (1980) working on the family Philomycidae.

D. reticulatum is classified as both a protandric and simultaneous hermaphrodite because the male system develops first and in mature slugs both the male and female systems are functional at the same time (Runham & Hunter, 1970). The sequence of development has been described in detail at the histological level (Runham & Laryea, 1968). These authors found that there was considerable overlap between the male and female-phases; stages of development 'graded into each other'. Depending on the extent of this overlap it is possible for mature gametes of both types to be present in the same place at the same time which provides a cellular basis for self-fertilisation.

Given, therefore, that most other members of the genus *Deroceras* are able to self-fertilise either as their main method of reproduction or in addition to cross-fertilisation, that there is a cellular basis for the process in *D. reticulatum* and that this is one of the most successful species at thriving in disturbed habitats such as agricultural environments, whilst it may predominantly use cross-fertilisation, it would be surprising if it were incapable of self-fertilisation.

The data presented in this chapter explore the potential of this species to self-fertilise and the development of viable offspring is monitored. It is an extension of the work described in *Chapter 3* on the growth and survival of *D. reticulatum*.

4.2 Materials and Methods

4.2.1 Materials

4.2.1.1 Eggs

The eggs used in these experiments were laid by the autumn hatching slugs described in *Chapter 3* (i.e. generation one slugs). Generation one slugs were reared in isolation hence any eggs that hatched were self-fertilised and constitute generation two.

Only batches of five or more eggs were used. Up to a maximum of 20 such batches were collected from generation one slugs per rearing temperature (ambient, 12°C or 15°C). There were no significant differences in batch size between treatments (Kruskal-Wallis: N = 51, df = 2, H = 0.224, *n.s.*). Each batch was placed on fine grade netting and rinsed with distilled water to remove any soiling before being transferred into a Petri dish lined with moist laboratory tissue. The number of eggs per batch was recorded.

4.2.1.2 Food

Hatched slugs were fed on a mixed diet of Chinese cabbage and carrot *ad libitum.* Cuttlefish bone was provided as a source of calcium.

4.2.2 Methods

4.2.2.1 Experimental treatments

The temperature treatments described in *Chapters 2* (*section 2.2.2.1*) and *3* (*section 3.2.2.1*) were maintained in this third phase of the study i.e. ambient temperature, 12°C or 15°C. Methods of temperature control and photoperiod remained unchanged. Egg batches and subsequent hatchlings were incubated and reared at the same temperature at which they were laid by their generation one 'parent'.

4.2.2.2 Egg maintenance and hatching

This was largely as described in *Chapter 2* (*section 2.2.2.2*). Egg batches were prevented from drying out by remoistening with distilled water as required and hatching was checked weekly. After it had commenced, monitoring continued until two full weeks had elapsed since the last slug hatched. On each checking occasion any offspring were removed from the Petri dishes and cultured as in *section 4.2.2.3*. The development time and number of slugs hatching per batch were recorded.

4.2.2.3 Culturing

As described in *Chapter 3* (*section 3.2.2.2*).

4.2.2.4 Weighing regime

As described in *Chapter 3* (*section 3.2.2.3*).

4.2.2.5 Monitoring period

Slugs were monitored for a total of 20 weeks.

4.2.3 Statistical analyses

Continuous data were tested for normality and transformed if necessary. Percentages were arcsine transformed. In all cases this resulted in parametric data which were then analysed using an independent sample t-test or analysis of variance (ANOVA). ANOVA was followed by Tukey post-hoc tests as appropriate.

Regression was used to analyse variables between which a cause-effect relationship was postulated; for binomial data binary logistic regression was carried out, otherwise linear regression was used.

Discrete counts were compared using chi-squared goodness of fit.

Continuous weight data were analysed using a repeated measures analysis of variance (ANOVA). To account for the non-parametric nature of the data, as confirmed by Mauchley's test of sphericity and Box's M-test, the ANOVA was

adjusted by applying the lower-bound epsilon correction. Tukey post-hoc tests were performed as appropriate.

Survival data were analysed with the Kaplan-Meier procedure, using the Breslow test to compare between treatments. A hazard ratio was calculated.

4.3 Results

Although egg batches were collected for all three temperature treatments, appreciable numbers of generation two slugs hatched only in the 15°C treatment (*Table 4.1*). Furthermore, of these, the majority were from the same egg batch. Analyses are, therefore, interpreted with caution and treated as exploratory.

Table 4.1: Number of egg batches collected, mean number of eggs (± S.E.) and total number hatching at ambient, 12°C and 15°C incubation temperatures.

	Incubation Temperature		
	Ambient	*12°C*	*15°C*
Number of batches collected (>=5 eggs)	11	20	20
Mean number of eggs (± S.E.)	10.27 ± 1.97	11.90 ± 2.68	14.90 ± 3.96
Number of eggs hatching	0	1	18

Analyses of parental effects on egg laying use data from all three rearing temperatures (*section 4.3.1*). All other analyses are confined to slugs reared at 15°C due to insufficient numbers at the other temperatures. Since these slugs hatched in May-July 2003 they are analogous to the spring hatching generation one slugs reared at 15°C and inter-generational comparisons are based on these groups.

4.3.1 Parental effects on egg laying and batch size in generation two

Parental weight at laying differed significantly depending on the rearing temperature (ANOVA: $F_{2, 48} = 5.725$, $P < 0.01$). Tukey post-hoc tests showed that parents reared at 15°C were heavier at laying than those at 12°C or ambient temperature, but

there were no differences between these latter two treatments (*Table 4.2*). There were also significant differences in mean parental age at laying between all three rearing temperatures (ANOVA: $F_{2, 48} = 50.910$, $P < 0.001$). Parents reared at 15°C were youngest and those at ambient temperature were oldest (*Table 4.2*). There was no correlation between parental weight and batch size (linear regression: N = 51, $R^2 = 0.036$, *n.s.*).

Table 4.2: Mean (± S.E.) parental age and weight at laying batches of 5 or more eggs at each of three incubation temperatures for Deroceras reticulatum *reared in isolation.*

Incubation Temperature (°C)	*Parental weight at laying (mg)*	*Parental age at laying (wks)*
Ambient	1004.10 ± 65.63	31.82 ± 0.67
12	992.83 ± 56.35	24.60 ± 0.72
15	1217.44 ± 46.00	21.30 ± 0.39

4.3.2 Relationship between hatching and batch size for generation two eggs

At 15°C batch size was not a significant predictor of whether any of the eggs in a batch hatched for generation two eggs (binary logistic regression: N = 20, Z = 0.37, *n.s.*, percent concordant pairs = 37.3%). When compared with spring hatching generation one slugs it was seen that, for batches of five or more eggs, there was a significant difference between batch size; generation two batches were smaller (ANOVA: $F_{1, 38} = 10.74$, $P < 0.01$) (*Table 4.3*). In addition, fewer of these batches had at least one egg hatching when compared to generation one (Fisher's Exact Test: N = 40, P = 0.04).

There was a significant relationship between the batch size and the number of eggs hatching in generation two (Linear regression: N = 20, $R^2 = 0.39$, $P < 0.01$), however this was mainly due to an outlier. This outlier was a particularly large batch of eggs which produced a large proportion of the slugs that hatched. When this was removed, the relationship was not significant (Linear regression: N = 19, $R^2 = 0.04$,

n.s.) (*Fig. 4.1*). In both cases, the regression line was forced through the origin as the number of eggs hatching must equal zero when batch size is zero.

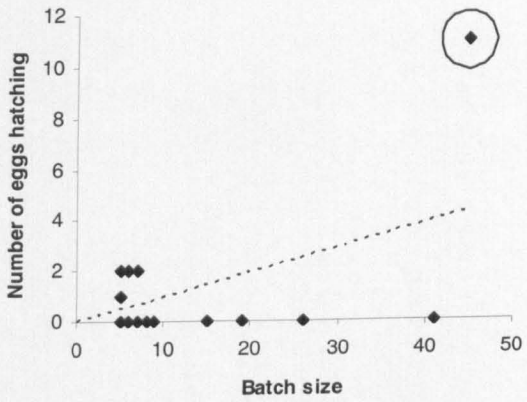


Figure 4.1: Scatter plot of the number of eggs hatching and batch size for generation two eggs reared at 15°C. The circled data point is an outlier. The dotted line shows the relationship between batch size and number of eggs hatching when the outlier was included. Regression equation: y = 0.096x.

4.3.3 Development time and hatching rate of generation two eggs

At 15°C generation two slugs took longer to develop than spring hatching generation one slugs, i.e. the parental population (t-test: N = 25, df = 23, t = -6.503, $P < 0.001$). Similarly, there was a significant difference in hatching rate between these groups of slugs, with generation two slugs having a lower hatching rate (t-test: N = 25, df = 23, t = 6.830, $P < 0.001$). Mean values are summarised in *Table 4.3*.

Table 4.3: Mean (± S.E.) development time (weeks), hatching rate (%) and batch size for spring hatching generation one and generation two Deroceras reticulatum *reared at 15°C.*

Generation	*Development time (wks)*	*Hatching rate (%)*	*Batch Size (No. eggs)*
1	3.53 ± 0.09	74.5 ± 4.72	22.3 ± 2.1
2	5.00 ± 0.27	29.2 ± 3.48	11.9 ± 2.7

4.3.4 Growth of self-fertilised slugs

Although the sample size was considerably smaller, at a rearing temperature of 15°C generation two slugs were consistently heavier at all weeks from 0-20 compared to spring hatching generation one slugs. This became most marked from week 4 onwards (*Fig. 4.2*). This was a highly significant difference (ANOVA: $F_{1, 99}$ = 185.41, $P < 0.001$). The mean weight at week 20 (± S.E.) was 121.55 ± 11.56 mg (N = 94) and 794.22 ± 186.12 mg (N = 7) for generation one and two slugs respectively.

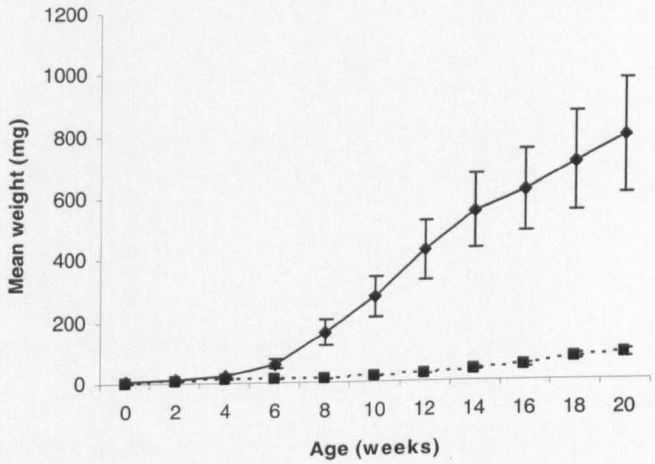


Figure 4.2: Mean weight (mg) of Deroceras reticulatum *from spring hatching generation one (dotted line) and self-fertilised generation two (solid line) reared at 15°C. Bars represent S.E.*

4.3.5 Survival of self-fertilised slugs

At a rearing temperature of 15°C there was a significant difference in the survival of self-fertilised generation two slugs and spring hatching generation one slugs at all times from week 0-20 (Kaplan-Meier (Breslow test): N = 101, df = 1, χ^2 = 18.22, $P<0.001$) (*Fig. 4.3*). The self-fertilised slugs had a lower chance of survival from weeks 0-20 (hazard ratio = 6.56); the mean survival times (± S.E.) during this period are 17.36 ± 0.55 weeks and 11.56 ± 1.92 weeks for generation one and two slugs respectively.

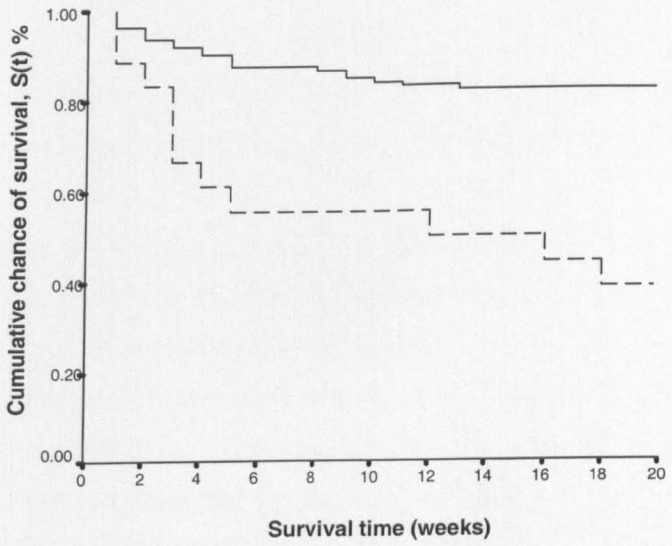


Figure 4.3: Kaplan-Meier estimators for Deroceras reticulatum *from spring hatching generation one slugs (solid line) and self-fertilised generation two slugs (dashed line) reared at 15°C.*

4.3.6 Egg laying by self-fertilised slugs

One of the self-fertilised generation two slugs laid three batches of eggs, i.e. a third generation. None of these eggs hatched.

4.4 Discussion

4.4.1 Self-fertilisation in *D. reticulatum*

The current experiment contradicts published reports that *D. reticulatum* is an obligate outcrosser and that eggs laid by individuals reared in isolation are infertile (South, 1982; Nicholas, 1984); some such eggs were found to be viable and the offspring were, by definition, produced by a self-fertilised parent. Consequently, this is the first study that contrasts the relative development of self-fertilised individuals of this species (generation two) with those representing an 'average population' (generation one, *Chapter 3*), hitherto assumed to be cross-fertilised. It was

hypothesised in *Chapter 3* that, whilst many of the individuals comprising generation one were probably cross-fertilised by allosperm, some may have been self-fertilised by autosperm. Formally testing this was beyond the scope of this thesis and therefore these slugs are considered an 'average population' to avoid ambiguity.

It is reiterated that the analyses presented in this chapter are exploratory and further work is required to test the hypotheses they generate. In comparisons between generations one and two, the analyses concerning events prior to hatching (i.e. *sections 4.3.2* and *4.3.3*) were balanced as the same number of egg batches were collected for both generations. The number of generation two offspring that hatched, however, was small and hence inter-generational comparisons of growth and survival (*sections 4.3.4* and *4.3.5*) are highly unbalanced. This was unavoidable, but means that the results of these analyses must be treated with caution.

4.4.2 Parental effects on egg laying and batch size

Parent slugs reared at 15°C were significantly heavier when they began laying batches of five or more eggs than those at 12°C or ambient temperature; there was no difference between these latter two groups. This finding is in broad agreement with South (1982) who found that the mean weight at egg laying tended to increase with temperature for *D. reticulatum*, although this was somewhat obscured at particularly high or low temperatures. It is not clear why there was no difference between parents reared at 12°C and ambient temperature. The mean ambient temperature was, unfortunately, not recorded due to malfunction of the Tinytalk® datalogger, but it could have been close to 12°C and thus the actual differences in treatments may have been small. South (1982) however, found that cycling and equivalent mean temperatures had different effects on the weight at egg laying implying that other factors must also have influenced the current results. One such factor may be photoperiod, which differed between constant and ambient treatments; there is evidence that photoperiod is involved in initiating reproductive maturation in *Limax maximus* (L.) with the transition to long-days promoting male-phase development (Sokolove & McCrone, 1978). It seems, therefore, that a combination of factors is

likely to modify the onset of egg laying in *D. reticulatum* and additional work is needed before any further conclusions can be drawn.

There was a clear inverse relationship between rearing temperature and age at laying; the higher the temperature, the younger were the parent slugs when they began laying eggs, i.e. the shorter the lifecycle. Again, this supports the findings of South (1982). Since it was shown in *Chapter 3, section 3.3.1.3*, however, that rearing temperature was positively associated with growth (i.e. weight) for the parent slugs and that weight was a poor indicator of age, it would seem likely that weight may be confounding this apparent association between rearing temperature and age.

There was no correlation between parental weight and batch size. This may be partly because parent slugs had only recently begun to lay eggs and the process can be somewhat sporadic initially (*pers. obs.*). In retrospect it may have been more appropriate to collect batches nearer to the mean size reported for this species (twenty two) (Carrick, 1938). It was seen, however, that for the wild caught slugs that laid the eggs constituting generation one the correlation between parental weight and batch size was only significant in spring and even here the R^2 values were very low. Taken together, it would seem that parental weight has a very weak influence, if at all, on batch size and other factors are more important.

4.4.3 Relationship between hatching and batch size

For generation two eggs incubated at 15°C batch size was not a significant predictor of whether any of the eggs in the batch hatched. This is not surprising given that the majority of those hatching were from the same batch (*Fig. 4.1*). Although batch size was 'artificially manipulated' to a certain extent by only collecting those with five or more eggs, *Fig. 4.1* suggests that it is unlikely that this would unduly bias the results; it can be seen that for batches smaller than 10 eggs, similar numbers had at least one egg hatching as had none hatch at all and very much larger batches also had no eggs hatching. A more likely explanation is that many of the eggs collected may not actually have been fertilised. It has been suggested that sperm production may vary among individuals in the snail *Arianta arbustorum* (L.) (Locher & Baur, 1999) and

this may also be the case for *D. reticulatum*, particularly for those that have just reached maturity.

It has been suggested that in *D. reticulatum* copulation usually precedes egg laying and that, as a consequence, egg laying is delayed in slugs reared in isolation (Runham & Laryea, 1968), perhaps due to a shift in reproductive physiology. A basic assumption of sex-allocation theory in simultaneous hermaphrodites is that there is a trade-off between male and female function because the individual has a fixed amount of resources to allocate to each gender (Charnov, 1982). Locher and Baur (2000) suggest that the optimal allocation to male versus female function in *A. arbustorum* may depend on density such that at higher densities there is a higher risk of sperm competition and therefore a higher mating frequency would lead to a larger allocation to the male reproductive function. The converse of this argument, which mirrors the situation in the current experiments, is that when there is a low density and therefore low mating frequency, there is a similarly low risk of sperm competition leading to a shift in resource allocation favouring the female reproductive function, i.e. egg laying. If this is the case in *D. reticulatum* then perhaps the eggs collected soon after oviposition commences are unfertilised by autosperm because reproductive physiology is still stabilising.

Generation two batches were significantly smaller than those of generation one. Whilst this may indicate that self-fertilisation is a 'back-up strategy' and not the most effective means of reproduction in this species, it could also be explained by initial sporadic laying and reflect a change in reproductive physiology as described above.

4.4.4 Development time and hatching rate of self-fertilised eggs

Of the generation two slugs that hatched, the development time was slower and the hatching rate was more than 50% lower than for generation one slugs. This supports the suggestion of Duncan (1975) that allosperm is usually more effective in fertilisation. He speculates that this is due to a biochemical barrier to self-fertilisation or to the prostatic secretions that allosperm receive following copulation.

To confirm the preliminary observations of the current study a larger sample of generation two eggs would be required along with a greater number of different parents. If this were to bear out these findings it would support the suggestion that self-fertilisation is a secondary reproductive strategy in *D. reticulatum* acting as a buffer against adverse conditions affecting the main cross-fertilised population. By developing more slowly, and consequently hatching later, the self-fertilised slugs may provide a background 'pool' to replace cross-fertilised slugs that hatch earlier and subsequently suffer mortality due to harsh conditions or predation, for example. The reduction in hatching rate indicates that it is not the predominant mode of fertilisation, but would, nevertheless, maintain a small pool if individuals to rebuild the population after a catastrophic decline in numbers.

4.4.5 Growth of self-fertilised slugs

Although many of the self-fertilised slugs were from the same batch, it is known that variation in growth rate is considerable even between slugs hatching from the same batch (Prior, 1983; Shibata & Rollo, 1988; South, 1992). Whilst, therefore, taking into account the low numbers of generation two slugs in this analysis, it may be that the range of weights observed are still representative of self-fertilised *D. reticulatum* generally.

That the growth of self-fertilised slugs was much faster than that of generation one slugs reared under the same conditions supports the hypothesis proposed in *Chapter 3* that the division of *D. reticulatum* into slow and fast growers may be due to whether the eggs are fertilised with allosperm or autosperm. Moreover, these results go further and indicate that if this is the case, then the faster growing slugs would probably be fertilised with autosperm.

There were much wider standard errors in the growth data for self-fertilised generation two slugs compared to generation one which are due to the markedly smaller sample size, although these still do not overlap between generations. The similarity in mean weight from weeks 0-4, regardless of the rearing treatment as

reported in *Chapter 3*, is also apparent in these results. After this time, again, there is a rapid divergence in weight between the generations which increases with time.

Unfortunately, as for generation one, the egg size in generation two was not measured. Despite the slower development time of the self-fertilised generation two eggs, the significantly higher growth rate of the offspring would predict that the eggs were smaller than those of generation one, according to Shibata and Rollo (1988).

4.4.6 Survival of self-fertilised slugs

The cumulative chance of survival of self-fertilised *D. reticulatum* was much lower than for generation one slugs at all ages, the difference between the generations increasing with time. By week 20, self-fertilised slugs had a less than 50% chance of being alive compared to more than 80% for generation one slugs. The difference in survival between these generations was much greater than between any of the treatments compared within generation one (*Chapter 3*). There was, however, the same apparent trade-off between growth and survival.

It was seen in *D. agreste,* which frequently self-fertilises, that self-fertilised slugs had a longer lifespan than those that were cross-fertilised (Chen *et al.*, 1984). The reverse was seen in the current experiment with *D. reticulatum* suggesting, again, that self-fertilisation is a 'back-up strategy' in this species. Whilst self-fertilised slugs are generally not as 'fit' as generation one slugs in terms of hatching rate, longevity etc. they may at least provide a 'stop gap' cohort. If these individuals were then to mate, resulting in cross-fertilised offspring, this may explain the sudden explosion in *D. reticulatum* populations reported in areas where numbers had previously been severely reduced by adverse conditions (Miles *et al.*, 1931; South, 1989b).

4.4.7 Third generation eggs

One of the second generation self-fertilised slugs laid a batch of eggs. This third generation batch failed to hatch and did not show any signs of development during

the 10 week monitoring period. Although no conclusions can be drawn from the results of a single batch, it may be speculated that if self-fertilisation acts as an emergency 'back up strategy', it is not an effective long-term coping mechanism and might only have the potential to 'buffer' a population against adverse conditions for one generation.

4.5 Conclusions and Future Work

This study explored the potential of *D. reticulatum* to self-fertilise and monitored the growth and survival of such offspring, which was compared with that of slugs hatching from eggs laid by field collected parents, reared under the same conditions (generation 1, *Chapter 3*).

In conclusion it was found that:

1. Generation one *D. reticulatum* laid eggs when reared in isolation (*Chapter 3*). Some of these hatched giving rise to a self-fertilised second generation.
2. For the generation one parental population there was no clear relationship between mean weight and age at egg laying.
3. For the second generation eggs development time was longer and hatching rate was lower compared to the parental population. The egg batches were also smaller and batch size was a poor predictor of whether any of the eggs in a batch hatched.
4. The growth rate of self-fertilised *D. reticulatum* showed marked variation under identical conditions and was significantly faster than that of the parental population. It is suggested that growth rate in field populations may be connected to whether eggs are fertilised by autosperm or allosperm.
5. Survival of self-fertilised *D. reticulatum* was significantly lower at all ages than the parental population.
6. Fast growth and longevity seem to be mutually exclusive.
7. Eggs laid by the self-fertilised *D. reticulatum* did not hatch.

To investigate the hypotheses put forward in this chapter further and to confirm preliminary results it is necessary to increase the sample size of the second generation slugs. Second generation eggs were laid readily and it would be informative to collect them for a longer period after laying commences, (e.g. for 2 weeks, 4 weeks, 6 weeks etc.) to see if there is an increase in viability with time. It would also be interesting to develop a 'breeding programme' to alternate between crossing and selfing generations to see whether the trends in hatching and development revert to generation one patterns (*Chapter 3*), and whether the population as a whole can be maintained this way. It would help in interpreting results if it could be determined whether eggs laid by isolated individuals have actually been fertilised, but for some reason the embryos are not viable, or are simply failing to develop because they have not been fertilised by either allo- or autosperm.

It has been consistently seen in both generation one (*Chapter 3*) and generation two slugs that fast growth and longevity seem to be mutually exclusive; individuals that grow quickly also die quickly regardless of the mode of fertilisation. A natural extension to this finding would be to try and model the relationship between growth and longevity which may be of value in predictions of population dynamics.

Chapter 5

The Relationship between Weight and Female-Phase Sexual Maturity in *Deroceras reticulatum* (Müller)

Abstract

Sexual maturity of *Deroceras reticulatum* (Müller) was classified using a five category system based on body, ovotestis and albumen gland weights. This categorical system was shown to be efficient in allocating individuals to a maturity class. There was a significant relationship between body weight and female-phase maturity for laboratory reared individuals and this was described with a probability equation. The relationship was validated against field-collected slugs and found to predict female-phase maturity with 86% accuracy. The probability of slugs being mature females was relatively low, even at high body weights. There may be a male-phase bias in populations of *Deroceras reticulatum* with less than 20% physiologically female individuals at any time during the year. This requires further testing in other locations. The implications of these findings for control are discussed.

5.1 Introduction

Growth rate in slugs has been shown to be a very 'plastic' characteristic varying considerably even between individuals from the same egg batch (e.g. Hunter, 1978; Shibata & Rollo, 1988; South, 1992). All size classes of slugs may, as a result, be found in a population at any one time (Haynes *et al.*, 1996) and it is of interest to know whether this is reflected in the maturity structure.

There is considerable disagreement in the literature concerning the relationship between body weight and maturity, in large part due to differences in the way that maturity is defined and assessed. For example, Runham and Laryea (1968) defined maturation on a gland by gland basis, Smith (1966) divided maturity into male and female-phase, Hunter (1968b) considered slugs to be mature if the hermaphrodite duct contained sperm or eggs and Haynes *et al.* (1996) based their conclusions on combined gland and body weight analyses. Assessments of maturity have, consequently, been based on one of two approaches: histological classifications (Smith, 1966; Hunter, 1968b; Runham & Laryea, 1968; Parivar, 1978; Runham, 1978) or the use of gland indices, i.e. gland/body weight ratios (Sokolove & McCrone, 1978; Duval & Banville, 1989; Haynes *et al.*, 1996). The former are time consuming and difficult to quantify reliably whereas the latter, while remedying the disadvantages of the former, result in numerical values that are not particularly intuitive to interpret and do not allow the separation of body weight from gland weights.

The experiments presented in this chapter were designed to explore the relationship between body weight and female-phase maturity in *Deroceras reticulatum* and used multivariate techniques to develop a new system of maturity classification. This was based on laboratory reared slugs described in *Chapter 3* and was validated against field collected slugs. By focusing on the female-phase the results relate to that section of the population capable of egg laying and may, therefore, be of applied use in the indirect estimation of population egg banks. Such estimates are particularly difficult to obtain by direct methods and would be of considerable benefit in studies of population dynamics.

5.2 Materials and Methods

5.2.1 Materials

The slugs used in these experiments were preserved specimens from the study of growth and survival described in *Chapter 3*. They had been laid in autumn by field

collected 'parents'. After hatching they were reared in isolation and fed *ad libitum* on Chinese cabbage and carrots. Their growth was monitored regularly; when monitoring ceased they were preserved in 70% ethanol. This was changed and replaced with fresh 70% ethanol two weeks after they were preserved. Their weight at preservation was recorded.

Field collected specimens were used to validate the results from laboratory reared slugs. These were preserved in the same way as the laboratory specimens and came from Close House Field Station, Heddon-on-the-Wall, Northumberland (Grid reference NZ 127659).

5.2.2 Methods

5.2.2.1 Dissection Procedure

Forty two preserved laboratory reared slugs and 50 preserved field collected specimens were dissected to remove the ovotestis (also known as the hermaphrodite gland) and albumen gland. Both samples comprised a similar range of body weights over 200 mg; the glands in slugs weighing less than this were too small to be identified with certainty. Each slug was weighed before dissection using a Mettler MT 5 balance to an accuracy of 0.01 mg. Due to the evaporation of ethanol from the body surface on exposure to air the recorded weight fluctuated and therefore a standard 'settling period' of five seconds was allowed between placing the slug on the balance and taking the reading.

The glands were dissected from each individual according to the procedure outlined in Bullough (1970), augmented with modifications from Reise and Hutchinson (2001) and Block (1967), the latter being a method for snail dissection. The final protocol was as follows:

1. Any mucus was wiped from the surface of the slug. The slug was then placed in a wax filled dissection tray.
2. The first incision was made by cutting the integument along the left foot fringe using a pair of small spring loaded scissors. This resulted in less damage to the

distal genitalia than following the usual recommendation of a median cut (J Hutchinson, *pers. comm..).*

3. The mantle was peeled back over to the right hand side and pinned out, taking care not to puncture any of the internal organs (*Fig. 5.1).*

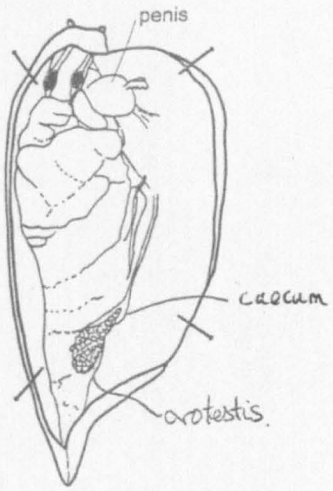


Figure 5.1: Pinning out the slug for dissection (after Reise & Hutchinson, 2001).

4. The dissection tray was filled with 70% ethanol to completely immerse the slug. [*The ovotestis is immediately visible lying to the posterior of the animal, dorsal to the visceral mass. It is a brown/purple coloured structure. The reproductive system runs very close to the digestive system and the two are entwined*] *(Appendix C).*
5. The reproductive and digestive system were separated by carefully teasing apart, beginning at the ovotestis and working forwards.
   [The narrow hermaphrodite duct emerges from the ovotestis. It is unconvoluted and passes between the crop and the digestive gland, entering the large, creamy-coloured albumen gland].
6. The hermaphrodite duct was cut from between the ovotestis and the albumen gland (*Appendix D*). The ovotestis was placed into a glass tube containing 70% ethanol prior to weighing.

[The convoluted common duct arises from the albumen gland and continues towards the anterior of the animal where it separates into two distinct male and female ducts].

7. The emerging common duct was cut from the albumen gland at the point of entry and the gland was placed into a second glass tube containing 70% ethanol prior to weighing.

5.2.2.2 Weighing dissected glands

The dissected ovotestis and albumen gland were weighed using a Mettler MT 5 balance to an accuracy of 0.01 mg. A small plastic dish (3.5 cm diameter) was used as a weighing receptacle and the balance was tared to zero before the gland was added. As for weighing the intact slug, a standard settling time of five seconds was allowed to account for fluctuation in the reading due to the evaporation of ethanol from the glands upon exposure to air.

5.2.3 Statistical analysis

The relationship between fresh and preserved weight was assessed using linear regression.

For laboratory reared slugs principle components analysis (PCA) was used to explore the extent to which the measured body and gland weights accounted for the overall differences between individuals in the sample. The outcome of this was used to define a 'maturity score' which classified slugs into one of five groups (immature, early maturation, mid maturation, late maturation and mature).

Discriminant analysis (DA) was carried out to assess the accuracy of the maturity scoring system derived from PCA and binary logistic regression was used to evaluate the relationship between maturity score and body weight.

Data from field collected slugs were used to validate the binary logistic regression equation.

5.3 Results

5.3.1 Relationship between preserved and fresh weight of specimens

Linear regression showed that there was a strong significant relationship between the fresh and preserved weight of laboratory reared slugs (Linear regression: N = 42, $R^2 = 0.993$, $P < 0.001$) (*Fig. 5.2*).

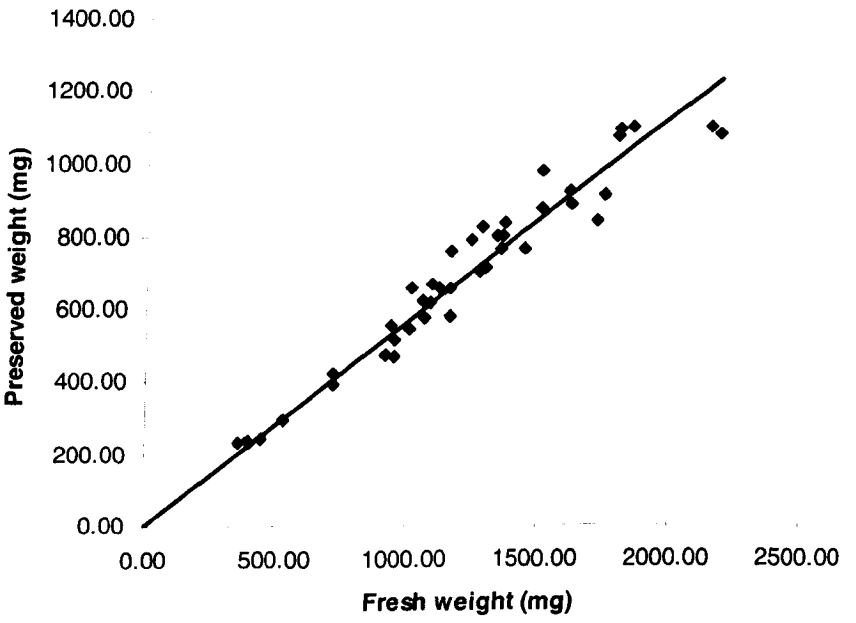


Figure 5.2: Relationship between fresh and preserved weight of laboratory reared Deroceras reticulatum. *Regression equation (line intercepts at origin): Preserved weight= 0.565 x fresh weight.*

A sample of 20 field collected slugs were also weighed before and after preservation and the relationship was almost identical (Linear regression: N = 20, $R^2 = 0.996$, $P < 0.001$; Regression equation: preserved weight = 0.567 x fresh weight).

It was assumed that the change in intact weight due to preservation would also apply approximately to the gland weights. The regression equation of the laboratory reared

slugs was, therefore, used to estimate the fresh weights of dissected glands (*Table 5.1*).

5.3.2 Derivation of relationship between body weight and maturity in laboratory reared specimens

5.3.2.1 Dissected gland weights

Table 5.1 shows the body and gland weights of the laboratory reared slugs. There were no significant differences in weight of selected slugs that were reared at different temperatures and therefore the data were pooled (ANOVA: $F_{2, 39} = 1.222$, *n.s.*). In one specimen (slug 9) there was no albumen gland. *Table 5.2* shows similar data for the field collected slugs.

5.3.2.2 Classification of slugs according to a maturity score

Principle components analysis showed that all three weight variables were required to adequately describe the differences between individuals in the sample (*Table 5.3*).

Table 5.3: Eigenvalues and component weightings for principle components analysis on body weight, ovotestis and albumen gland weights in Deroceras reticulatum.

	Principle Component		
Variable	*1*	*2*	*3*
Body weight	-0.577	0.576	0.579
Ovotests weight	-0.568	-0.793	0.221
Albumen gland weight	-0.586	0.201	-0.785
Eigenvalue	2.182	0.441	0.377
% total variance accounted for	72.7	14.7	12.6

Pearson correlation confirmed that body weight and both gland weights significantly contributed to principle component 1. In addition to body weight, component 2 was correlated significantly with ovotestis weight and component 3 was correlated significantly with albumen gland weight (*Table 5.4*).

Table 5.1: Body and gland weights of laboratory reared Deroceras reticulatum.

Slug ID	Body Weight (mg)		Gland Weight (mg)			
			Ovotestis		Albumen Gland	
	Fresh	Preserved	Fresh[§]	Preserved	Fresh[§]	Preserved
1	440.42	243.37	1.29	0.73	1.66	0.94
2	528.61	297.09	5.26	2.97	6.85	3.87
3	719.57	391.74	28.55	16.13	6.96	3.93
4	915.29	471.70	10.80	6.10	78.65	44.44
5	934.95	554.07	8.25	4.66	94.85	53.59
6	1015.20	654.49	9.96	5.63	168.42	95.16
7	1092.63	666.13	11.06	6.25	110.46	62.41
8	1117.35	655.28	17.75	10.03	125.20	70.74
9	1161.11	759.09	31.66	17.89	n/a[†]	n/a[†]
10	1271.20	700.37	11.40	6.44	120.64	68.16
11	1293.14	714.88	6.92	3.91	153.40	86.67
12	1444.05	764.13	13.47	7.61	126.60	71.53
13	1720.34	840.69	14.97	8.46	70.34	39.74
14	1745.30	911.59	12.51	7.07	105.70	59.72
15	358.96	233.16	0.44	0.25	0.27	0.15
16	403.22	235.11	1.27	0.72	4.53	2.56
17	1057.03	623.13	24.04	13.58	132.41	74.81
18	1159.26	657.73	15.49	8.75	140.25	79.24
19	1161.10	579.20	23.10	13.05	128.42	72.56
20	1282.78	825.69	21.13	11.94	168.16	95.01
21	1341.26	798.06	30.39	17.17	187.96	106.20
22	1351.40	762.34	27.49	15.53	197.26	111.45
23	1503.96	877.24	20.62	11.65	207.33	117.14
24	1611.62	921.67	32.39	18.30	191.04	107.94
25	1621.20	885.23	26.97	15.24	165.58	93.55
26	1803.63	1093.84	14.78	8.35	100.37	56.71
27	2147.36	1100.33	17.79	10.05	151.43	85.56
28	2181.35	1078.76	25.75	14.55	189.22	106.91
29	356.18	233.21	2.16	1.22	2.81	1.59
30	397.41	237.73	3.93	2.22	2.85	1.61
31	721.75	420.66	6.48	3.66	91.91	51.93
32	948.80	468.22	15.56	8.79	161.10	91.02
33	952.26	512.39	18.41	10.40	185.70	104.92
34	1008.49	546.25	22.99	12.99	60.21	34.02
35	1064.35	576.72	17.73	10.02	236.16	133.43
36	1082.12	615.85	13.03	7.36	183.84	103.87
37	1238.46	789.84	15.59	8.81	170.30	96.22
38	1360.47	797.38	17.91	10.12	44.57	25.18
39	1367.16	835.91	19.15	10.82	199.27	112.59
40	1509.59	977.60	17.93	10.13	170.50	96.33
41	1796.23	1073.58	12.71	7.18	156.73	88.55
42	1850.78	1098.72	16.00	9.04	129.26	73.03

§ These weights were predicted from the regression described in section 5.3.1.1, Fig. 5.2.
† There was no albumen gland in this specimen.

Table 5.2: Body and gland weights of field collected Deroceras reticulatum *from Close House Field Station, Northumberland.*

Slug ID	Body Weight (mg)		Gland Weight (mg)			
			Ovotestis		Albumen Gland	
	Fresh	Preserved	Fresh §	Preserved	Fresh §	Preserved
1	363.29	213.84	18.74	10.30	n/a†	n/a†
2	410.04	238.91	11.72	6.44	26.66	14.65
3	410.19	238.99	16.54	9.09	21.45	11.79
4	411.18	239.52	16.32	8.97	n/a†	n/a†
5	416.53	242.39	10.24	5.63	24.09	13.24
6	442.00	256.05	19.47	10.70	23.76	13.06
7	443.03	256.60	7.22	3.97	41.90	23.03
8	457.07	264.13	29.06	15.97	15.43	8.48
9	468.01	270.00	9.10	5.00	26.47	14.55
10	470.60	271.39	28.04	15.41	17.61	9.68
11	484.05	278.60	8.64	4.75	30.33	16.67
12	493.91	283.89	9.17	5.04	19.27	10.59
13	497.03	285.56	18.18	9.99	13.79	7.58
14	501.82	288.13	37.97	20.87	15.99	8.79
15	511.16	293.14	13.99	7.69	23.62	12.98
16	540.19	308.71	17.01	9.35	18.81	10.34
17	558.50	318.53	23.82	13.09	21.02	11.55
18	567.10	323.14	18.65	10.25	40.63	22.33
19	568.11	323.68	15.10	8.30	47.73	26.23
20	575.23	327.50	11.75	6.46	36.15	19.87
21	590.07	335.46	10.90	5.99	38.03	20.90
22	596.04	338.66	15.81	8.69	50.64	27.83
23	607.92	345.03	8.66	4.76	49.60	27.26
24	627.46	355.51	13.57	7.46	26.46	14.54
25	645.25	365.05	16.28	8.95	29.86	16.41
26	651.16	368.22	7.68	4.22	34.57	19.00
27	651.73	368.53	8.11	4.46	45.41	24.96
28	692.18	390.22	12.95	7.12	31.48	17.30
29	708.59	399.02	14.26	7.84	36.19	19.89
30	763.28	428.35	21.80	11.98	23.60	12.97
31	812.24	465.95	16.05	8.82	52.98	29.12
32	815.68	459.43	25.86	14.21	68.85	37.84
33	871.11	477.41	22.62	12.43	84.59	46.49
34	873.10	477.34	13.23	7.27	80.75	44.38
35	875.26	446.57	16.27	8.94	83.70	46
36	896.29	551.80	17.94	9.86	64.23	35.30
37	925.54	526.85	27.55	15.14	85.43	46.95
38	928.28	485.20	30.99	17.03	58.10	31.93
39	957.46	591.57	12.72	6.99	55.95	30.75
40	961.26	571.60	14.63	8.04	167.92	92.29
41	961.85	535.2	19.21	10.56	111.46	61.26
42	981.96	567.59	34.15	18.77	80.68	44.34
43	1012.41	610.49	10.70	5.88	112.52	61.84
44	1016.80	530.47	22.05	12.12	94.43	51.90
45	1020.04	596.05	26.11	14.35	36.90	20.28
46	1025.36	656.28	14.94	8.21	55.70	30.61
47	1146.24	616.93	6.15	3.38	170.14	93.51
48	1156.72	689.04	17.69	9.72	108.70	59.74
49	1189.52	614.51	8.66	4.76	117.78	64.73
50	1264.75	705.54	23.67	13.01	76.64	42.12

§ These weights were predicted from the regression described in section 5.3.1.1(Fig. 5.2).
† There was no albumen gland in this specimen.

Table 5.4: Pearson correlation coefficients between weight variables and each principle component with associated P-values.

Principle Component	*Body Weight*	*Ovotestis Weight*	*Albumen Gland*
1	-0.856	-0.841	-0.878
	P < 0.001	*P < 0.001*	*P < 0.001*
2	0.406	-0.517	0.100
	P < 0.01	*P < 0.01*	*n.s.*
3	0.322	0.182	-0.469
	P < 0.05	*n.s.*	*P < 0.01*

Each of the principle components was plotted against the weight variables with which it was significantly correlated. The plots were inspected to determine where there were divisions between clusters of data points and individuals were given a maturity code for each plot according to the cluster they belonged to. If there were two clusters the codes were 1 for immature and 2 for mature; if there were three clusters the codes were 1 for immature, 2 for maturing and 3 for mature. An example is shown in *Fig. 5.3.* These codes were then combined to assign each individual a single overall value for that principle component based on the protandric sequence of reproductive development in this species. There is some overlap between developmental stages and hence some judgement had to be exercised, but *Table 5.5* summarises the general 'decision matrix' for the assignment of these codes. Finally, the three principle component scores for each individual were used to categorise it in one of five overall 'maturity groups' (*Table 5.6*). The first digit in the three figure codes shown in *Table 5.6* refers to the maturity score for the first principle component (as detailed in *Table 5.5*), the second digit is the score for the second principle component and finally the third digit is that assigned for the third principle component.

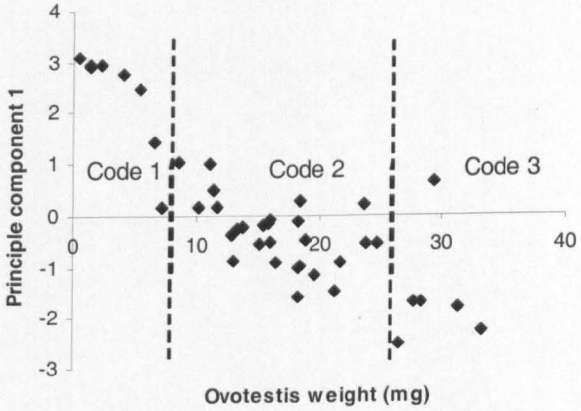


Figure 5.3: An example to show how individuals were assigned maturity codes. Weight variables were plotted against each principle component with which they were significantly associated. The plots were then divided into clusters by visual inspection and each individual was assigned a code according to the cluster it belonged to.

Table 5.5: Maturity scores for each principle component. Brackets indicate codes where judgement most commonly had to be exercised due overlapping between gland developmental stages.

Principle Component	*Maturity Code*	*Body Weight*			*Ovotestis Weight*			*Albumen Gland Weight*		
		Sml	*Med*	*Lge*	*Sml*	*Med*	*Lge*	*Sml*	*Med*	*Lge*
1	1	+			+			+		
	2		+			+			+	
	3			+			+			+
2	1	+			+					
	2		+		+	(+)				
	3			+			+			
3	1	+						+		
	2		+					(+)	+	
	3			+						+

Table 5.6: Final 'maturity group' categories.

Category	*Maturity*	*Separate PCA Codes*
1	Immature	111
2	Early maturation	222
3	Mid maturation	232/231
4	Late maturation	223
5	Mature	233/333

5.3.2.3 Accuracy of maturity classification

Discriminant analysis was used to assess how well the classification of weight variables into five maturity groups distinguished between individuals. This analysis generates a 'discriminant function' based on combinations of predictor variables (in this case weight) that best divide individuals into a requested number of groups (in this case five). This was compared with the five maturity groups derived from the principle components analysis and there was 95.1% agreement between the two (*Table 5.7*).

Table 5.7: Results of discriminant analysis to assess accuracy of maturity classification.

Maturity group assigned by PCA	*Group predicted from Discriminant Analysis*				
	1	*2*	*3*	*4*	*5*
1	6	0	0	0	0
2	0	12	0	1	0
3	0	0	4	0	0
4	0	1	0	12	0
5	0	0	0	0	5
Total	6	13	4	13	5
Number Correct	6	12	4	12	5
% Correct	100.0	92.3	100.0	92.3	100.0

The two individuals misclassified in the maturity groups assigned by principle components analysis were numbers 10 and 17 (*Table 5.1*). These had been put into groups 2 and 4 respectively (early and late maturation stages) and the

misclassification is likely to have arisen due to overlap between the development of the ovotestis and albumen gland making them difficult to classify. The probabilities of them belonging to the alternative groups assigned by the discriminant analysis (4 and 2 respectively) were only marginally higher than those of the groups they had been classified in, but values were, in any case, reassigned.

5.3.2.4 Relationship between weight and maturity

Binary logistic regression showed that there was a significant relationship between body weight and maturity (Binary logistic regression: N = 41, Z = 1.06, $P < 0.05$; percent concordant pairs = 71.3%). The curve predicted from the regression describes the probability that a slug of a given body weight is female-phase mature (*Fig. 5.4*). As can be seen, this curve is smooth and flat, which reflects the gradual nature of maturation in *D. reticulatum*. Although age data were available for each slug, this was not included in the binary logistic regression as it cannot be used in classifying field collected specimens, whose ages are unknown.

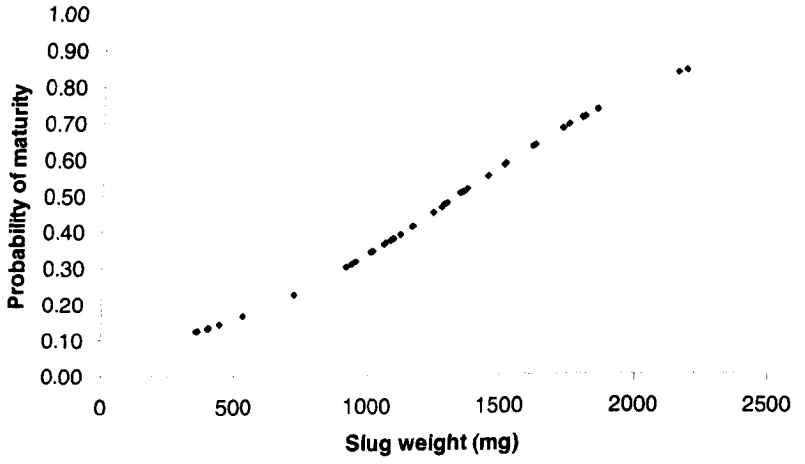


Figure 5.4: The relationship between weight and maturity for laboratory reared Deroceras reticulatum *as predicted by binary logistic regression. Binary logistic regression equation: Probability of maturity = (exp (-2.654 + 0.001981 x weight)) / (1 + exp (-2.654 + 0.001981 x weight)).*

5.3.3 Validation of relationship between body weight and maturity in laboratory reared slugs with field collected specimens

The 50 field collected *D. reticulatum* were classified as female-phase mature or immature based on gland weights. The criteria upon which this decision was made are in accordance with the known protandric sequence of development of this species, namely that the ovotestis develops first and enlarges, followed by development and enlargement of the albumen gland and gradual reduction in size of the ovotestis as the slug enters the egg laying, female-phase (Runham & Laryea, 1968).

The weights of albumen glands were plotted against ovotestis weight to observe the spread in sizes. Data were divided into 'large' and 'small' groups for each gland; this was only an approximate division as there were no very clear delineations between individuals and some judgement had to be exercised to decide whether a slug was mature. In general, all slugs with a small albumen gland were considered immature and those with a large albumen gland were assessed as mature. For individuals on the borderline between small and large, the weight of the ovotestis was taken into account; if this was small, the slug was classed as mature (*Fig. 5.5*).

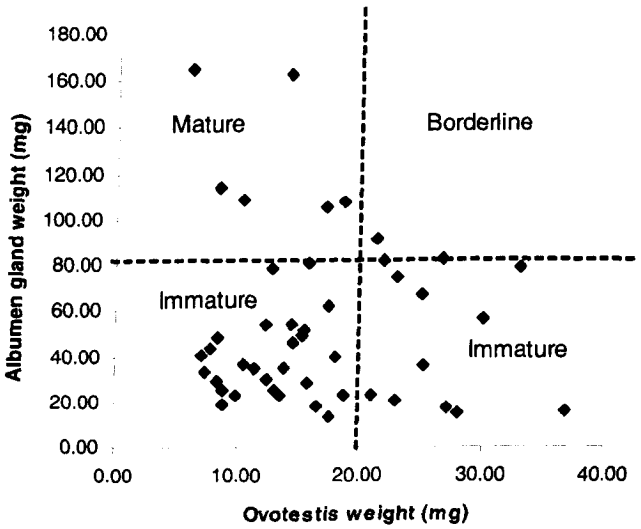


Figure 5.5: Approximate divisions of field collected Deroceras reticulatum *into mature and immature groups. A value judgement was exercised for borderline individuals (see text).*

The logistic regression equation based on the relationship derived in the laboratory (*Fig. 5.4*) was applied to this field collected sample to indicate the probability of a slug being mature. Since probability is a continuous variable, it needed to be dichotomised in order to compare agreement between the predicted and actual categorisation of field collected specimens which were assigned a binary classification (mature/immature). Various thresholds were applied to divide the laboratory reared slugs into mature and immature groups based on their probability of being mature, according to Fielding & Bell (1997). Calculations of the percentage of correct classifications, sensitivity (false negative errors) and specificity (false positive errors) were performed to select the optimum threshold that minimised false negative errors whilst giving the highest agreement between classifications and as low a false positive rate as possible (false negatives errors were regarded as more serious than false positive errors with respect to predicting the need for control in the field). Results are summarised in *Table 5.8* and indicate that a probability threshold of 0.25 optimised the performance of the model with 86% agreement and 100% sensitivity. The minimum weight corresponding to this threshold was approximately 700 mg, i.e. 700 mg was the minimum weight at which a slug is considered female-phase mature.

Table 5.8: Performance of binary logistic regression model.

Probability Threshold	*% Agreement with visual classification of field specimens*	*Sensitivity*	*Specificity*
0.15	40	1.00	0.81
0.20	74	1.00	0.35
0.25	86	1.00	0.19
0.30	86	0.77	0.11
0.35	82	0.31	0.00
0.40	82	0.31	0.00
0.45	76	0.08	0.00
0.50	74	0.00	0.00

Probability thresholds refer to the cut-off points tested to categorise slugs as mature and immature (binary classification) based on the predicted probability of maturity.

5.3.4 Implications of binary logistic regression equation for field populations of *D. reticulatum*

The binary logistic regression equation *(Fig. 5.4)* was applied to a database of weights from field populations of *D. reticulatum* collected from the same site between August 1997 & June 1999 (MAFF project CSA 3396). Data for each month were pooled for all years and the minimum slug weight for each 10% increase in the chances of being mature was calculated. The numbers of the population in each maturity bracket are shown in *Table 5.9.*

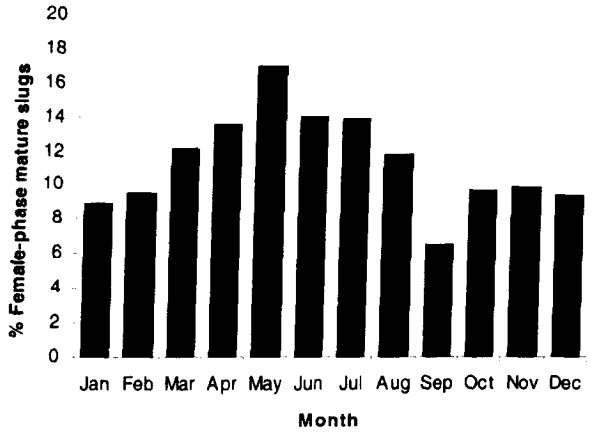
Table 5.9: Numbers of field collected Deroceras reticulatum *of the minimum weight required for sequential 10% increases in the chances of maturity as predicted by binary logistic regression equation derived from laboratory reared individuals (Fig. 5.4). (Based on data from Heddon Banks Farm, Northumberland 1997-1999,* MAFF project CSA 3396*).*

Month	*Total catch*	*Minimum weight (mg) for given percentage chance of maturity (in brackets)*									
		0- (<10%)	*230 (10%)*	*640 (20%)*	*920 (30%)*	*1140 (40%)*	*1340 (50%)*	*1550 (60%)*	*1770 (70%)*	*2040 (80%)*	*2450 (>90%)*
Jan	706	457	227	16	5	1					
Feb	566	383	158	2	5	12	3	2	1		
Mar	792	429	293	19	14	13	11	6	4	3	
Apr	200	89	87	8	8	3		3	1	1	
May	1011	375	452	61	23	20	28	22	22	5	3
Jun	3856	672	3002	119	36	14	4	3	2	1	3
Jul	2060	418	1447	180	13	2					
Aug	897	301	589	5			1				1
Sep	2487	2120	363		1	2					1
Oct	1253	666	582	2		3					
Nov	1501	871	553	52	19	5	1				
Dec	611	375	207	23	4	2					

By taking the mid-point of each maturity bracket the number of mature slugs of the required minimum weight expected to be found can be estimated. For example, in January 457 slugs in the 0-230 mg weight range have a 0-10% chance of being mature, hence approximately 5% of these are actually likely to be mature, i.e. 23 slugs. Summing the numbers of mature slugs estimated for each month suggests that less than 20% population are predicted to be female-phase mature at any time, i.e. the

majority of the field population are immature, and the pattern of the percentage of mature slugs appears to be cyclical (*Fig. 5.6*).

Figure 5.6: Monthly percentages of female-phase mature Deroceras reticulatum *in the field (1997-1999 combined) as predicted by binary logistic regression equation derived from laboratory reared individuals (Fig. 5.3). (Based on data from Heddon Banks Farm, Northumberland 1997-1999,* MAFF project CSA 3396*).*



This trend was also consistent when years were looked at separately; data for part-years in 1997 and 1999 followed the corresponding pattern for the same period shown in *Fig. 5.6* and the complete data for 1998 mirrored the cyclical pattern for combined years extremely closely (*Fig. 5.7*).

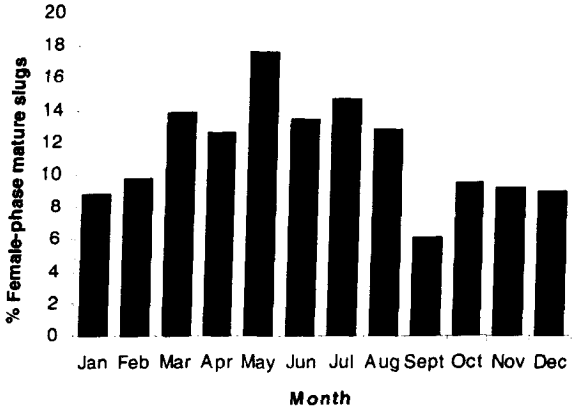


Figure 5.7: Monthly percentages of female-phase mature Deroceras reticulatum *in the field (1998) as predicted by binary logistic regression equation derived from laboratory reared individuals (Fig. 5.3). (Based on data from Heddon Banks Farm, Northumberland 1997-1999, MAFF project CSA 3396).*

5.4 Discussion

5.4.1 General methodology

The few studies in the literature concerning the effect of temperature on the reproductive development of terrestrial slugs concern the species *Arion ater* (L.). They report that temperature can affect the speed of development of the reproductive tract, but not the sequence (Smith, 1966) and that constant very high (27°C) or very low (4°C) temperatures affect the development of gametes (Lusis, 1966), but temperatures between these extremes do not markedly disrupt the process. Although there is nothing published that has investigated this in *D. reticulatum*, it is known to be less sensitive to temperature effects than many other temperate slugs, including *Arion* species (Mellanby, 1961). In the current experiment there were, in addition, no differences in the mean weights between individuals sampled from each rearing temperature and it seems unlikely that pooling the slugs would have unduly confounded the results.

There was a very consistent relationship between the fresh and preserved weight of laboratory reared slugs across the whole range examined; the relationship was almost identical for those collected from the field. Slugs lost just under half their body weight due to preservation and this reflects the movement of body fluids from the animals to the more concentrated ethanol preserving medium. It was assumed that the relationship shown for body weights also applied to gland weights. The glands had different surface area/volume ratios and water content compared to the intact body and, therefore, this assumption may be criticised. However, these differences in surface area/volume ratio and water content also apply to different body sizes, and since the relationship held across the full range of intact weights, it is considered to be a reasonable approximation in the absence of any other data.

The field specimens were collected during routine trapping work and individual weights were, unfortunately, not recorded for all slugs. Of the sample whose weights were available, the relationship between fresh and preserved weight was remarkably similar to that for laboratory reared slugs implying that they lost body fluids at a

similar rate. The equation for laboratory reared slugs was used to estimate the fresh gland weights of all slugs; since the equations for this group and the sample of field collected slugs were virtually identical it would make little difference which was used, but for consistency, the equation derived from the laboratory reared slugs was applied as it was based on a larger sample.

Many authors have highlighted the difficulty of dissecting glands from very small slugs (e.g. Lusis, 1961; Runham & Laryea, 1968; Duval & Banville, 1989). In the current experiments it was found that for slugs below a preserved weight of ~200 mg (fresh weight ~350 mg) the glands, particularly the albumen gland, could not be identified with certainty. It is reported that very early on in maturation, whilst body size increases, glands remain completely undifferentiated and the albumen gland and common duct are impossible to separate (Smith, 1966). Furthermore, once differentiation begins, early changes in glands occur at a cellular level without an accompanying increase in gland weight (Runham & Laryea, 1968). These factors, in addition to the 'shrinking' effect of ethanol on the weights of already small glands, probably explain the problems encountered with small slugs in this study and it can be confidently inferred that such individuals were immature.

This study may be criticised on the grounds that some analyses have been based on the results of other analyses rather than empirical data and hence any uncertainties in the outcome of one are propagated in those that follow. This is a valid consideration, but is inevitable in exploratory work that generates hypotheses. The limitations of the study presented here are, therefore, acknowledged, but it is emphasised that it should be regarded as investigative. The possibilities it raises pose interesting questions, but these require further examination in order to take this work further.

5.4.2 Maturity classification

The present study used principle components analysis to separate the effects of body, ovotestis and albumen gland weights arriving at a more flexible classification system than histological or gland indices approaches used previously with the particular advantage that overlap between male and female-phases could be interpreted in

relation to the overall body size. A disadvantage is that the decisions regarding classification are subjective and may, therefore, be less reproducible than a strict numerical system, requiring knowledge of *D. reticulatum* biology. It does, however, result in a higher proportion of correct classifications as assessed by discriminant analysis than the gland indices approach where this has been explicitly reported (Duval & Banville, 1989; Haynes *et al.*, 1996).

The five group system used to discriminate between different maturity stages was found to be optimum, which agrees with Haynes *et al.* (1996). A classification system with fewer groups did not distinguish sufficiently between individuals to divide them into classes that were significantly different from one another. The basis of this five group system (*Table 5.4*) reflects broadly the development of the two glands as revealed by histological analysis (Runham & Laryea, 1968) and, therefore, has an empirical foundation. In this study the authors showed that there is an initial very slow and small increase in weight of both organs, the albumen gland lagging the ovotestis, followed by a rapid increase in the ovotestis size due to spermatogenesis and male-phase maturation. This is followed by an increase in the weight of the albumen gland due to accumulation of secretory products in the female-phase. The size of the albumen gland continues to increase to a maximum whilst that of the ovotestis decreases. This is also comparable with the stages described for *A. ater* (Smith, 1966).

5.4.3 Relationship between weight and maturity

In the current study, maturity was defined anatomically as having a large albumen gland following an increase in ovotestis size (maturity class 5), i.e. being physiologically female. There was a significant relationship between body weight and this measure of maturity for laboratory reared *D. reticulatum,* agreeing with the anatomical assessment of Haynes *et al.* (1996). The work of Lusis (1961) and Parivar (1978) on gland volumes in *A. ater* also supports this finding, showing a strong relationship between albumen gland volume and body weight in laboratory reared specimens. The study of Smith (1966) found no relation between body weight and maturity, but assessed this at a cellular level, resolving maturity into a much

larger number of stages some of which involved changes only discernable by histological techniques.

If egg laying had commenced in individuals when they were preserved it had done so only recently. In most individuals classified as mature, therefore, the albumen gland was reaching its peak size. It has been shown that the processes of growth and reproduction are antagonistic; adult *D. reticulatum* generally lose weight during reproduction even when food is plentiful and death ensues very rapidly after egg laying is completed (Rollo, 1988). It may be speculated that the relationship between body weight and maturation would have been modified had egg laying been a little more advanced in the mature slugs.

Sokolove and McCrone (1978), working with *Limax maximus* (L.), showed that male-phase maturation in this species was initiated by a short to long day-length transition in photoperiod whilst female-phase maturation seemed to be independent of the light regime. Although this has not been specifically tested in *D. reticulatum* it is suggested that it is less important in this species which is capable of breeding at any time of the year if temperature and humidity are favourable (South, 1989a). Other environmental factors such as quality of diet (Rollo & Shibata, 1991) and humidity (Lusis, 1966) have been shown to influence reproductive development, but it seems probable that these are mediated not directly, but rather through effects on feeding and growth and should not, therefore, alter the applicability of the relationship derived in the current study to different populations; for the derivation of the relationship the diet of the laboratory reared slugs was uniform and relative humidity remained high due to the culture conditions so there should be no confounding effect between samples.

5.4.4 Validation against field collected specimens

There was 86% agreement between those field collected slugs judged to be mature through visual inspection of gland weights and those predicted to be mature by the binary logistic regression equation based on body weight alone. This ostensibly suggests that the binary logistic regression equation is a very reliable means of

estimating female-phase maturity. The probability threshold at which its performance was optimised was, however, low at just 25%, i.e. the minimum weight at which *D. reticulatum* were considered to be female-phase mature by visual inspection (~700 mg) corresponded to just 25% chance as predicted by the binary logistic regression equation and, therefore, only the largest slugs were considered female-phase mature. It may be that there are differences in the weight at maturity between field and laboratory reared slugs. This could be tested by performing binary logistic regression on field collected slug data. Conventional wisdom would suggest that *D. reticulatum* of approximately 700 mg would be highly capable of laying eggs. Perhaps, therefore, field slugs mature at lower weights and the chances of them laying eggs at 700 mg are, in reality, much greater than 25%. On the other hand, independent studies that have explicitly investigated female-phase slugs in the population support the suggestion that numbers in this group may be relatively low. Lusis (1961) reports a consistent rarity of female-phase individuals in random samples of a wild population of *A. ater* and suggests that this is due to the female-phase being much shorter than the male-phase. Furthermore it has been shown in *D. reticulatum* that once egg laying commences the parent is 'sacrificed to augment reproduction' (Rollo, 1988) and hence the mature female-phase is short-lived and at any single point in time the chances of a slugs being female-phase mature is probably fairly low.

Application of the binary logistic regression equation to routinely collected weight data from field-caught specimens (MAFF project CSA 3396) indicated that at any time of the year less than 20% of the field population were mature by the definition used in the current study, i.e. in the female-phase. This requires further testing, but is supported by Haynes *et al.* (1996) who found that at any month of the year wild populations of *D. reticulatum* from a nearby site were rarely comprised of more than about 20-30% in maturity class 5 (large albumen glands and therefore in the female-phase). Application of the equation to routine data also suggested that changes in the percentage of mature slugs were cyclical, with approximately equal proportions of mature individuals in December as in the previous January. Moreover, the population of mature slugs is highest around summer and into autumn and then falls rapidly, coinciding with the known decline in adult numbers following egg laying

(South, 1992). The pattern was consistent between years, indicating that there may be some underlying natural pattern increasing confidence in the result. Further study is required to explore this finding further, for example, at different sites. It seems, however, that such a scenario would be feasible on biological grounds. For example, the cumulative numbers of eggs laid by individuals are high (Carrick, 1938) suggesting large numbers of mature females are not necessarily required to maintain the egg bank of a population. Furthermore, there may be selective disadvantages to being large such as the need for more food to maintain metabolic processes and greater vulnerability to environmental stress if adequate refuge sites cannot be found. This result, therefore, raises the possibility that populations of *D. reticulatum* may naturally be male biased, but since there is always a small chance that low weight slugs may be physiologically mature 'females', a small pool of egg-producing individuals would be maintained in the population at all times.

5.4.5 Implications for control

Directly estimating the 'egg bank' in slug populations is notoriously problematic due to the small size of eggs and the difficulties in extracting them from soil samples without damage. The relationship between body size and maturity demonstrated in this study may help to overcome this problem indirectly; the size structure of a population can be ascertained through sampling and the probability that slugs in different size classes will be mature may then be estimated from the binary logistic regression equation. This would indicate the proportion of slugs likely to be female-phase mature and hence capable of contributing to the egg bank of that population. Relative comparisons between years could help to predict an increased risk of population expansion which would help to refine our understanding of population dynamics in this species, aiding decisions regarding the timing of control measures.

If the growth rate of slugs in the field could be predicted and the minimum weight at which slugs become female-phase mature were to be confirmed by further investigations, it may be possible to 'back calculate' in order to estimate the size of slugs in spring that consequently reach egg laying condition by autumn. If there are large numbers of such slugs then control measures could be applied in a more

targeted way before they lay eggs, drastically reducing the potential population in the following year.

5.5 Conclusions and Future Work

In conclusion, it was found that:

1. A five-category system of maturity classification for *D. reticulatum* based on body, ovotestis and albumen gland weight was found to be very efficient in distinguishing between individuals.
2. There was a significant relationship between body weight and female-phase maturity for laboratory reared *D. reticulatum* and this could be described by a probability equation (binary logistic regression model).
3. The relationship between weight and maturity was validated against field data and was shown to predict female-phase maturity with 86% accuracy. The minimum weight at which *D. reticulatum* is considered to be female-phase mature by visual inspection of gland weights, however, corresponds to a probability of just 25% as predicted by the binary logistic regression equation.
4. There may be a male-phase bias in natural populations of *D. reticulatum* in Northumberland with fewer than 20% of physiologically female individuals throughout the year. This requires further testing in other locations.

As discussed in *section 5.4.5* the exploratory work in this chapter needs testing further. In particular, in order to evaluate concerns regarding whether laboratory reared and field collected *D. reticulatum* differ in their weight at maturity the equation could be validated with data from more laboratory reared specimens or, conversely, the binary logistic regression equation could be derived from field collected specimens. The results could then be compared with the current experiment to gauge its general applicability.

An interesting extension to this work would be to divide up the population of slugs studied into male-phase mature and female-phase mature to see whether it is possible

to derive a relationship between body weight and maturity, specific to each stage in reproductive development.

Section B

Control Efficacy using Molluscicide Pellets

The chapters presented in this section address aspects of control efficacy using molluscicide pellets.

Chapter 6 focuses on the behaviour of *D. reticulatum* in relation to different methods of pellet application and assesses whether this is affected by pellet condition.

Chapter 7 characterises the surface activity patterns of *D. reticulatum* on coarse and fine seedbeds. The influence of molluscicide pellets on these movement patterns is reported.

Chapter 6
Behaviour of *Deroceras reticulatum* (Müller) in Response to Broadcast, Drilled and Soil Contaminated Molluscicide Pellets

Abstract

The foraging, feeding and post-feeding behaviour of *Deroceras reticulatum* (Müller) in response to broadcast and drilled molluscicide pellets was investigated. The effect of pellet condition was evaluated by comparing 'clean' and soil contaminated pellets. After the onset of activity slugs began feeding on broadcast pellets much sooner than drilled pellets and were more likely to feed on the first pellet encountered. Methiocarb poisoned more slugs than metaldehyde when pellets were broadcast, but there was no difference between active ingredients when drilled. No recovery from poisoning was observed in the twenty four hours following pellet exposure for either method of application. Soil contamination did not reduce the efficacy of the pellets and had no differential effect on poisoning.

6.1 Introduction

Control of slugs in arable crops currently relies largely on the use of molluscicide pellets (Iglesias *et al.*, 2002). These are applied either to the soil surface (broadcasting) or beneath the soil surface with the seed (drilling) (Glen & Wilson, 1995). Despite an increase of almost fourfold in pellet use during the last decade (Garthwaite & Thomas, 2003) there has not been a concomitant reduction in levels of damage. For example, slug damage in wheat alone is estimated at £4 million per annum (Shirley *et al.*, 2001) compared to £2.69 million in 1985 (Port & Port, 1986).

Much research in recent years has been directed towards understanding factors that influence the foraging and feeding behaviour of slugs. This includes the role of climatic factors, such as temperature and humidity, in determining activity levels (South, 1989b; Young & Port, 1989), the processes involved in meal initiation and termination (Wedgwood & Bailey, 1988; Bailey, 1989) and the means by which slugs locate food items (Howling, 1991). There has, however, been no work that specifically investigates the foraging and feeding behaviour of slugs in relation to the method of pellet application (i.e. broadcasting versus drilling). Furthermore, although it has been suggested that heavy rainfall may render molluscicides ineffective due to pellets becoming covered in mud splash (Hass *et al.*, 1999; Simms *et al.*, 2002) this has never been formally tested; these aspects are investigated in the experiments presented here. Subsequent mortality and recovery were also monitored.

Time-lapse, infra-red video techniques were employed to create permanent records of slug behaviour. This established methodology has a number of strengths. It allows whole nights of activity to be observed remotely and does not disturb the slugs themselves. The approach has been used successfully in several previous studies (e.g. Howling & Port, 1989; Bailey & Wedgwood, 1991; Hommay *et al.*, 1998; Grimm & Schaumberger, 2002).

The species used in these experiments was *Deroceras reticulatum*, the most serious slug pest of arable crops in the United Kingdom (Schley & Bees, 2003).

6.2 Materials and Methods

6.2.1 Materials

6.2.1.1 Slugs

D. reticulatum used in the experiments were collected from under refuge traps at Close House Field Station, Heddon-on-the-Wall, Northumberland (Grid reference NZ 127659). They were maintained at 12°C in ventilated plastic containers (18 x 12 x 7 cm) filled with moist laboratory tissue for up to five days prior to use. This

ensured that any slugs in the sample that were unhealthy following collection could be excluded from the experiments. During this time they were provided with Chinese cabbage and carrot *ad libitum*.

6.2.1.2 Arenas

The experiments were carried out under controlled conditions at 12 ± 2°C in plastic arenas measuring 57 x 36 x 16 cm, the rims of which were painted with a Fluon® (polytetrafluoroethylene) (Whitford Plastics, Cheshire, England) barrier to prevent slugs escaping. The arenas were filled to a depth of 8-10 cm with loamy soil dug from an agricultural plot at Close House Field Station and any stones, large lumps of organic matter and soil organisms were removed prior to use. The soil surface was raked to a fine tilth. Fresh soil was used for each replicate and it was watered such that the surface appeared damp at the start of each recording. Samples of soil from each replicate were dried in an oven at 80°C and the mean moisture content was found to be 28.6 ± 0.5%.

6.2.1.3 Recording equipment and lighting

Slug activity was recorded using a Panasonic AG-6040 time lapse VHS video recorder. This was set to record 80 times more slowly than normal speed so that a total of 240 hours of activity could be stored on a single cassette. Recordings were played back at normal speed. The camera used was a Sanyo VCB3572 IRP ½" high resolution 570TVL infra-red sensitive camera, fitted with a Computar 8-48 mm lens and infra red bandpass filter.

The arenas were illuminated at night using a Computar Uniflood LED infra red lamp (serial number CL057787). Light of this wavelength does not appear to disrupt slug activity yet permits recording to take place during darkness (Howling, 1990). Daytime lighting was provided by two 400 Watt halogen lamps suspended 1.55 m above the arena. These were controlled by a timer to match the prevailing sunrise and sunset times, which were updated weekly.

6.2.1.4 Molluscicide pellets

Two types of commercial grade molluscicide pellets were used in the experiments; 3% a.i. methiocarb (Bayer, UK) and 5% a.i. metaldehyde (De Sangosse, UK). These were applied at the manufacturers recommended rate.

6.2.2 Methods

6.2.2.1 Pellet application

For each pellet type two application methods were assessed; broadcasting and drilling. The pellets were not pre-treated in any way prior to application. They were placed equidistant from each other in the arena either on the soil surface (broadcast) or 1 cm below the soil surface in drill lines 15 cm apart, lightly covered in soil (drilled). The configuration of the pellets in the arena is shown in *Fig. 6.1 (a)* and *(b)*.

6.2.2.2 Pellet condition

Pellets can become splashed with soil as a result of heavy rainfall which may influence the behavioural response of slugs towards them. This was investigated by comparing clean and soil contaminated pellets of each type. The effect of soil contamination was achieved by rolling the pellets in dishes filled with wet soil. The pellets were applied to the arena as for broadcast pellets *(section 6.2.2.1)*.

6.2.2.3 Acclimatisation and recording

Five slugs were used per arena which is equivalent to a field density of 25 slugs per m^2. It has been suggested that when slugs are disturbed they exhibit an immediate, atypical feeding response and therefore it is advisable to allow them one day to acclimatise to their new environment before conducting experiments (Whelan, 1982). Accordingly the slugs were placed in the arena 24 hours prior to the commencement of recording. During this time they were starved to ensure that they were motivated to forage and feed. Following the acclimatisation period pellets were applied to the arena, recording began and continued overnight until the following morning, giving a total of 16 hours recording time per replicate. The six pellet application regimes

were recorded in blocks. Each block was replicated six times and different slugs were used for each recording.

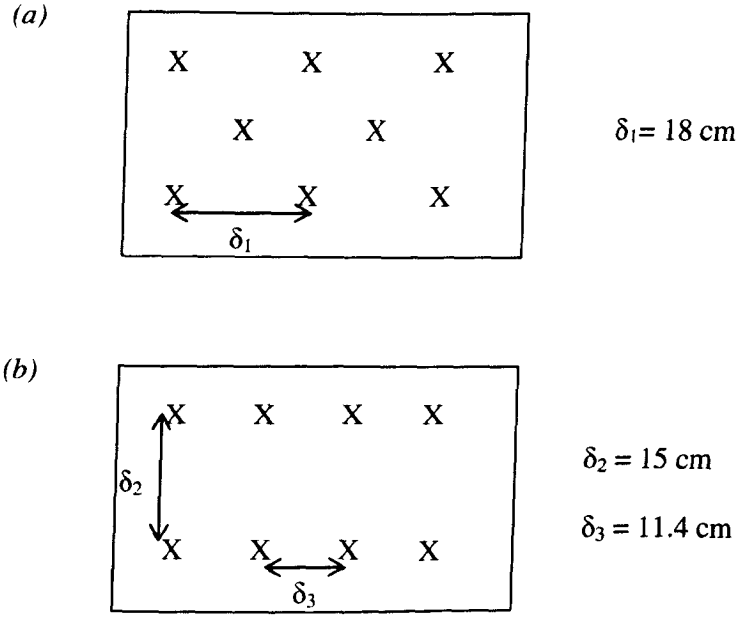


Figure 6.1: Configuration of pellets in arenas (a) broadcast and soil splashed pellets (b) drilled pellets. X indicates the position of a pellet.

6.2.2.4 Mortality assessment

After each night of recording slugs were removed from the arena and weighed. The appearance of each slug was recorded as poisoned or unpoisoned. Poisoned slugs showed a characteristic appearance depending upon the active ingredient; methiocarb poisoning tends to cause bloating of the body and may induce eversion of the buccal mass and head tentacles whereas metaldehyde poisoning is typically manifested by a shrivelled body and retracted buccal mass and tentacles (Frain & Newell, 1983). Individuals were then placed into separate Petri dishes lined with moist laboratory tissue at 20°C and supplied with Chinese cabbage *ad libitum*. After 24 hours the state of each slug was again recorded as poisoned or unpoisoned.

6.2.2.5 Weighing slugs

Slugs were weighed prior to acclimatisation, at the end of recording and 24 hours later using a Mettler MT5 balance.

6.2.3 Video analysis

Video recordings were analysed using a computer programme called Inchworm™. This semi-automated system allowed pellets to be defined as zones within the arena. The path of the slug was then followed manually using the computer cursor. The software digitised the track output and resolved it into time and distance data for zoned and non-zoned areas. To discern feeding from non-feeding contact with a pellet, a threshold contact duration criterion of 0.5 minutes was used. If the slug was in contact with the pellet for more than this time it was assumed to be feeding; if it was in contact with the pellet for less than this time, it was categorised as having ignored the pellet. Feeds on the same pellet separated by less than three minutes were treated as a single meal. If consecutive feeds were on different pellets they were counted as separate meals regardless of the intervening time. The number of pellets ignored by slugs between the onset of activity and the first pellet feed was counted along with the number of pellets eaten after the first feed.

6.2.4 Statistical analysis

Continuous data on times and distances travelled were non-parametric. They were analysed using the Mann-Whitey U-test.

Discrete counts of events were assessed using the Chi-squared test with Yates' correction.

6.3 Results

6.3.1 Time elapsed between onset of activity and first pellet feed

For both methiocarb and metaldehyde the method of pellet application significantly affected the time elapsed between the onset of slug activity and the first feed. It took considerably more time for feeding to begin when pellets were drilled compared to broadcast (Mann-Whitney U-test: methiocarb: $N = 48$, $W = 436.0$, $P < 0.001$;

metaldehyde: N = 56, W = 604.0, $P < 0.001$). In the case of methiocarb, this difference was almost 10-fold whereas for metaldehyde it took six times as long (*Table 6.1*).

Pellet condition did not significantly affect the time taken to start feeding on a pellet after the onset of activity for methiocarb, with no difference between soil contaminated and 'clean' broadcast pellets (Mann-Whitney U-test: N = 55, W = 773.5, *n.s.*). This difference was, however, significant for metaldehyde (Mann-Whitney U-test: N = 57, W = 946.0, $P < 0.001$), with it taking almost half the time for a slug to start feeding on a soil contaminated compared to a 'clean' pellet (*Table 6.1*).

Table 6.1: Median times (mins) elapsed between onset of activity and first feed (with interquartile range) for broadcast, drilled and soil contaminated methiocarb and metaldehyde pellets.

Active Ingredient	*Pellet Treatment*	*N*	*Median Time (mins)*	*Interquartile Range*	
				Q1	*Q3*
Methiocarb	Broadcast	27	27.20	10.40	43.85
	Drilled	21	268.90	143.30	496.70
	Soil contaminated	28	21.30	7.68	61.58
Metaldehyde	Broadcast	28	30.25	9.70	83.30
	Drilled	28	182.40	40.08	424.28
	Soil contaminated	29	16.50	4.20	26.50

6.3.2 Distance travelled between onset of activity and first pellet feed

There were highly significant differences in the distance travelled before the first feed for the different methods of pellet application. For both pellet types slugs travelled much further before feeding on a drilled compared to a broadcast pellet (Mann-Whitney U-test: methiocarb: N = 48, W = 413.0, $P < 0.01$; metaldehyde: N = 56, W = 574.0, $P < 0.01$). In the case of methiocarb they travelled six times as far when pellets were drilled and for metaldehyde they travelled almost five times

further (*Table 6.2*). This difference is illustrated in *Fig. 6.2* which shows typical slug tracks for broadcast and drilled pellets.

Pellet condition had no effect on the distance travelled before the first feed for methiocarb (Mann-Whitney U-test: N = 55, W = 768.0, *n.s.*). Although, in the case of metaldehyde, the median distance slugs travelled before the first feed was greater for 'clean' rather than soil contaminated pellets this difference was not statistically significant (Mann-Whitney U-test: N = 57, W = 921.5, *n.s.*) (*Table 6.2*).

Table 6.2: Median distances (cm) travelled by Deroceras reticulatum *between the onset of activity and first feed (with interquartile range) for broadcast, drilled and soil contaminated methiocarb and metaldehyde pellets.*

Active Ingredient	*Pellet Treatment*	*N*	*Median Distance (cm)*	*Interquartile Range*	
				Q1	*Q3*
Methiocarb	Broadcast	27	33.70	20.85	62.05
	Drilled	21	211.40	98.60	415.10
	Soil contaminated	28	33.05	13.03	57.23
Metaldehyde	Broadcast	28	49.95	17.93	139.03
	Drilled	28	238.05	85.13	412.23
	Soil contaminated	29	18.40	9.10	52.80

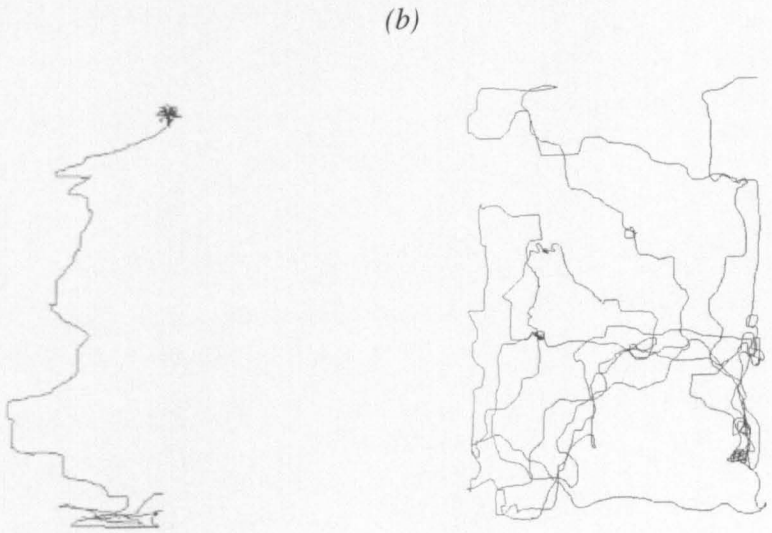


Figure 6.2: Typical slug tracks for (a) broadcast and (b) drilled pellets.

6.3.3 Number of pellets ignored before the first feed

The results are displayed in *Fig. 6.3.*

The number of pellets ignored before the first feed was categorised as 'zero' or 'one or more'. For both methiocarb and metaldehyde there was a significant difference between these categories for broadcast and drilled pellets. In the broadcast applications most slugs fed on the first pellet they encountered. This is in contrast to the drilled applications where considerable numbers of slugs were observed to ignore one or more pellets before the first feed of the night (Chi-Square: methiocarb: N = 53, df = 1, $\chi^2_c = 9.228$, $P < 0.01$; metaldehyde: N = 56, df = 1, $\chi^2_c = 8.590$, $P < 0.01$).

The condition of the pellet did not affect the number of pellets ignored before the first feed; there were no significant differences in the number of 'clean' and soil contaminated pellets ignored for either active ingredient (Chi-Square: methiocarb: N = 55, df = 1, $\chi^2_c = 0.315$, *n.s.*; metaldehyde: N = 57, df = 1, $\chi^2_c = 0.228$, *n.s.*).

6.3.4 Number of subsequent pellets eaten after the first feed

The method of pellet application had no significant effect on the number of slugs feeding on subsequent pellets after the first feed for methiocarb (Chi-Square: N = 48, df = 1, $\chi^2_c = 0.066$, *n.s.*). For metaldehyde, however, this difference was significant with more slugs eating two or more pellets when drilled compared to broadcast (Chi-Square: N = 56, df = 1, $\chi^2_c = 16.556$, $P < 0.001$).

The condition of the pellet did not influence the number of slugs contacting and eating subsequent pellets after the first feed with no significant differences between soil contaminated and 'clean' pellets for either active ingredient (Chi-Square: methiocarb: N = 55, df = 1, $\chi^2_c = 0.444$, *n.s.*; metaldehyde: N = 57, df = 1, $\chi^2_c = 0.809$, *n.s.*).

These results are shown in *Fig. 6.4.*

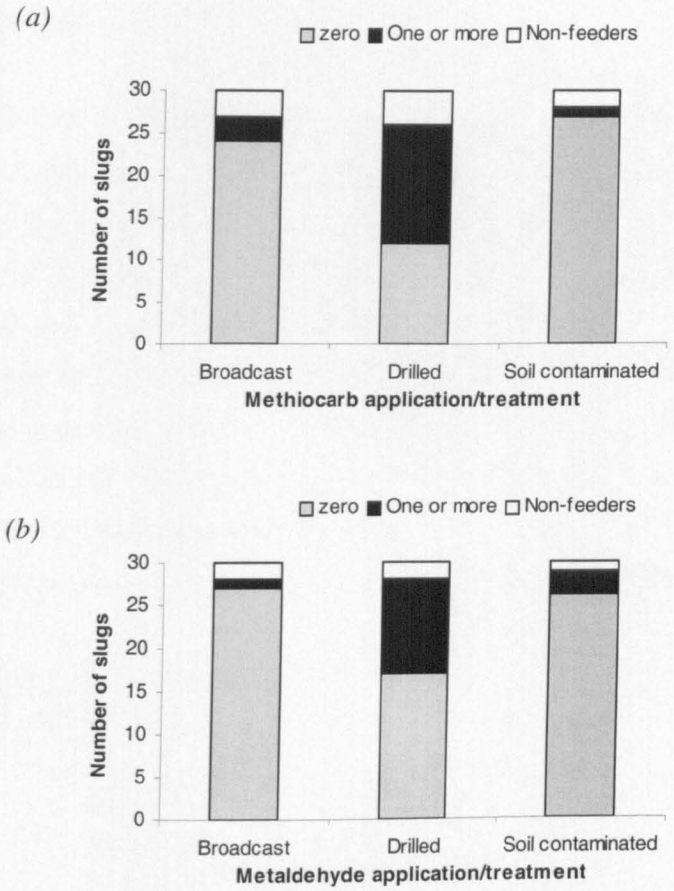


Figure 6.3: The number of slugs ignoring 'zero' or 'one or more' pellets before the first feed for (a) methiocarb pellets and (b) metaldehyde pellets.

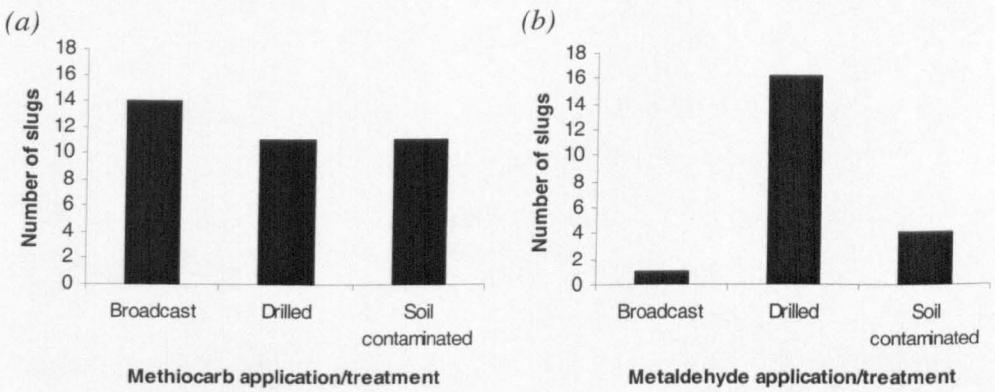


Figure 6.4: The number of slugs consuming subsequent pellets after the first feed for (a) methiocarb pellets and (b) metaldehyde pellets.

6.3.5 Mortality and recovery

The effect of the application method and pellet condition on mortality and recovery was assessed by observing numbers of poisoned and unpoisoned slugs at the end of the night's recording (T_0) and 24 hours later (T_{24}). Homogeneity chi-square tests indicated that, for both metaldehyde and methiocarb, there were no significant differences between replicates for either pellet application method or condition at T_0 or T_{24}. Results were therefore pooled over arenas. *Table 6.3* summarises the numbers of slugs in each of these categories for both active ingredients. It was not possible to recover every slug from the arena as some became obscured by a soil covering. Additionally, some reassessments at T_{24} were missed for logistical reasons; hence the numbers in *Table 6.3* do not represent a strictly a 'before and after' analysis.

Table 6.3: The numbers of poisoned and unpoisoned Deroceras reticulatum *at T_0 & T_{24} with metaldehyde and methiocarb pellets applied in different ways.*

Method of Application/ Pellet Condition	*Methiocarb*				*Metaldehyde*			
	T_0		*T_{24}*		*T_0*		*T_{24}*	
	UP	*P*	*UP*	*P*	*UP*	*P*	*UP*	*P*
Broadcast	2	23	1	19	12	13	10	15
Drilled	11	18	9	10	12	7	7	7
Soil contaminated	2	28	1	19	9	11	4	16
Total	15	69	11	48	33	31	21	38

UP = unpoisoned; P = Poisoned

6.3.5.1 Mortality according to application method and pellet condition

The numbers of poisoned and unpoisoned slugs were compared for application method and pellet condition, using a Contingency Chi-square test, applying the tests for each active ingredient at T_0 and T_{24}. The results are shown in *Table 6.4.*

At both T_0 and T_{24} the only significant difference in relation to the method of pellet application was between drilled and broadcast methiocarb with fewer slugs poisoned when pellets were drilled. There were no such differences observed for metaldehyde.

Pellet condition did not affect the numbers of poisoned and unpoisoned slugs for either metaldehyde or methiocarb with no significant differences between soil contaminated and 'clean' pellets at T_0 or T_{24}.

Table 6.4: Contingency Chi-square values comparing, between methods of application and pellet condition, the numbers of Deroceras reticulatum *poisoned by metaldehyde or methiocarb pellets at T_0 and T_{24}.*

	Chi-Square Value	
Comparison	*Metaldehyde*	*Methiocarb*
T_0		
Broadcast vs. Drilled	0.484	*5.049
Broadcast vs. Soil contaminated	0.010	0.111
T_{24}		
Broadcast vs. Drilled	0.072	**7.094
Broadcast vs. Soil contaminated	1.243	0.526

*Significance level: *P < 0.05; **P < 0.01*

6.3.5.2 Mortality according to active ingredient

The difference in mortality caused by metaldehyde and methiocarb was directly compared at T_0 and T_{24} for each of the two methods of application and for pellet condition. At T_0 methiocarb poisoned significantly more slugs than metaldehyde when broadcast yet there were no differences when the pellets were drilled. This pattern was also observed for soil contaminated as opposed to 'clean' pellets where methiocarb poisoned significantly more slugs at T_0 than metaldehyde. By T_{24} the disparity between numbers of slugs poisoned by the two active ingredients had declined with only broadcast methiocarb pellets poisoning significantly more slugs than metaldehyde pellets applied in the same way.

When results were combined for all methods of application/pellet condition there were highly significant differences between active ingredients at T_0 with methiocarb poisoning more slugs than metaldehyde. At T_{24} these differences had completely disappeared and both active ingredients were seen to be equally effective.

The results are summarised in *Table 6.5.*

Table 6.5: Contingency Chi-squared values comparing, between metaldehyde and methiocarb, the numbers of Deroceras reticulatum *poisoned when pellets are applied in different ways or are soil contaminated at T_0 and T_{24}.*

Application Method/ Pellet Condition	T_0				χ^2	T_{24}				χ^2
	MA		*MC*			*MA*		*MC*		
	UP	*P*	*UP*	*P*		*UP*	*P*	*UP*	*P*	
Broadcast	12	13	2	23	**8.036	10	15	1	19	*5.066
Drilled	12	7	11	18	2.011	7	7	9	10	0.042
Soil contaminated	9	11	2	28	**8.163	4	16	1	19	0.914
Total	33	31	15	69	***17.315	21	38	11	48	3.474

*UP = unpoisoned; P = Poisoned. Significance levels: *P < 0.05; **P < 0.01; ***P < 0.001*

Crawford-Sidebotham (1970) expressed the differences in mortality between active ingredients as an 'Index of Efficiency', *I*. This is defined as $I=R_D/R_M$ where:

$$R_D = \frac{\text{Number of slugs poisoned by methiocarb}}{\text{Total number of slugs in methiocarb experiment}}$$

$$R_M = \frac{\text{Number of slugs poisoned by metaldehyde}}{\text{Total number of slugs in metaldehyde experiment}}$$

The index of efficiency serves as a useful guide to illustrate the magnitude of the differences observed between active ingredients. Of the results presented here, the indices of efficiency for those comparisons where differences between methiocarb and metaldehyde were found to be significant are given in *Table 6.6.*

It can be seen that, for example, at T_0 1.35 times as many slugs were poisoned by broadcast methiocarb as compared to metaldehyde. By T_{24} this had declined although methiocarb was still causing the higher mortality of the two active ingredients. For soil contaminated pellets methiocarb was observed to poison almost twice as many slugs as metaldehyde at T_0.

Table 6.6: Values for the index of efficiency of methiocarb as compared with metaldehyde for methods of application/pellet condition where there were significant differences in mortality between active ingredients.

Application Method/ Pellet Treatment	*Time*	*Index*
Broadcast	T_0	1.35
Broadcast	T_{24}	1.27
Soil Contaminated	T_0	1.94

6.3.5.3 Recovery according to method of application and pellet condition

Recovery was defined as an improvement in the appearance of the slug from poisoned to unpoisoned during the 24 hour observation period. There was no significant recovery for slugs consuming either active ingredient, regardless of the method of application or condition of the pellets. The results are summarised in *Table 6.7.*

Table 6.7: Contingency Chi-squared values comparing, for different methods of pellet application/condition, the number of Deroceras reticulatum *poisoned at T_0 and T_{24} for methiocarb and metaldehyde.*

Application Method/	*Metaldehyde*					*Methiocarb*				
	T_0		*T_{24}*			*T_0*		*T_{24}*		
Pellet Condition	*UP*	*P*	*UP*	*P*	χ^2	*UP*	*P*	*UP*	*P*	χ^2
Broadcast	12	13	10	15	0.082	2	23	1	19	0.042
Drilled	12	7	7	7	0.160	11	18	9	10	0.120
Soil contaminated	9	11	4	16	1.822	2	28	1	19	0.133

6.3.5.4 Weight change during recovery period

There were no clear trends in the weight change of poisoned and unpoisoned slugs between T_0 and T_{24} regardless of the active ingredient or method of application/pellet condition.

6.4 Discussion

An understanding of how control measures influence the behaviour of pest species provides a valuable insight into their ecology and can lead to better pest management strategies resulting in more precise targeting of pesticides and a reduction in collateral damage.

The aim of the experiments presented here was to investigate how different methods of pellet application and pellet condition affect the foraging and feeding behaviour of *D. reticulatum*. Specifically foraging was assessed in terms of the time and distance travelled between the onset of activity and the first pellet feed. Analysis was confined to the first feed only because it was assumed that at least some active ingredient would be ingested during this feed and its toxic effects may influence subsequent behaviour. Since it was not possible to quantify these effects the interpretation of further foraging activity would have been compromised. Feeding behaviour was resolved into the number of pellets ignored before the first feed and the number of slugs that then went on to consume at least one further pellet. This gave a measure of how pellet attractiveness and sequential acceptability, as defined by Howling (1990), are affected by the method of application and condition. Slug activity was recorded overnight and consequent poisoning and recovery were monitored during the following twenty four hour period.

Control of slugs in arable crops is most crucial at the time of seed germination and emergence when the most serious damage is caused (Duthoit, 1964). This follows a period of relative food scarcity in the arable environment when fields have lain bare after ploughing and, therefore, no alternative food source was provided in these experiments. The addition of germinating seedlings to the arena would, in any case, have severely reduced the clarity of the video recordings.

It has been shown that food deprivation increases the locomotor activity of *D. reticulatum* (Airey, 1987) and it is common in studies investigating feeding activity to starve slugs for a short period prior to experiments (e.g. Briggs & Henderson, 1987; Bailey *et al.*, 1989). In the current work this practice was adopted to increase

the motivation of slugs to forage and feed and to ensure that all individuals were at a similar level of food deprivation thereby reducing within-group variation.

6.4.1 Method of pellet application

6.4.1.1 Foraging behaviour

Since *D. reticulatum* is largely surface dwelling (South, 1965; Hunter, 1968c) and feeds on leafy vegetation in preference to roots (Runham & Hunter, 1970) it might be expected that pellets broadcast on the soil surface would be encountered more readily by this species than drilled pellets and, indeed, this is supported by these results. After the onset of activity *D. reticulatum* travelled a shorter distance and began feeding on broadcast pellets much sooner than drilled pellets regardless of the active ingredient. This contrasts, however, with a study by Hogan (1985). Although Hogan's experiments are not directly comparable, as they assessed drilled wheat seeds versus those presented in a central seed pile, she found *D. reticulatum* fed on drilled seeds sooner. This observation may be explained by considering the means by which slugs encounter food items. Evidence regarding this has been contradictory in the past with some studies suggesting olfaction plays a key role (Gelperin, 1974; Pickett & Stephenson, 1980) whilst other conclude that it is an entirely random process (Duval, 1970). Most recent work indicates that slugs are attracted to food only when they are in close proximity to it, i.e. within 3-4 cm, and not over larger distances (Bailey *et al.*, 1989; Howling, 1991). It would seem, therefore, a likely explanation of Hogan's result is that the slugs simply did not follow a path that took them near enough to the central seed pile to detect its presence. In the current experiments the broadcast pellets were distributed evenly throughout the arena and within the optimum density for control (Hunter & Symonds, 1970) thereby representing a more realistic field situation.

An inherent limitation of any video based assessment of individual slug activity is that animals must be contained within an arena of fixed size. This is relevant in studies of foraging in that it restricts movement along the margins and may, therefore, cause atypical behavioural patterns. These so called edge effects have been investigated in studies using arenas of various sizes and it was found that slug

movement was not significantly affected by the area of the arena (Bailey, 1989; Howling, 1990). It is not, therefore, thought that edge effects would have had a major impact on the foraging behaviour observed in the experiments described here.

6.4.1.2 Feeding behaviour

When pellets of either active ingredient were broadcast most slugs fed on the first one they encountered whereas when they were drilled considerable numbers of slugs ignored one or more pellets before commencing feeding. As discussed in *section 6.4.1.1* this is likely to be because broadcast pellets are more immediately accessible to *D. reticulatum* than drilled pellets. Since soil contamination was clearly shown not to affect the ability of slugs to detect pellets (*section 6.4.2*) it would seem unlikely that the loose covering of soil over shallow drilled pellets would itself markedly reduce their attractiveness to slugs. This is supported by a study which compared the efficacy of broadcast and drilled methiocarb pellets in protecting wheat seedlings from damage (Glen *et al.*, 1992). It was seen that, although slugs fed on and were killed by pellets drilled at the same depth as those used in the current study, they were not as effective as broadcast applications in reducing damage suggesting that slugs feeding activity was arrested sooner by broadcast pellets. Physical inaccessibility is probably the more important factor in explaining both the increase in damage and greater number of pellets ignored when drilling is used to apply molluscicides.

It has been suggested that drill lines are used as 'motorways' by slugs with foraging beginning at one end of the furrow and continuing along to the other as the looser soil in the furrow is easier to travel through (Allen 1981 in Martin & Kelly, 1986). The study presented here did not find any evidence in support of this. Slug tracks were seen to meander randomly across the arena both before and after feeding on pellets, as shown in the example of a typical track in *Fig. 6.2 (b)*. The soil surface was, however, a uniform fine tilth and pellets were deposited in hollows rather than furrows before being loosely covered with soil. Had furrows been used, or if the tilth had been coarser slugs may have been able to detect drill lines more easily.

Analysis of the number of slugs eating at least one further pellet after the first feed gave mixed results depending both on the active ingredient and the application method. For methiocarb there was no difference between drilled and broadcast pellets whereas for metaldehyde more slugs went on to consume subsequent pellets when they were drilled compared to broadcast. A combination of two factors may explain these results; difference in the amount of pellet consumed and in the action of the active ingredient. When pellets are drilled, after the soil covering has been removed by a foraging slug, it is only the top of the pellet that is exposed the rest remaining in a soil depression. This contrasts with the broadcast situation where the entire pellet is accessible. It is likely, therefore, that a smaller quantity of drilled pellet is eaten. Feeding activity is also inextricably linked to the means by which the active ingredients in molluscicides exert their toxic effects and this may account for the differences between methiocarb and metaldehyde. Both work by disrupting the control of the feeding apparatus of slugs (Mills *et al.*, 1989) and there is evidence that the effect of metaldehyde is cumulative (Kemp & Newell, 1985). It is possible that in eating a smaller quantity of pellet when drilled the amount of active ingredient ingested on the first feed with metaldehyde was not sufficient to interfere with further feeding. In the case of methiocarb toxic effects are not thought to be cumulative and so, despite less drilled pellet being consumed, enough active ingredient may nevertheless have been ingested for there to be no difference between methods of application. Further support for these results comes from a study that shows young *D. reticulatum* have a tendency to develop an aversion to methiocarb, refusing subsequent pellets after the initial feed (Kemp & Newell, 1985). No such effect was observed for metaldehyde which also helps to explain why slugs were seen to consume further drilled pellets of this active ingredient and not methiocarb.

A contact duration threshold of 0.5 minutes was used to distinguish whether slugs were feeding as distinct from simply resting next to pellets. Other studies vary as to how feeding has been determined. For example, Grimm and Schaumberger (2002) considered any time spent at the food constituted feeding whereas Howling (1990) used movement of the pellet as his criterion. It was observed in the recordings made in the experiments presented here that, if contact was made with a pellet, slugs tended either to move off relatively quickly, subsequently behaving normally, or they

stayed in contact for a prolonged period of time, ultimately exhibiting signs of poisoning. It seemed, therefore, that a useful distinction could be made here rather than assuming any contact equated to feeding. Since it took some time for slugs to locate drilled pellets, however, involving removal of the loose covering of soil and inevitably movement of the pellet in the process Howling's criterion would have been inappropriate. Frequency plots of the numbers of slugs in contact with pellets for different time intervals showed that there was no clear boundary where transient contact changed to prolonged contact for either drilled or broadcast pellets i.e. there was no bimodal distribution. The majority of slugs that went on to exhibit signs of poisoning were in contact with the pellet for 0.5 minutes or longer and, therefore, this value was chosen as a compromise. Had a lower threshold been applied there would have been a greater chance of misclassifying feeds on drilled pellets yet a higher threshold could have underestimated feeds on broadcast pellets.

6.4.1.3 Mortality and recovery

It is known that metaldehyde and methiocarb are, themselves, repellent to slugs (Wright & Williams, 1980) and this is why they are incorporated with attractants amongst other substances in bait formulations (Bailey, 2002). The amount of an active ingredient consumed is therefore influenced by its repellency and efficacy is a trade-off between there being enough active ingredient to poison the slug and not so much that it is deterred from consuming a toxic dose. Evidence suggests that metaldehyde is more repellent to slugs than methiocarb (Wedgwood & Bailey, 1988; Bailey *et al.*, 1989) and this is supported by these experiments. When broadcast, metaldehyde poisoned fewer slugs than methiocarb which would be explained by a higher repellency of the former, resulting in fewer slugs consuming a lethal dose before terminating the meal. The experiments were carried out at 12 ± 2°C. This temperature was chosen to represent conditions typical of a mild spring or cool autumn in the UK. There is strong evidence to suggest, however, that temperature affects the toxicity of metaldehyde (e.g. Cragg & Vincent, 1952; Webley, 1965; Wright & Williams, 1980). Had the experiments been carried out at a higher temperature the mortality due to metaldehyde might have been enhanced. That there was no difference between active ingredients when drilled suggests that although slugs fed on more metaldehyde pellets than methiocarb when applied in this way,

overall lethal amounts of each were ingested during the night of activity further underlining the differences in repellency and time course of action.

When comparing the mortality of slugs between methods of application for a given active ingredient it was found that broadcast methiocarb poisoned more slugs than when this molluscicide was drilled. The smaller amount of pellet consumed when drilled (as explained in *section 6.4.1.2*) may account for this difference. Furthermore, methiocarb pellets were slightly smaller than metaldehyde pellets which could explain why this effect was observed for the former and not the latter along with the cumulative action of metaldehyde.

There was no recovery noted for slugs poisoned by either active ingredient over twenty four hours regardless of application method despite provision of moist humid conditions said to be conducive to recovery (Bourne *et al.*, 1988). This may be because the observation period was relatively short, although other studies where slugs were monitored for forty eight hours after feeding on molluscicide also showed no significant recovery for *D. reticulatum* (Crawford-Sidebotham, 1970; Airey, 1986). All these studies were, however, laboratory based. Results in the field would be expected to show more variation due, for example, to fluctuations in temperature and other factors (Glen & Orsman, 1986) or the availability of shelter under clods of earth (Bailey, 2002). It is, however, difficult to assess mortality of individuals in field conditions due to problems of containing the experimental population, scavenging of moribund and dead individuals by other animals and the rapid decay of poisoned slugs.

Although the results on mortality and recovery presented here are informative, this assessment was a secondary aim of these experiments. Ideally more slugs would have been used and the study could be extended to investigate the effects of temperature and different recovery periods more fully.

6.4.2 Pellet condition

It has been suggested that soil contamination of broadcast pellets may reduce their efficacy due to slugs being unable to detect them (Hass *et al.*, 1999; Simms *et al.*, 2002). In formally testing this it was found not to be the case. There was no reduction in the performance of soil contaminated pellets relative to 'clean' pellets of either active ingredient for any of the parameters assessed. Indeed, in one case soil contaminated pellets were shown to be more effective; slugs began feeding on soil contaminated metaldehyde pellets in half the time it took them to start feeding on clean pellets. An explanation for this could be that the increase in moisture content of the pellet imparted by the soil covering made it more palatable to the slug. It is not clear, however, why this was observed only with metaldehyde and not methiocarb. Bait pellets contain, in addition to the active ingredient and other substances to reduce deterioration, a bulking material which is often a cereal base (Bailey, 2002). Perhaps the increased moisture had a differential effect on this base constituent in metaldehyde and methiocarb increasing the attractiveness of the former more than the latter. In summary these results indicate that soil contamination of molluscicide pellets is, of itself, unlikely to impede the feeding, foraging or mortality of *D. reticulatum*.

6.5 Conclusions and Future Work

In conclusion, it was found that:

1. *D. reticulatum* travel shorter distances and feed on broadcast pellets sooner than drilled pellets.
2. *D. reticulatum* are more likely to feed on the first pellet encountered when they are drilled compared to broadcast.
3. Methiocarb poisoned more *D. reticulatum* than metaldehyde when pellets were broadcast, but there was no difference between active ingredients when drilled.

4. No recovery from poisoning was observed during the 24 hour period following pellet exposure for either active ingredient.
5. Soil contamination did not reduce the efficacy of pellets and had no differential effect on poisoning compared to 'clean' pellets.

With respect to the control of *D. reticulatum* the results of these experiments imply that broadcasting is a more effective method of molluscicide pellet application than drilling. Since slugs locate broadcast pellets sooner there may be less opportunity for them to cause damage, providing they consume a toxic dose. Furthermore, because soil contamination of broadcast pellets does not diminish their efficacy, re-application following heavy rainfall is unnecessary.

These experiments have shown how certain aspects of the feeding and foraging of *D. reticulatum* are influenced by the method of pellet application and pellet condition under a defined set of conditions. Activity is affected by temperature (e.g. Crawford-Sidebotham, 1972; Young & Port, 1989) and it would be interesting to see whether changing the temperature at which the experiments were carried out would alter the results. It has been suggested that in the presence of alternative food the susceptibility of *D. reticulatum* to molluscicides is reduced (Airey, 1986). Although it would be impractical to introduce seedlings to the arena due to problems with the clarity of recordings mentioned previously it would be feasible to introduce seeds, e.g. wheat. This would permit an assessment of whether any observed differences in susceptibility to poisoning could be due to changes in the foraging or feeding behaviour.

Chapter 7

Characterisation of the Surface Activity of *Deroceras reticulatum* (Müller) on Coarse and Fine Seedbeds in the Presence and Absence of Metaldehyde Pellets

Abstract

Temporal and spatial activity of *Deroceras reticulatum* (Müller) on coarse and fine seedbeds was assessed in the presence and absence of metaldehyde pellets. Seedbed conditions did not affect the time activity started. The total distance travelled per night was also comparable between seedbeds and was significantly reduced by metaldehyde pellets. Slugs that fed generally did so on the first pellet encountered and travelled similar distances in comparable times to reach it, regardless of seedbed type. The small number of slugs ignoring pellets before feeding or failing to feed at all was, again, unaffected by seedbed conditions. The only difference in slug activity observed between seedbeds was in the effect they had upon utilisation of available space. Fractal dimension analysis showed that, without metaldehyde pellets, trail complexity was between truly random and constrained on both coarse and fine seedbeds, but trails covered a larger area of the arena on the latter. The presence of pellets significantly reduced trail complexity on both types of seedbed.

7.1 Introduction

The extent of cultivation applied to arable land in preparation for sowing is determined by a combination of the soil type and requirements of the crop to be grown. For example, loamy soils may be cultivated to achieve a firm, fine seedbed suitable for oilseed rape, or may be less extensively treated resulting in coarser seedbeds appropriate for wheat. On heavy soils, in contrast, it can be difficult and

very costly to produce a reasonable seedbed which limits the choice of crops available to the farmer (Martin & Kelly, 1986). The extent of cultivation, i.e. minimum tillage versus full tillage, has a marked effect on slug numbers and the consequent damage they cause.

Cultivations may kill slugs directly by mechanical injury (Hunter, 1967), physically displace individuals (Port, 1989) or bring them to the soil surface where they are exposed to predators and adverse weather conditions (Martin & Kelly, 1986). This latter effect is particularly critical to the survival of eggs as they are highly vulnerable to desiccation (South, 1989a).

The damage caused to seeds and seedlings by slugs remaining after cultivation is related to seedbed conditions. It is generally perceived to be worse in coarse seedbeds with large aggregates because slugs can easily move between them and reach seed which is not completely covered by soil (Glen *et al.*, 1992; Green *et al.*, 1992). Whilst consolidation may reduce such damage by restricting vertical movement (Gould, 1961), the success of this approach depends on the extent to which soil aggregates can be broken down; in some circumstances, e.g. clay soils, it may, in fact, simply push aggregates deeper into the soil, leaving behind large pockets in which slugs can shelter and move freely, making the situation worse (Stephenson, 1975; Glen *et al.*, 1989; Port, 1989). Molluscicides are, as a result, often routinely applied on fields considered to be at high risk of slug damage (Port & Port, 1986).

Studies of the effect of seedbed conditions on slug activity have largely made indirect assessments by evaluating resultant damage in field conditions (e.g. Gould, 1961; Glen *et al.*, 1992; Green *et al.*, 1992). They therefore take into account both vertical and horizontal movement, but cannot quantify individual behaviour, nor have they considered the response of slugs to molluscicide pellets on different seedbeds.

The experiments described in this chapter were designed to complement existing studies by characterising surface activity patterns of individual *Deroceras*

reticulatum on coarse and fine seedbeds in the presence and absence of metaldehyde pellets. Although the time-lapse video techniques used do not allow assessment of vertical activity they are a valuable means of making permanent records of horizontal surface movement, which is particularly critical with respect to damage (Hommay *et al.*, 1998). The extent to which slugs used the area available was assessed using fractal dimension analysis. This technique categorises trail paths on a continuum from straight to constrained (i.e. criss-crossing due to some limiting barrier) and assigns them a score such that they may be compared to each other and to a true 'random' path.

7.2 Materials and Methods

7.2.1 Materials

7.2.1.1 Slugs

D. reticulatum (mean weight ± S.E. = 466.13 ± 17.50 mg) used in the experiments were collected from under refuge traps at Close House Field Station, Heddon-on-the-Wall, Northumberland (Grid reference NZ 127659). There were no significant differences in the weight of slugs between treatments (ANOVA: $F_{3, 156} = 5.07$, *n.s.*). They were maintained at 12°C in ventilated plastic containers (18 x 12 x 7 cm) filled with moist laboratory tissue for up to five days prior to use. This ensured that any slugs in the sample that were unhealthy following collection could be excluded from the experiments. During this time they were provided with Chinese cabbage and carrot *ad libitum*.

7.2.1.2 Arenas

The experiments were carried out under controlled conditions at 12 ± 2°C in plastic arenas measuring 57 x 36 x 16 cm, the rims of which were painted with Fluon® (polytetrafluoroethylene) (Whitford Plastics, Cheshire, England) which acts as a barrier to prevent slugs escaping.

7.2.1.3 Soil

The arenas were filled with two layers of loamy soil dug from an agricultural plot at Close House Field Station from which any stones, large lumps of organic matter and soil organisms were removed prior to use. The base layer was 8-10 cm deep and its surface was lightly consolidated by packing with the back of a trowel. The top layer consisted of an even covering of fine or coarse aggregates, according to seedbed type. The fine aggregates were obtained by sieving soil through a 5 mm grade mesh; the coarse aggregates comprised selected large clods of soil as collected from the field with a diameter of approximately 5-6 cm *(see also section 7.3.1).* Fresh soil was used for each replicate and it was watered such that the surface appeared damp at the start of each recording.

7.2.1.4 Recording equipment and lighting

Slug activity was recorded using a Panasonic AG-6040 time lapse VHS video recorder. This was set to record 60 times more slowly than normal speed so that a total of 180 hours of activity could be stored on a single cassette. Recordings were played back at normal speed. The camera used was a Sanyo VCB3572 IRP ½" high resolution 570TVL infra-red sensitive camera, fitted with a Computar 8-48mm lens and infra red bandpass filter.

The arenas were illuminated at night using a Computar Uniflood LED infra red lamp (serial number CL057787). Light of this wavelength does not appear to disrupt slug activity yet permits recording to take place during darkness (Howling, 1990). Daytime lighting was provided by two 400 Watt halogen lamps suspended 1.55 m above the arena. These were controlled by a timer to match the prevailing sunrise and sunset times, which were updated weekly.

7.2.1.5 Molluscicide pellets

Commercial grade 5% a.i. metaldehyde pellets (De Sangosse, UK) were applied at the manufacturers recommended rate in molluscicide treatments.

7.2.2 Methods

7.2.2.1 Experimental treatments

Slug activity was recorded on each of two seedbed types (coarse and fine) both with and without molluscicide pellets applied, resulting in four experimental treatments (*Table 7.1*).

Table 7.1: Experimental treatments ('+' indicates molluscicide present; '-' indicates molluscicide absent).

Treatment	*Seedbed*	*Molluscicide*
1	Coarse	+
2	Coarse	-
3	Fine	+
4	Fine	-

7.2.2.2 Pellet application

Pellets were not pre-treated in any way prior to application. They were broadcast equidistant from each other in the arena on the soil surface. Their configuration is shown in *Fig. 7.1*.

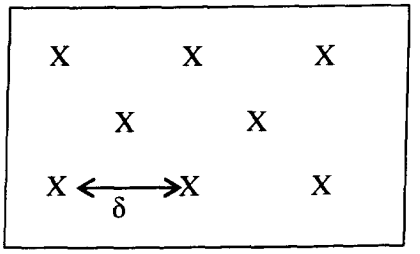


Figure 7.1: Configuration of pellets in the arena (experimental treatments 1 & 3 only).

7.2.2.3 Acclimatisation and recording

Eight slugs were used per arena which is equivalent to a field density of 40 slugs per m^2. These were weighed using a Mettler MT5 balance to an accuracy of 0.01 mg.

To allow for atypical activity in response to a new environment slugs were placed in the arena for 24 hours prior to the commencement of recording to allow them to acclimatise (Whelan, 1982). During this period they were starved. Following acclimatisation, pellets were applied to the soil surface for molluscicide treatments (*Table 7.1*); for treatments without molluscicide nothing further was added to the arena. Recording then commenced and continued overnight until the following morning. The four treatments, each of which was replicated five times, were recorded in blocks and different slugs were used for each replicate.

7.2.3 Soil aggregate size analysis

Samples of soil aggregates from fine and coarse seedbeds were photographed using a Canon G3 Powershot digital camera, 4 mega pixel resolution. The images were then analysed using a computer programme called Image-J to obtain data on aggregate size.

7.2.4 Video analysis

Video recordings were analysed using a computer programme called Inchworm™, as described in *Chapter 6, section 6.2.3.* Pellets were defined as zones and slug trails were resolved into time and distance data for zoned and non-zoned areas. Counts were made of the number of pellets ignored by slugs between the onset of activity and the first pellet feed. To discern feeding from non-feeding contact with a pellet a threshold contact duration criterion of 0.5 minutes was used. If the slug was in contact with the pellet for more than this time it was assumed to be feeding; if it was in contact with the pellet for less than this time, it was categorised as having ignored the pellet. Feeds on the same pellet separated by less than three minutes were treated as a single meal. If consecutive feeds were on different pellets they were counted as separate meals regardless of the intervening time.

The extent to which slug trails filled the arena space in the different treatments was assessed by calculating the fractal dimension according to the procedure of Katz and George (1995). For each slug trail, distance data from the standard Inchworm™

output were resolved into 50 steps ('sticks') of equal length and the fractal dimension, D, was calculated according to *Equation 7.1*:

$$D = \frac{\log(n)}{\log(n) + \log(d / L)}$$

Equation 7.1

where n is the number of sticks per trail, L is the total trail length and d is Feret's diameter (i.e. greatest distance between two points on the trail). These calculations were carried out using an in-house computer programme (written by Dr. Mark Shirley).

7.2.5 Statistical analysis

Continuous data were tested for normality and transformed if necessary. Where this resulted in parametric data, independent sample t-tests were used in one-factor analyses; otherwise the non-parametric Kruskal-Wallis test or Mann-Whitney U-test was applied as appropriate. For the single two-factor analysis to compare the total distance travelled by slugs on different seedbeds with and without molluscicide present transformation failed to normalise the data and the Scheirer-Ray-Hare test was used.

Discrete counts were compared using the chi-square test of association with Yates' correction.

All analyses of fractal dimensions were based on log transformed data which conformed to a normal distribution. To assess whether the activity patterns in each treatment differed from that expected if slugs were moving randomly, a t-test was used to compare the mean fractal dimension with that for 501 simulated random walks defined by the same number of sticks (tabulated in Katz & George, 1995). Means were compared between treatments using analysis of variance (ANOVA).

7.3 Results

7.3.1 Soil aggregate diameter on coarse and fine seedbeds

Fig. 7.2 (a)-(d) shows representative coarse and fine seedbeds, along with samples of aggregates from each (with scale bar).

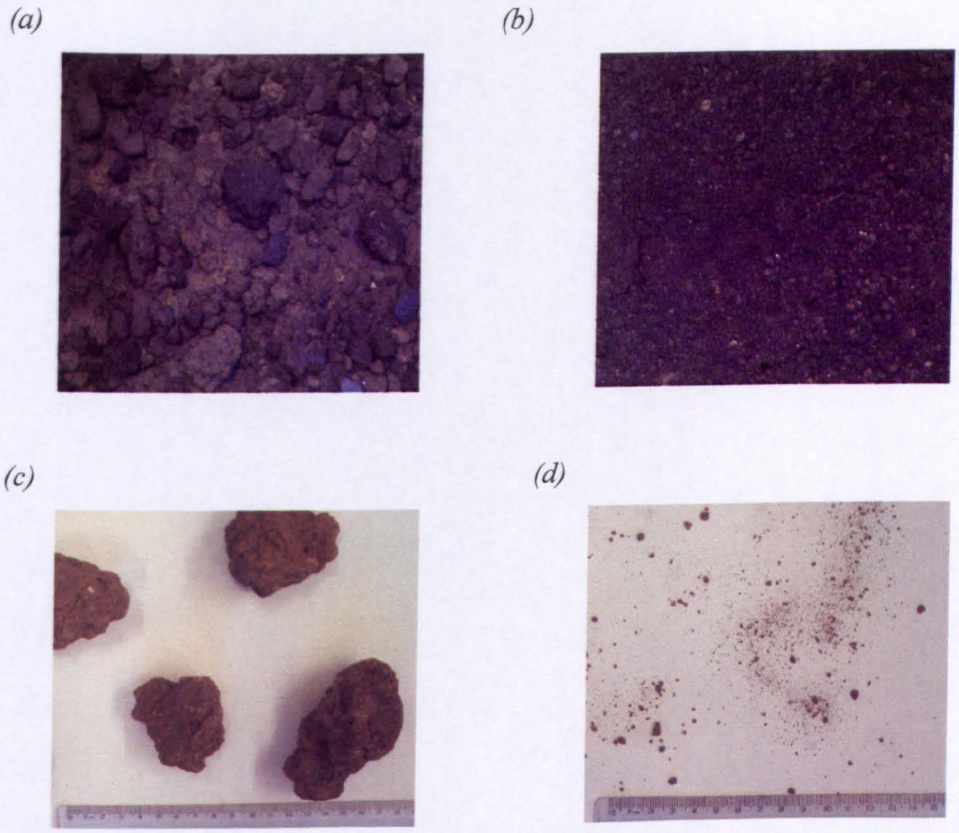


Figure 7.2: Representative seedbeds and soil aggregates (a) coarse seedbed; (b) fine seedbed; (c) coarse aggregates; (d) fine aggregates. Scale bar is in cm.

There were significant differences between the mean Feret's diameter of aggregates from each of the two seedbeds as judged by the Mann-Whitney U-test (*Table 7.2*).

Table 7.2: Results of Mann-Whitney U-tests to compare the Feret's diameter (mm) of soil aggregates from coarse and fine seedbeds.

Seedbed	*Number of samples*	*Number of aggregates measured*	*Mean ± S.E. (mm)*	*Z*	*P-value*
Coarse	5	22	64.84 ± 2.52	-8.139	*< 0.001*
Fine	2	6618	0.68 ± 0.01		

The diameter of aggregates from different samples of a given seedbed did not differ significantly for coarse seedbeds (Kruskal-Wallis: N = 22, df = 4, H = 1.15, *n.s.*) or fine seedbeds (Kruskal-Wallis: N = 6618, df = 1, H = 0.95, *n.s.*).

7.3.2 Influence of seedbed on activity onset time

Analyses in this section are based on treatments where no molluscicide was applied to the arena (i.e. treatments 2 and 4, *Table 7.1*).

On each type of seedbed some slugs commenced activity before darkness whereas others did not become active until afterwards. The time interval between darkness and the onset of activity was, therefore, compared separately for these two sets of slugs; in neither case were the differences between coarse and fine seedbeds significant (*Table 7.3*). The proportion of individuals active before and after darkness did not differ between seedbed types either; the majority of slugs began activity after darkness (N = 79, df = 1, χ^2_c = 0.195, *n.s.*) (*Fig. 7.3*).

Table 7.3: Results of t-tests to compare the mean time between the onset of activity and darkness for Deroceras reticulatum *on coarse and fine seedbeds.*

Variable	*Seedbed*	*N*	*Mean ± SE (mins)*	*df*	*t*	*P-value*
Time between onset of activity and darkness (slugs active before darkness)	Coarse	12	128.40 ± 21.60	19	-1.35	*n.s.*
	Fine	9	167.65 ± 17.13			
Time between darkness and onset of activity (slugs active after darkness)	Coarse	28	81.49 ± 21.51	56	-1.38	*n.s.*
	Fine	30	100.53 ± 17.42			

Figure 7.3: Numbers of active Deroceras reticulatum *on coarse and fine seedbeds (black bars = before darkness; white bars = after darkness).*

7.3.3 Influence of seedbed and molluscicide presence on total distance travelled

Results are summarised in *Fig. 7.4* and *Table 7.4.* The presence of pellets significantly reduced the mean distance travelled per night on coarse and fine seedbeds; seedbed type alone had no effect, however, and there was no interaction between seedbed and pellets, i.e. slugs did not respond differently to pellets according to seedbed type.

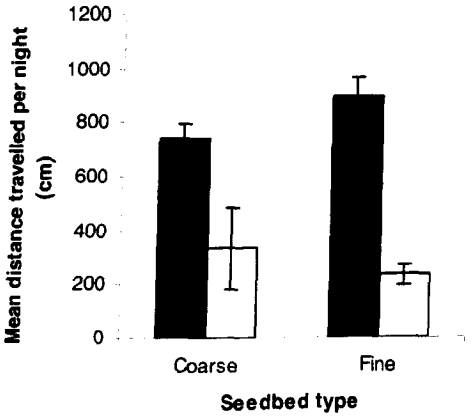


Figure 7.4: Mean distance travelled per night (± S.E.) on coarse and fine seedbeds with and without 5% metaldehyde pellets present (black bars = pellets absent; white bars = pellets present).

Table 7.4: Results of Scheirer-Ray-Hare test to compare the effect of seedbed type and presence of molluscicide on mean total distance travelled per night by Deroceras reticulatum.

Factor/Interaction	SS/MS_{total}	*df*	*P-value*
Seedbed	0.36	1	*n.s.*
Pellets	19.17	1	*< 0.001*
Interaction	0.04	1	*n.s.*

7.3.4 Initiation of feeding on molluscicide in relation to seedbed type

For both types of seedbed, the majority of slugs fed on at least one pellet during the night. Although the proportion of slugs that failed to feed was higher on coarse than fine seedbeds, this difference was not significant (Chi-square test: N = 80, df = 1, χ^2_c = 1.792, *n.s.*) (*Fig. 7.5*).

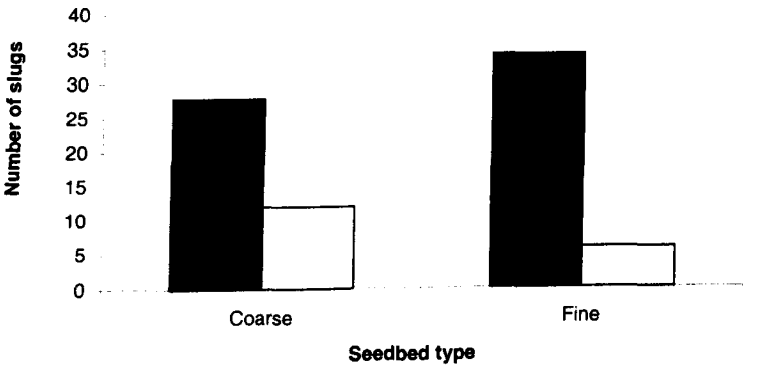


Figure 7.5: Numbers of Deroceras reticulatum *feeding on at least one pellet during the night on coarse or fine seedbeds compared to numbers that did not feed at all (black bars = slugs that fed; white bars = slugs that did not feed).*

For those slugs that fed, most did so on the first pellet they encountered. Where any pellets were ignored, this occurred on coarse seedbeds, but again, differences between seedbed types were not significant (Chi-squared test: N = 62, df = 1, χ^2_c = 1.855, *n.s.*) (*Fig. 7.6*).

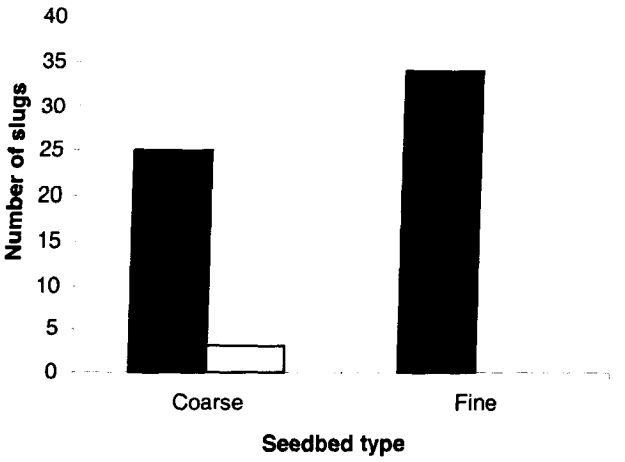


Figure 7.6: Number of pellets ignored before the first feed on coarse and fine seedbeds (black bars = zero pellets ignored; white bars = one or more pellets ignored).

The distance travelled before the first pellet feed did not differ with seedbed type (Independent sample t-test: N = 62, df = 60, t = -0.467, *n.s.*). This was also the case for the time between the onset of activity and the first pellet feed (Independent sample t-test: N = 62, df = 60, t = -0.499, *n.s.*). Data are summarised in *Table 7.5.*

Table 7.5: Mean distance travelled (± S.E.) and time taken between the onset of activity and the first pellet feed.

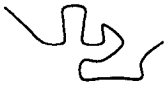
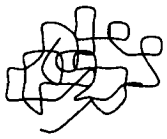
Seedbed	*Mean values (± S.E.) between onset of activity and first pellet feed*	
	Distance (cm)	*Time (mins)*
Coarse	84.0 ± 20.4	64.3 ± 23.5
Fine	109.2 ± 24.1	76.9 ± 17.6

7.3.5 Influence of seedbed and molluscicide on trail patterns

The fractal dimension, *D*, measures spatial patterns of movement within a defined area. As applied in the current experiments it denotes the extent to which slug trails fill the arena. Theoretically *D* can take any value from one to infinity, but in practice

it usually ranges from one to three. These values may be used to indicate the characteristics of the trail path (*Table 7.6*).

Table 7.6: Classification of Deroceras reticulatum *trail paths using their fractal dimension (after Katz & George, 1995).*

Fractal Dimension, D	1	2	3
Path Characteristic	Straight	Random	Constrained
Example Path			

Since, however, *D* depends upon the number of sticks per trail (*n*) (*Equation 7.1, section 7.2.3*) and is a continuum the values in *Table 7.6* are only considered a general guide. To test specifically whether experimental trails differ from a 'true' random path, mean fractal dimension values must be compared to the tabulated mean value for simulated random paths with a similar number of sticks (Katz & George, 1995, Table 1). Since *D* follows a lognormal distribution, standard parametric statistics may be applied and the detailed methodological procedure may be found in this paper.

From *Table 7.6*, it can be seen that the fractal dimension of a random walk approaches two. Thus, inspection of the mean fractal dimensions for each of the experimental treatments shown in *Table 7.7* would suggest that in the absence of metaldehyde pellets, slugs follow trails that can broadly be described as between random and constrained on coarse and fine seedbeds whereas in the presence of metaldehyde pellets they may be categorised as between straight and random. Comparisons with the mean fractal dimension for simulated random paths of a similar number of sticks (Katz & George, 1995) confirmed these trends (*Table 7.8*). All trails were significantly different from a 'true' random path; when the untransformed mean fractal dimensions for the experimental treatments (*Table 7.7*) are contrasted with that of the comparable simulated random paths (1.81) it can be seen that the differences are in the direction initially indicated.

Table 7.7: Mean fractal dimensions for coarse and fine seedbeds in the presence and absence of metaldehyde pellets.

Treatment			*Mean Fractal*
Seedbed	*Pellets*	*N*	*Dimension (± S.E.)*
Coarse	-	38	2.04 ± 0.07
	+	38	1.40 ± 0.04
Fine	-	35	2.37 ± 0.10
	+	37	1.48 ± 0.06

(-)Metaldehyde absent; (+) Metaldehyde present

Table 7.8: Results of t-tests to compare mean fractal dimensions of Deroceras reticulatum *on coarse and fine seedbeds in the presence and absence of metaldehyde pellets (Table 7.7) with the mean fractal dimension of 501 simulated random paths of a similar number of sticks (Katz & George, 1995, Table 1).*

			Log10 (Fractal Dimension)				
Treatment				*Standard*			
Seedbed	*Pellets*	*N*	*Mean*	*Deviation*	*df*	*t*	*P-value*
Coarse	-	38	0.30	0.09	537	13.88	*< 0.001*
	+	38	0.14	0.06	537	21.84	*< 0.001*
Fine	-	35	0.36	0.11	534	10.46	*< 0.001*
	+	37	0.16	0.09	536	20.38	*< 0.001*

For simulated random paths with 49 sticks: N = 501, mean $\log_{10}$fractal dimension = 0.59, standard deviation $\log_{10}$fractal dimension = 0.13. ('-' indicates metaldehyde absent; '+' indicates metaldehyde present).

When experimental treatments were compared with each other it was seen that on a given seedbed, the presence of metaldehyde significantly reduced the trail complexity, as indicated by a lower fractal dimension (ANOVA: Coarse seedbed: $F_{1, 74} = 76.54$, $P < 0.001$; Fine seedbed: $F_{1, 70} = 70.60$, $P < 0.001$) (*Table 7.7*). In the absence of metaldehyde slug trails on fine seedbeds filled more space than on coarse seedbeds (ANOVA: $F_{1, 71} = 6.49$, $P < 0.05$) (*Table 7.7*) whereas when it was present seedbed type had no effect (ANOVA: $F_{1, 73} = 1.30$, *n.s.*).

7.4 Discussion

The primary aim of the experiments presented in this chapter was to characterise the surface activity patterns of *D. reticulatum* on coarse and fine seedbeds both with and without molluscicide present. The aspects of surface activity assessed were those related to the time spent in locomotion, distance travelled and the extent to which slugs use the available arena space, i.e. temporal and spatial activity. The effect of seedbed conditions on the initiation of feeding could be evaluated in treatments using molluscicide pellets; it was not, however, intended to examine feeding and post feeding behaviour on molluscicides *per se*, some aspects of which were described previously in *Chapter 6*.

7.4.1 Soil aggregate size

In order to ensure that the soil aggregates used in this study were representative of fine and coarse arable seedbeds, advice was taken from a local farmer prior to commencing experiments (S. Vernon, *pers. comm.).* There were significant differences in the aggregate size of samples from these two seedbed types, verifying that the conditions being tested were genuinely different from each other, the coarse aggregates being markedly larger than the fine. Samples of aggregates from a given seedbed type did not differ significantly from one another, confirming reproducibility of experimental conditions between replicates.

7.4.2 Seedbed conditions and activity onset time

It has been reported that coarse seedbeds provide more shelter for slugs than fine seedbeds as they can rest in moist pockets between large soil aggregates (Martin & Kelly, 1986). It might be expected, therefore, that slugs on such seedbeds would commence activity later than those on fine seedbeds as the transition between light and darkness, which usually stimulates activity (Rollo, 1991) would be detected less directly. The current experiments did not, however, find any evidence to support this; there was no difference in the interval between darkness and the onset of activity for *D. reticulatum* on coarse and fine seedbeds.

Daily activity cycles are thought to result from an interaction between endogenous rhythms and external conditions (Lewis, 1969b), e.g. photoperiod (Wareing & Bailey, 1985) and temperature (Crawford-Sidebotham, 1972). In the words of Grimm *et al.* (2000), '...whereas endogenous rhythms determine when slugs are ready to become active, it is exogenous conditions which determine whether this readiness is expressed'. As the only variable 'exogenous condition' in the current experiments, it would seem that the extent of shelter is not a strong determinant of activity when conditions are otherwise favourable. It may be that in a harsher environment activity onset behaviour may differ, e.g. field conditions where temperatures fluctuate and air movement is greater.

Overall levels of activity were high; this is not surprising since *D. reticulatum* is a surface dwelling species (Duval, 1970) and individuals were motivated to feed following a short period of starvation. Although activity was essentially nocturnal, a small proportion of slugs commenced activity before artificial sunset. This may partly be due to individuals differing in the time required to acclimatise to the imposed photoperiod, i.e. adapt the endogenous rhythm; whilst 24 hours is thought to be sufficient for most (Whelan, 1982), some may take a little longer and hence emerge from their resting sites when it would previously have become dark. Daytime activity has also been shown to increase with temperature and day-length for *D. reticulatum* (Wareing & Bailey, 1985). In the current experiment, the day-length matched that of the prevailing conditions (approximately 17L:7D), i.e. long days, which could have induced earlier activity onset. That this was only the case for a small proportion of slugs could be because the temperature, at 12 ± 2°C, was relatively low compared to the long day optimum of 17°C reported by Wareing and Bailey (1985); this may have suppressed the 'willingness' of some slugs to express the endogenously determined readiness to become active. Certainly, in another study of *D. reticulatum* activity where the photoperiod was still relatively long at 10-12 hours and temperatures were higher (15-18°C) the onset of activity more closely corresponded with the start of the dark period (Hommay *et al.*, 1998).

7.4.3 Influence of seedbed and molluscicide on the total distance travelled

D. reticulatum travelled similar total distances per night on both coarse and fine seedbeds when pellets were absent suggesting that soil aggregate size had little influence on overall mobility. Presumably, provided the soil surface is moist enough for activity, slugs will travel equally willingly around or between large aggregates as directly across a flat surface.

In the field situation, greater air movement than in the current experiments may mean that conditions on fine seedbeds are more drying than on coarse seedbeds where slugs can rest in sheltered pockets between large aggregates and conserve moisture (Martin & Kelly, 1986). Contact rehydration is a mechanism used to replenish water loss, for example that due to the production of mucus trails during locomotion, whereby individuals pause foraging, assume a flattened posture on a moist substrate and uptake water through the integument of the foot (Prior, 1989). It could be postulated that if conditions on fine seedbeds are, indeed, more drying than coarse seedbeds, pauses for rehydration during foraging would be more frequent on the former. If so, the distances travelled may be shorter and hence, the results could differ from those of the laboratory based study described in this chapter. Confirmation requires further investigation.

The total distances travelled per night in the current experiment when pellets were absent (mean ± S.E. was 7.4 ± 5.7 m on coarse seedbeds and 9.0 ± 6.7 m on fine seedbeds) were longer than those reported in studies where food was provided, albeit that overall food abundance was shown to have little effect, for example Bailey and Wedgwood (1991), where mean ± S.E. = 4.6 ± 3.1 m and Hommay (1998), where mean ± S.E. = 4.0 ± 2.9 m. This is likely simply to reflect the continued foraging due to hunger not being satiated.

The presence of metaldehyde pellets significantly decreased the total distance travelled by slugs on both coarse and fine seedbeds. This is not surprising given the action of this active ingredient; as a stomach or contact poison it induces immobilisation through disruption of the nervous system central pattern generator

(Mills *et al.*, 1989) which ensues within approximately 45 minutes of feeding (Bailey & Wedgwood, 1991) and, even with moderate doses, may persist for over twenty four hours (Cragg & Vincent, 1952).

There was no interaction between seedbed type and pellets, indicating that *D. reticulatum* were responding to pellets in a similar way, regardless of seed bed conditions and that, by implication, aggregate size does not interfere with pellet encounters provided they are accessible.

7.4.4 Initiation of feeding on molluscicide in relation to seedbed type

Most *D. reticulatum* fed at least once during the night regardless of seedbed conditions and of these most did so on the first pellet encountered, supporting the findings of Bailey (1989) and Howlett and Port (2003); this is not surprising since individuals had been starved for 24 hours prior to recordings to ensure they were motivated to forage. Too few slugs fed on a second pellet to allow statistical analysis. As discussed in *section 7.4.3*, within about 45 minutes of ingesting metaldehyde slugs exhibit clear symptoms of poisoning (Bailey & Wedgwood, 1991). Individuals in the current experiment evidently consumed a sufficient quantity of active ingredient to effectively prevent further feeding for the remainder of the night.

The mean distance travelled before the first pellet feed did not differ between seedbeds and was broadly comparable with the distances recorded for *D. reticulatum* feeding on broadcast metaldehyde reported in *Chapter 6* (87.6 ± 18.6 cm). Similarly, the time between the onset of activity and the first feed did not differ on coarse and fine seedbeds and, again, agreed with recorded values for broadcast metaldehyde in *Chapter 6* (89.0 ± 29.3 min). These findings support the suggestion that olfaction does not play a strong role in attraction to food items over long distances and that slugs tend to encounter them by random (S.E.R. Bailey in Howling, 1991). Were long distance olfaction to be important then it might be that slugs would have located pellets sooner on fine than coarse seedbeds where the larger soil aggregates could act as baffles, dampening olfactory cues, but this was not observed.

Where any pellets were ignored, i.e. slugs were in contact with the pellet for less than 0.5 minutes, this occurred slightly more frequently on coarse than fine seedbeds possibly because only part of some pellets are exposed on coarse seedbeds, the remainder being hidden in a soil crack or under a clod of earth. These differences, however, were not statistically significant. Similarly the numbers of *D. reticulatum* that failed to feed were slightly higher on coarse than fine seedbeds, but again, the difference was not significant. These results suggests that, whilst it may be harder for slugs to locate broadcast pellets in between the large soil aggregates of coarse seedbeds compared to fine seedbeds, this does not reduce overall efficacy. This situation contrasts with that in the study of Glen *et al.* (1992) where damage to seeds was assessed on coarse and fine seedbeds. In this case, the seeds were covered in soil on fine seedbeds, but were more accessible to slugs on coarse seedbeds where they fell in between large aggregates; slugs fed more readily on the latter. Taken together, this suggests sowing seeds into fine seedbeds, followed by a broadcast application of molluscicide affords a high level of protection.

7.4.5 Influence of seedbed and molluscicide on trail patterns

The extent to which slug trails filled the space in the arena was assessed using fractal dimensions analysis. This measure is easy to interpret and was originally developed to categorise cell growth patterns (Katz & George, 1995), but has since been very usefully applied to studies of animal movement (Erlandsson & Kostylev, 1995; Bascompte & Vilà, 1997). It does not give information on angular deviations so cannot be used to infer whether animals are orienting in response to particular stimuli in their environment, e.g. a pellet, unlike other measures of movement such as correlated random walk. It does, however, take account of the total structure of the trail, even if it spans an entire day, rather than being limited to small sub-trails (Erlandsson & Kostylev, 1995).

In the absence of metaldehyde pellets slug trails on both coarse and fine seedbeds were more constrained than a 'true' random path. i.e. they criss-crossed over each other. This is likely to reflect the arena size. Whilst it has been shown that arena size does not affect total distance travelled (Bailey, 1989; Howling, 1991), it is not

surprising that it influences spatial movement patterns; the arenas were small compared to the mean total distance travelled by *D. reticulatum* per night of approximately four to six metres (Bailey, 1989; Hommay *et al.*, 1998) and in the absence of factors that arrest activity it is almost inevitable that an individual will re-cross earlier parts of its trail. It may be speculated that in larger arenas the fractal dimensions of trails may approach that of a truly random path. Trails filled significantly more of the available space on fine than coarse seedbeds in the absence of metaldehyde. This may reflect a tendency of slugs to travel between as opposed to over larger soil aggregates; if so, parts of the soil surface beneath large aggregates in coarse seedbeds are unavailable to the slugs which may explain the lower fractal dimension of trails.

Metaldehyde pellets significantly reduced the space filled by *D. reticulatum* trails. Spatial patterns tended to be straighter than a 'true' random path. It is assumed that further movement is prevented after slugs feed on a pellet (*section 7.4.3*) and so this 'straighter than random' pattern relates to movement before the first feed. As stated previously, the fractal dimension cannot indicate whether slugs begin to orient differently when they approach stimuli in their environment and therefore it is not possible to say whether these relatively straight movement patterns are due to pellet attraction. As described earlier, however, other studies suggest that this is unlikely to be the case since olfaction is believed to play a role in detection of food only at very close range (Bailey, 1989; Howling, 1991) and in the current experiments *D. reticulatum* travelled reasonably far before first contacting a pellet (~80–100 cm). It may instead be that slugs were moving in an undirected manner, but because pellets were easy to locate, being broadcast and evenly spaced, they encountered them without covering a large area which would result in the lower fractal dimension and this result is, therefore, interpreted with caution. It would be interesting to see whether paths became more random if pellets were more widely spaced. An alternative non-toxic food source may also critically affect spatial movement patterns as slugs would be able to continue foraging after feeding.

7.5 Conclusions and Future Work

In conclusion, it was found that:

1. The activity onset time of *D. reticulatum* did not differ between coarse and fine seedbeds.
2. The total distance travelled per night by *D. reticulatum* was comparable between coarse and fine seedbeds; in both cases this was significantly reduced when metaldehyde pellets were present, but there was no interaction between seedbed and pellets.
3. The initiation of feeding on metaldehyde pellets was not affected by seedbed conditions; on both coarse and fine seedbeds *D. reticulatum* travelled similar distances in comparable times before the first feed and most slugs fed on the first pellet encountered.
4. The number of *D. reticulatum* ignoring metaldehyde pellets or failing to feed altogether did not differ between coarse and fine seedbeds.
5. *D. reticulatum* trails filled significantly more of the available space on fine than coarse seedbeds in the absence of metaldehyde pellets; in both cases trail complexity was between a 'truly random' and constrained path.
6. The fractal dimension was reduced on coarse and fine seedbeds in the presence of metaldehyde pellets; trail complexity was between a straight and random path and there were no significant differences between seedbed types.

In field conditions, seedbeds are less uniform than those in the present experiment, consisting of aggregates of mixed sizes and several species of slugs are present simultaneously. This may result in more complex behaviours than those observed in this study (Glen *et al.*, 1992). It would be difficult to discern different species on monochromatic video recordings, unless they were markedly different sizes, but experiments could be repeated using variable proportions of fine and coarse soil aggregates in the seedbed. The current results would suggest that this would have minimal effect on the timings of activities, but it may alter trail patterns, which would be reflected in the fractal dimension calculations. Recovery from poisoning was not assessed in this study. It is reported that under moist, humid conditions,

such as would be found between large soil aggregates on coarse seedbeds, slugs frequently recover from metaldehyde poisoning (Cragg & Vincent, 1952; Crawford-Sidebotham, 1970). It would be useful from the perspective of control to know whether there was significantly greater recovery rates of slugs on coarse seedbeds compared to fine. Fractal dimension analysis has great potential to analyse the effects of a host of factors on spatial movement patterns; an alternative food source has already been suggested. It could, for example, also be used to assess the effects of different shelter materials or novel repellents on surface activity.

Section C

Population Assessment using Refuge Traps

This section comprises a single chapter and focuses on the efficacy of refuge traps in estimating surface active slug populations.

Chapter 8 compares refuge trap catches with those of defined area traps under field conditions. The behaviour of different sized *D. reticulatum* in response to refuge traps is investigated using time lapse video techniques.

Chapter 8
Estimation of Surface Active Slug Populations using Refuge Traps

Abstract

The efficacy of refuge traps and defined area traps was compared in field conditions. The most abundant species were *Deroceras reticulatum* (Müller) and *Arion subfuscus* (Draparnaud); refuge traps tended to under represent small individuals compared to defined area traps. To investigate whether size related differences in slug behaviour might explain this observation, time-lapse video techniques were used with a novel refuge trap made of infra-red transparent material to record individual movement of small (< 100 mg) and large (> 500 mg) *Deroceras reticulatum* upon encountering the trap. Approximately one third of the slugs that entered the traps at some point in the night remained there at dawn. There were no size specific differences in the number, timing or duration of trap entries. Infra-red transparent refuge traps performed as well as standard opaque saucer traps under field conditions.

8.1 Introduction

Central to any decision regarding the implementation of control measures for crop pests is an estimation of population size (Port & Port, 1986). This is not an easy task in the case of slugs due to their patchy distribution and partly subterranean habitat (Hunter, 1966) along with the dependence of their activity on factors such as soil moisture, photoperiod, humidity and temperature (Rollo, 1991). Approaches comprise both indirect and direct methods. The former include searching at night (Barnes, 1944; Barnes & Weil, 1944), inference from damage levels (Duthoit, 1961) and trapping in artificial refuges (Getz, 1959; Schrim & Byers, 1980). These sample

slugs from an unknown area, but are relatively quick to complete. Direct methods, in contrast, sample slugs from a known area, but are more laborious and include soil washing (Hunter, 1968a), flooding (South, 1964) and defined area traps (DATs) (Ferguson *et al.*, 1989).

The different methods of population assessment vary in the type of estimate they provide, e.g. density, abundance or surface activity levels (Thomas, 1944; Oggier *et al.*, 1998). Their efficacy is affected by weather conditions (Hunter, 1968a) and factors such as construction material and the use of bait (Young *et al.*, 1996). In combination with the variable nature of slug activity this makes comparisons between studies inherently difficult; even within a study variation in conditions on different assessment dates can confound results.

Despite their limitations, there are no alternatives to the current methods of population estimation. Soil sampling is considered the 'gold standard' (Hunter, 1968a; Glen & Wiltshire, 1986), but is impractical for use by farmers to assess the need for control measures due to time and labour constraints. Refuge traps, however, are transportable, cheap, easy to use and store (Schrim & Byers, 1980). Of the direct methods DATs are the most convenient and least labour intensive (Ferguson & Hanks, 1990). For these reasons these two methods are among the most frequently used and are likely to remain so for the foreseeable future. The experiments presented in this chapter were carried out on this basis.

Refuge traps provide an estimate of surface activity. They consist of a shelter material which usually covers a food bait. Toxic baits, e.g. piles of molluscicide pellets, retain slugs in the traps, but are not desirable due to the hazard large amounts of pesticide under a single trap poses to wildlife and domestic animals (Voss *et al.*, 1998). In a trial of various shelter materials and non-toxic baits it was shown that hardboard squares baited with chicken layers mash was a very effective combination (Young *et al.*, 1996). Being lighter and more portable than hardboard squares, however, plastic flowerpot saucers are also commonly used (Clements & Murray, 1991).

DATs estimate population density and comprise a metal barrier sunk into the ground enclosing a known area, typically 0.1 m^2 (Ferguson *et al.*, 1989). A hardboard square or sacking is usually placed within the metal barrier to provide humid, sheltered conditions which encourage slugs to remain on the surface. The DATs are checked regularly and any slugs present are removed and counted until no further slugs are found; the area is then considered 'trapped out'.

Numerous studies have contrasted the efficacy of refuge traps with DATs (e.g. Byers *et al.*, 1989; Clements & Murray, 1991; Barratt *et al.*, 1993; Voss *et al.*, 1998). Since these assessment methods measure different aspects of the population (i.e. surface activity and density) the comparisons drawn have a relative rather than absolute meaning. It has been consistently reported that refuge traps under represent small slugs in relation to DATs and other direct methods of sampling (e.g. Glen & Wiltshire, 1986; Clements & Murray, 1991). The reasons for this are not clear; it has been suggested that large slugs may move further than small slugs and are, therefore, more likely to contact refuge traps (Glen & Wiltshire, 1986) or that small slugs are more easily overlooked (Archard *et al.*, 2004). It may also be that small and large slugs behave differently towards refuge traps when they encounter them (Howlett *et al.*, 2004, *In press*).

The experiments described in this chapter were designed to investigate the behaviour of different sized slugs towards refuge traps. There were three elements to the study. Firstly, locally collected trapping data from DATs and refuge traps were compared to ascertain whether the bias reported in the literature was also apparent in our study area. Secondly, activity studies of slugs using time-lapse video techniques were conducted to examine whether there were any size specific differences in their behavioural response to refuge traps; this involved using a novel saucer trap that was transparent to infra-red light. Thirdly the novel trap was compared to a standard saucer trap under field conditions to assess the general applicability of the behavioural study results.

8.2 Materials and Methods

8.2.1 Materials

8.2.1.1 Comparison of refuge traps and DATs

Defined area traps (DATs) comprised four galvanised steel sheets arranged to form a square measuring 42 cm x 42 cm, enclosing an area of 0.18 m^2. These were sunk into the ground to a depth of 5-7 cm, such that approximately 15 cm protruded above the surface. A sheet of hardboard was placed inside each DAT to provide sheltered, moist conditions suitable for slugs. Refuge traps comprised upturned terracotta coloured plastic flowerpot saucers of 25 cm diameter. Refuge traps were baited with 2.5 g chicken layers mash. DATs were unbaited.

8.2.1.2 Slug activity beneath refuge traps

8.2.1.2.1 Slugs

Small (< 100 mg) and large (> 500 mg) *D. reticulatum* used in the experiments were collected from under refuge traps at Close House Field Station, Heddon-on-the-Wall, Northumberland (Grid reference NZ 127659). They were maintained at 12°C in ventilated plastic containers (18 x 12 x 7 cm) filled with moist laboratory tissue for up to five days prior to use. This ensured that any slugs in the sample that were unhealthy following collection could be excluded from the experiments. During this time they were provided with Chinese cabbage and carrot *ad libitum*.

8.2.1.2.2 Arenas

The experiments were carried out in plastic arenas measuring 57 x 36 x 16 cm, the rims of which were painted with a Fluon® (polytetrafluoroethylene) barrier (Whitford Plastics, Cheshire, England) to prevent slugs escaping. The arenas were filled to a depth of 8-10 cm with loamy soil dug from an agricultural plot at Close House Field Station and any stones, large lumps of organic matter and soil organisms were removed prior to use. The soil surface was raked to a fine tilth. Fresh soil was used for each replicate and it was watered such that the surface appeared damp at the start of each recording.

8.2.1.2.3 Recording equipment and lighting

In indoor experiments slug activity was recorded using a Panasonic AG-6040 time-lapse VHS video recorder. The camera used was a Sanyo VCB3572 IRP ½ " high resolution 570TVL infra-red sensitive camera, fitted with a Computar 8-48 mm lens and infra red bandpass filter. The arena was illuminated at night using a Computar Uniflood LED infra red lamp (serial number CL057787). Light of this wavelength does not appear to disrupt slug activity yet permits recording to take place during darkness (Howling, 1990). Daytime lighting was provided by two 400W halogen lamps suspended 1.55 m above the arena. These switched on and off via a timer to match the prevailing sunrise and sunset times, which were updated weekly.

In outdoor experiments a Sanyo TLS-1600P/IR time-lapse VHS video recorder was used with a Baxall CD0242/IR ½ " high resolution infra-red sensitive camera. This was fitted with a Computar 4.5-10 mm lens and infra-red filter. The camera was housed in waterproof casing and mounted on a tripod. Night time illumination was provided by a waterproof infra-red LED unit and daytime lighting was not required. Power was supplied to the camera by a 12 volt car battery, otherwise mains electricity was used.

In both indoor and outdoor setups the video recorder was set to record 60 times more slowly than a standard machine so that a total of 180 hours of activity could be stored on a single cassette. Recordings were played back at normal speed.

8.2.1.2.4 Infra-red transparent refuge trap

Specially designed refuge traps comprised 18 cm diameter upturned saucers manufactured from black infra-red transparent plastic (Tracksys Ltd., Nottingham, England). These were opaque to visible light, but transparent to infra-red illumination (*Fig. 8.1 (a) & (b)*). The traps were sprayed with a fine anti-mist coating (Holts anti-mist and water repellent spray, product code HMC6) to prevent condensation forming and obscuring the image during recordings. They were baited with 2.5 g chicken layers mash.

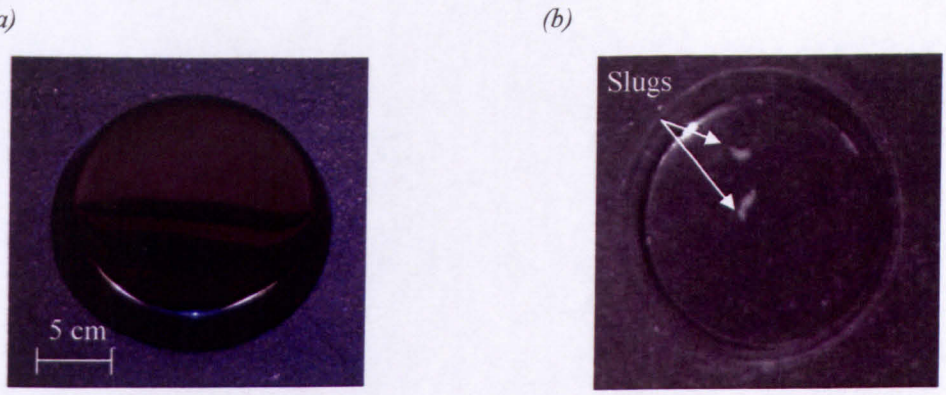


Figure 8.1: Infra-red transparent saucer (a) as viewed by the naked eye and (b) under infra-red illumination with slugs visible beneath.

8.2.1.3 Field scale comparison of infra-red transparent and standard refuge traps

Infra-red transparent refuge traps as described in *section 8.2.1.2.4* were compared with terracotta coloured standard plastic flowerpot saucers of the same diameter (18 cm). Where traps were baited, this was with 2.5 g chicken layers mash.

8.2.2 Methods

8.2.2.1 Comparison of refuge traps and DATs

8.2.2.1.1 Study site

Trapping data were collected from two arable fields (middle field and bottom field) at Heddon Banks Farm, Heddon-on-the-Wall, Northumberland (NZ 138 657) on ten, two-week sampling periods in 2002-03. Prior to and during trapping both fields were sown with wheat.

8.2.2.1.2 Trap deployment

Sixteen DATs were deployed in each field at a distance of 10 m apart in a grid arrangement. These remained in-situ for two weeks. Tall vegetation protruding above the rim of the DAT was trimmed. Any slugs that were on this vegetation were removed and replaced into the DAT. Sixteen refuge traps were deployed at the midpoint between DAT rows (i.e. a distance of 5 m between two DATs) on a single

night during the two week trapping period when weather conditions were appropriate for surface activity (*Fig. 8.2*). After this the refuge traps were removed again. After each two week trapping period the DATs were removed and redeployed according to a rotation system (*Fig. 8.3*).

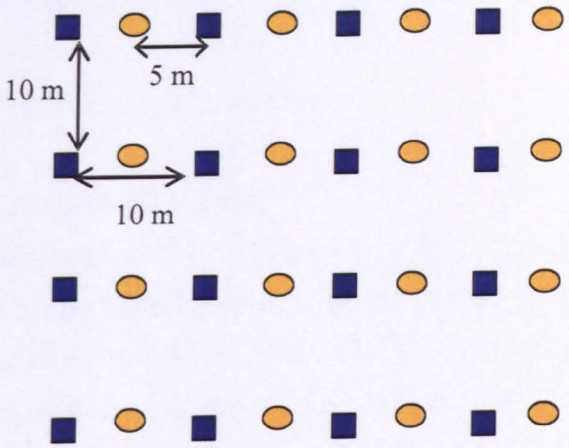


Figure 8.2: 10 m x 10 m grid arrangement of 16 DATs at Heddon Banks Farm showing the position of 16 refuge traps 5 m between DAT rows (blue squares represent DATs; yellow circles represent refuge traps).

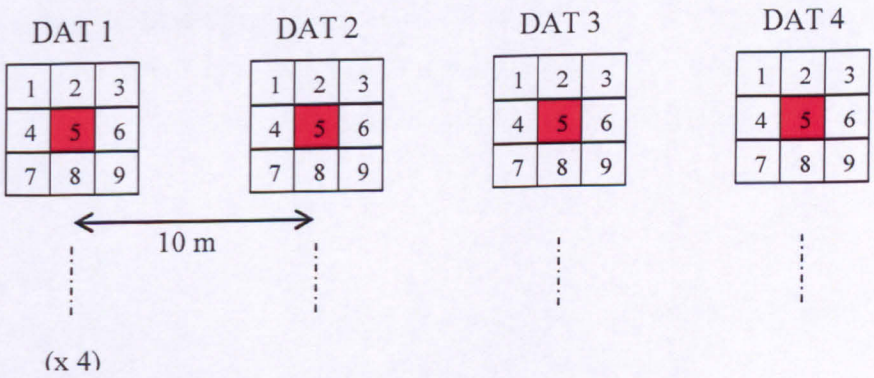


Figure 8.3: Rotation of DATs at Heddon Banks Farm.
At each DAT site within the 10 m x 10 m grid (Fig. 8.2) there is a second grid arrangement of adjacent squares numbered 1-9. A pole is placed in the centre of each grid (marked in red). The distance between poles in adjacent grids is 10 m. Each of the 16 DATs is placed at the same numerical position within its own grid (e.g. all DATs are placed at position 1 in their own grid). At the end of each two week sampling period, all DATs are moved to the next numerical position within their own grid (e.g. all DATs are moved from position 1 to position 2) etc.

8.2.2.1.3 Trap assessment

Traps were checked each morning by 10.30 a.m. and slugs of all species present were removed and counted. The first 30 individuals of each species were weighed using a Mettler MT5 balance to an accuracy of 0.01 mg. DATs were checked every working day during each two week trapping period (i.e. a total of ten occasions) and the refuge traps were checked on the morning following their deployment.

8.2.2.2 Slug activity beneath refuge traps

8.2.2.2.1 Acclimatisation and recording

Six *D. reticulatum* were used per arena, three small (< 100 mg) and three large (> 500 mg), which is equivalent to a field density of approximately 28 slugs per m^2. These were weighed using a Mettler MT5 balance to an accuracy of 0.01 mg. To allow for atypical activity in response to a new environment slugs were placed in the arena for 24 hours prior to the commencement of recording to allow them to acclimatise (Whelan, 1982). During this time they were starved to ensure that they were motivated to forage. Following the acclimatisation period the chicken layers mash bait was applied to the arena in a central pile and was then covered with the infra-red transparent refuge trap. Recording began and continued overnight until the following morning. A total of eight replicates were recorded for both indoor and outdoor setups and different slugs were used for each. The indoor experiments were carried out at 12 ± 2°C in a controlled temperature room. The ambient temperature and soil temperature during the outdoor experiments were recorded using Tinytalk™ data loggers.

8.2.2.2.2 Video data extraction

Video recordings were played back and for each slug the number and timing of trap entries and exits was noted, in addition to the activity start time.

8.2.2.3 Field scale comparison of infra-red transparent and standard refuge traps

8.2.2.3.1 Study site

Trapping was carried out in a grass plot within a walled garden at Close House Field Station, Heddon-on-the-Wall, Northumberland (Grid reference NZ 127659).

8.2.2.3.2 Trap deployment

A total of 12 assessments were completed. Traps were deployed on mild evenings when the soil surface was moist. Two standard terracotta and two infra-red transparent refuge traps were used. These were placed 5 m apart in a grid arrangement. One of each trap type had 20 ml chicken layers mash underneath; the other had no bait (*Fig. 8.4*). The traps were left *in-situ* for a single night before being redeployed 5 m to the left of the previous site according to a rotation system (*Fig. 8.5*).

8.2.2.3.3 Trap assessment

Traps were checked each morning by 10.30 a.m. and the numbers of slugs of all species present were counted. Any *D. reticulatum* were weighed on a Mettler MT5 balance to an accuracy of 0.01 mg. Traps were then wiped clean and any remaining chicken layers mash was disposed of such that there was no trace left on the soil surface.

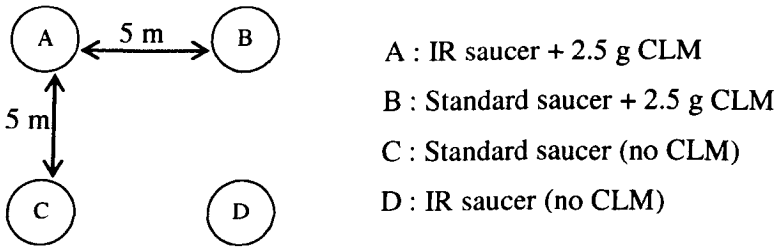


Figure 8.4: 5 m x 5 m grid arrangement of refuge traps at Close House Field Station (CLM = chicken layers mash).

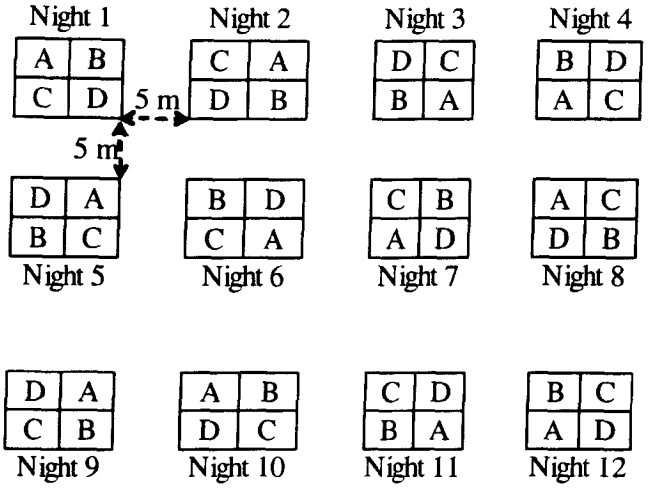


Figure 8.5: Rotation of refuge traps at Close House Field Station.

8.2.3 Statistical analysis

For comparisons between refuge traps and DATs the mean size of slugs in different trap types was analysed using the Wilcoxon Signed Ranks test as the data were non-parametric.

For assessments of slug activity beneath refuge traps count data was analysed using Fisher's Exact test or contingency Chi square tests as appropriate. For the latter the Yates' correction was applied if necessary. For non-parametric two factor comparisons of counts the Scheirer-Ray-Hare test was used. Data concerning the timing of events were assessed with one-way analysis of variance (ANOVA) following transformation.

For field scale comparisons of infra-red transparent and standard saucer refuge traps counts were analysed using the contingency chi-square test with Yates' correction. Continuous weight data were parametric; these were assessed by two-way analysis of variance (ANOVA).

8.3 Results

8.3.1 Comparison of refuge traps and DATs

The months and years corresponding to each of the ten sampling occasions are shown in *Table 8.1*. There are no data for August and September due to harvesting and cultivations which prevented access to the fields.

Table 8.1: Months and years corresponding to each sampling occasion.

Sample Occasion	*Month*	*Year*	*Season*
1	Oct	2002	Autumn
2	Nov	2002	
3	Dec	2002	Winter
4	Jan	2003	
5	Feb	2003	
6	Mar	2003	Spring
7	Apr-May	2003	
8	May-June	2003	Summer
9	July	2003	
10	Oct	2003	Autumn

8.3.1.1 Species abundance

Although there were some fluctuations in relative abundance on different sampling occasions, the most abundant species summed over the total growing season were generally *D. reticulatum* and *A. subfuscus* in both trap types and fields. The single exception to this is in the middle field refuge traps where *Deroceras panormitanum* (Lessona and Pollonera) was the second most numerous species after *D. reticulatum* (*Tables 8.2 & 8.3*).

Table 8.2: Species abundance in DAT traps in middle and bottom field on each of ten sampling occasions at Heddon Banks Farm (2002-03).

Field	Sampling Occasion	Arion circumscriptus	Arion distinctus	Arion subfuscus	Deroceras panormitanum	Deroceras reticulatum
Bottom	1	-	-	-	-	-
	2	-	-	2	-	7
	3	-	-	1	-	3
	4	-	-	-	-	4
	5	-	-	-	-	3
	6	-	-	2	-	13
	7	-	-	11	-	30
	8	-	-	8	-	94
	9	-	1	26	-	103
	10	-	-	1	-	-
	Total	0	1	51	0	257
Middle	1	-	-	3	6	23
	2	-	-	3	12	50
	3	-	2	4	1	22
	4	1	-	11	9	30
	5	-	-	7	4	19
	6	-	1	12	4	24
	7	-	1	34	8	69
	8	-	-	115	9	52
	9	-	1	178	6	19
	10	-	-	-	-	-
	Total	1	5	367	59	308

Dates for each sampling occasion are shown in Table 8.1.

Table 8.3: Species abundance in refuge traps in middle and bottom field on each of ten sampling occasions at Heddon Banks Farm (2002-03).

Field	Sampling Occasion	Arion subfuscus	Deroceras panormitanum	Deroceras reticulatum
Bottom	1	-	-	3
	2	-	-	1
	3	9	-	20
	4	4	-	12
	5	8	-	20
	6	1	-	10
	7	6	-	23
	8	1	-	2
	9	3	-	8
	10	-	-	-
	Total	*32*	*0*	*99*
Middle	1	1	8	24
	2	1	-	14
	3	17	53	114
	4	21	19	55
	5	26	17	66
	6	2	4	20
	7	6	2	26
	8	1	1	3
	9	5	2	1
	10	1	1	3
	Total	*81*	*107*	*326*

There were more species trapped in total in the middle field for both DATs and refuge traps, although it was only *D. panormitanum* that were found in appreciable numbers in addition to *D. reticulatum* and *A. subfuscus (Fig. 8.6).* Analyses are, therefore, restricted to *D. reticulatum* and *A. subfuscus* in order to make consistent comparisons between fields.

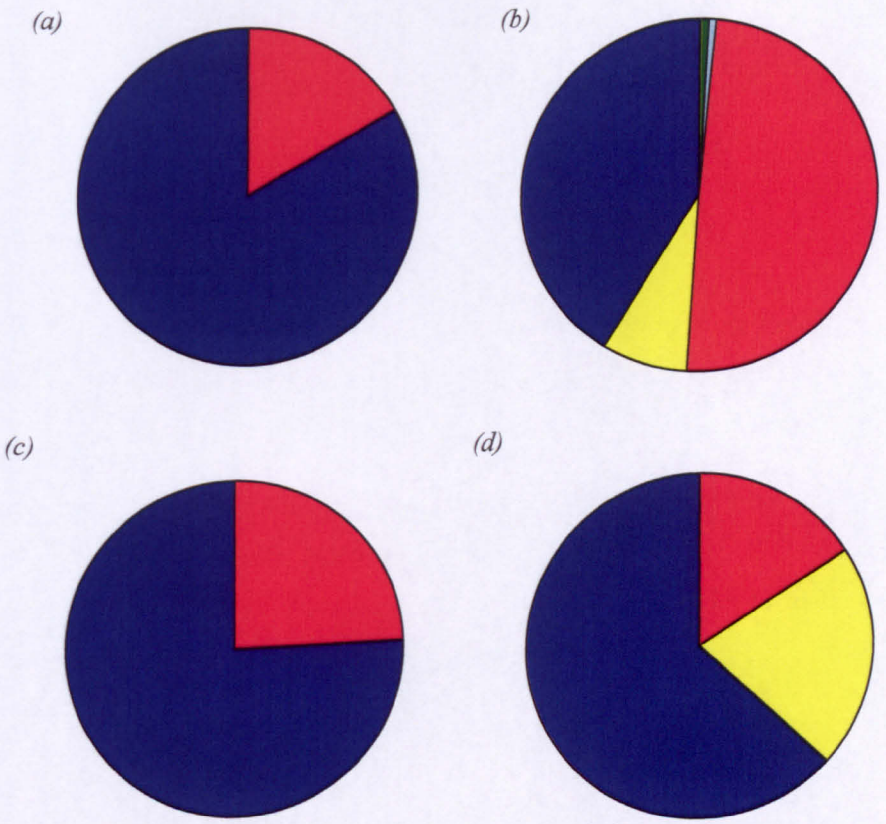


Figure 8.6: Slug species abundance at Heddon Banks Farm, Northumberland 2002-03 (a) DAT bottom field (b) DAT middle field (c) refuge trap bottom field (d) refuge trap middle field (blue segment = Deroceras reticulatum; *yellow segment* = Deroceras panormitanum*; red segment* = Arion subfuscus*; pale blue segment* = Arion distinctus*; green segment* = Arion ater*).*

8.3.1.2 Numbers of D. reticulatum and A. subfuscus

Results for both fields are summarised in *Table 8.4* for *D. reticulatum* and *Table 8.5* for *A. subfuscus.*

Table 8.4: Results of Wilcoxon Signed Ranks Test to compare mean weights of Deroceras reticulatum *between DATs and refuge traps in middle and bottom fields, Heddon Banks Farm, Northumberland (2002-03). Data refer only to those trap pairs where at least one slug was caught on a given sampling occasion († indicates that this is a single individual).*

Field	Sampling occasion	Number of trap pairs	Number of slugs		χ^2	P-value	Mean weight ± S.E. (mg)		Z	P-value
			Refuge trap	DAT			Refuge trap	DAT		
Middle	1	14	24	23	0.09	*n.s*	153.00 ± 21.94	85.36 ± 11.69	-1.977	*< 0.05*
	2	16	14	50	19.14	*< 0.001*	142.70 ± 38.14	162.33 ± 9.88	-0.414	*n.s.*
	3	12	114	22	63.60	*< 0.001*	111.51 ± 14.24	157.23 ± 27.12	-0.784	*n.s.*
	4	13	55	30	7.95	*< 0.01*	151.43 ± 20.30	263.00 ± 25.55	-1.572	*n.s.*
	5	12	66	19	27.11	*< 0.001*	217.14 ± 22.65	186.50 ± 28.79	-0.628	*n.s.*
	6	13	20	24	0.20	*n.s.*	415.26 ± 44.47	177.32 ± 33.76	-2.411	*< 0.05*
	7	16	26	69	18.57	*< 0.001*	419.35 ± 92.16	173.76 ± 14.49	-2.741	*< 0.01*
	8	15	3	52	41.89	*< 0.001*	79.69 ± 98.54	179.47 ± 18.66	-2.613	*< 0.01*
	9	10	1	19	14.45	*< 0.001*	32.17†	271.20 ± 50.34	-2.803	*< 0.01*
	10	3	3	0	5.33	*< 0.05*	235.82 ± 160.72	-	-	-
	TOTAL	*124*	*326*	*308*	*0.57*	*n.s.*	*199.10 ± 14.02*	*176.01 ± 10.03*	*-0.347*	*n.s.*
Bottom	1	2	3	0	5.33	*< 0.05*	441.11 ± 56.66	-	-	-
	2	6	1	7	3.13	*n.s.*	124.63†	277.78 ± 67.17	-1.153	*n.s.*
	3	10	20	3	14.09	*< 0.001*	316.39 ± 35.76	10.03 ± 12.65	-2.803	*< 0.01*
	4	9	12	4	5.06	*< 0.05*	129.15 ± 31.97	90.47 ± 71.52	-0.533	*n.s.*
	5	9	20	3	14.09	*< 0.001*	376.10 ± 47.65	55.47 ± 65.87	-2.429	*< 0.05*
	6	10	10	13	0.17	*n.s.*	321.52 ± 83.79	185.32 ± 61.44	-1.172	*n.s.*
	7	16	23	30	0.68	*n.s.*	266.10 ± 36.26	404.81 ± 27.33	-2.223	*< 0.05*
	8	15	2	94	86.26	*< 0.001*	49.50 ± 93.51	321.71 ± 20.83	-2.953	*< 0.01*
	9	12	8	103	79.60	*< 0.001*	167.07 ± 87.64	172.76 ± 10.18	-0.235	*n.s.*
	10	0	-	-	-	-	-	-	-	-
	TOTAL	*89*	*99*	*257*	*69.24*	*< 0.001*	*219.79 ± 22.26*	*201.44 ± 12.57*	*-0.305*	*n.s.*

Table 8.5: Results of Wilcoxon Signed Ranks Test to compare mean weights of Arion subfuscus *between DATs and refuge traps in middle and bottom fields, Heddon Banks Farm, Northumberland (2002-03). Data refer only to those trap pairs where at least one slug was caught on a given sampling occasion († indicates that this is a single individual).*

Field	Sampling occasion	Number of trap pairs	Number of slugs				Mean weight ± S.E. (mg)			
			Refuge trap	DAT	χ^2	P-value	Refuge trap	DAT	Z	P-value
Middle	1	3	1	3	0.25	*n.s*	47.41†	284.78 ± 164.42	-1.069	*n.s.*
	2	4	1	3	0.25	*n.s*	85.36†	55.09 ± 28.23	-0.365	*n.s.*
	3	12	17	4	9.33	*< 0.01*	225.26 ± 63.27	11.61 ± 12.40	-2.667	*< 0.01*
	4	14	21	11	3.78	*n.s*	140.96 ± 31.73	109.80 ± 55.83	-1.161	*n.s.*
	5	14	26	7	12.12	*< 0.001*	309.35 ± 56.61	36.34 ± 34.02	-3.296	*< 0.01*
	6	7	2	12	5.79	*< 0.05*	154.01 ± 241.00	118.62 ± 21.86	-0.507	*n.s.*
	7	14	6	34	18.23	*< 0.001*	274.37 ± 196.46	312.91 ± 35.48	-0.596	*n.s.*
	8	14	1	115	110.08	*< 0.001*	30.70†	219.01 ± 13.80	-2.794	*< 0.01*
	9	12	5	178	161.66	*< 0.001*	315.90 ± 188.02	175.96 ± 11.58	-0.706	*n.s.*
	10	0	-	-	-	-	-	-	-	-
	TOTAL	*95*	*81*	*367*	*181.31*	*< 0.001*	*296.62 ± 32.96*	*143.67 ± 9.06*	*-0.635*	*n.s.*
Bottom	1	0	-	-	-	-	-	-	-	-
	2	2	0	2	0.50	*n.s*	-	549.81 ± 388.77	-1.342	*n.s.*
	3	6	9	1	8.10	*< 0.01*	554.91 ± 149.44	12.01†	-1.992	*< 0.05*
	4	3	4	0	6.25	*< 0.05*	177.42 ± 68.49	-	-	-
	5	6	8	0	10.13	*< 0.01*	528.82 ± 160.46	-	-	-
	6	2	1	2	0.00	*n.s*	102.45†	423.94 ± 423.93	-0.447	*n.s.*
	7	11	6	11	0.94	*n.s*	364.38 ± 178.49	884.49 ± 164.47	-1.689	*n.s.*
	8	6	1	8	4.00	*< 0.05*	177.25†	1247.51 ± 147.85	-1.992	*< 0.05*
	9	12	3	26	16.69	*< 0.001*	341.40 ± 364.97	1327.26 ± 260.30	-3.059	*< 0.01*
	10	0	-	-	-	-	-	-	-	-
	TOTAL	*49*	*32*	*51*	*3.90*	*< 0.05*	*334.86 ± 82.83*	*718.45 ± 91.74*	*-2.711*	*< 0.01*

In general refuge traps caught more slugs in early autumn and winter (sampling occasions 1-5 and 10) whereas DATs caught larger numbers in spring and summer (sampling occasions 6-9). The significance of differences tended to be greater when the total numbers caught were higher. There were no clear differences between fields for either species.

8.3.1.3 Weight differences between slugs caught in DATs and refuge traps

As for *section 8.3.1.2* results for both fields are summarised in *Table 8.4* for *D. reticulatum* and *Table 8.5* for *A. subfuscus*. Note that some comparisons involve a single individual. Although the tests are valid, such results must be treated with caution.

The mean weight of slugs caught by refuge traps and DATs did not differ significantly on all sampling occasions. For both species, however, it can be seen that, where there are significant differences, the refuge traps generally caught heavier slugs in autumn, winter and early spring, whereas in summer these were found in the DATs.

For the growing season as a whole (i.e. all sampling occasions combined) the only instance where a significant difference in mean slug weight between trap types persisted was in the bottom field for *A. subfuscus* where the DATS caught heavier slugs.

8.3.2 Slug activity beneath refuge traps

A pilot study showed that the anti-mist spray used on infra-red transparent saucers to prevent condensation had no differential effect on the activity of slugs compared to an unsprayed saucer.

There were no block effects between replicates in either indoor or outdoor experiments and data were therefore pooled for each setup. In all analyses the results of the indoor experiments were confirmed by outdoor experiments.

8.3.2.1 First trap entry

The mean time elapsed between the onset of activity and the first occasion slugs entered the trap was shorter for large than small slugs in indoor experiments and the converse in outdoor experiments (*Table 8.6*). There was, however, considerable variation in entry times and these differences were not significant in either case. (ANOVA: Indoors: $F_{1,41} = 0.450$, *n.s.*; outdoors: $F_{1,30} = 0.058$, *n.s.*)

Table 8.6: Mean time (± S.E.) between onset of activity and first trap entry for small (< 100 mg) and large (> 500 mg) Deroceras reticulatum *in indoor and outdoor experiments.*

Experimental Setting	*Slug size*	*N*	*Mean time (mins) to first trap entry (± S.E.)*
Indoor	Small	19	107.84 ± 38.37
	Large	24	75.24 ± 20.48
Outdoor	Small	16	50.36 ± 26.42
	Large	16	129.31 ± 91.85

The number of small and large slugs entering the trap on at least one occasion did not differ significantly; for both size classes the majority of slugs entered the trap at least once during the night (Fishers Exact test: indoors: N = 47, P = 0.234, *n.s.*; outdoors: N = 41, P = 1.000, *n.s.*) (*Fig. 8.7*).

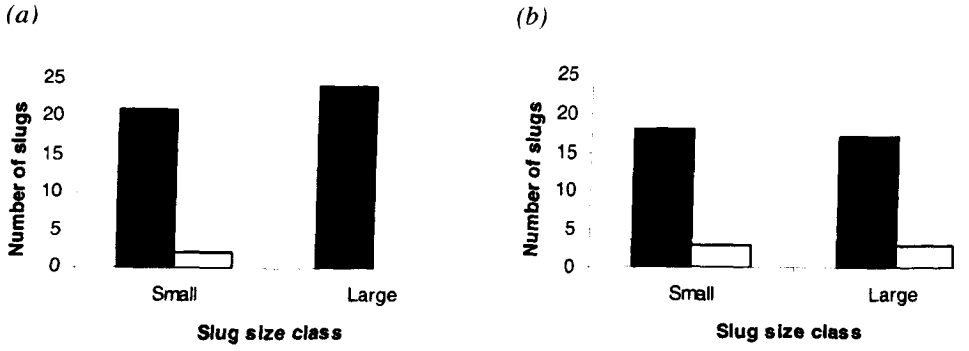


Figure 8.7: Number of small (< 100 mg) and large (> 500 mg) Deroceras reticulatum *entering the refuge trap at least once during the night (a) indoors and (b) outdoors (black bars = slugs entering trap; white bars = slugs not entering trap).*

There was no significant difference in the mean time slugs of different sizes spent under the trap on their first entry (ANOVA: indoors: $F_{1, 39} = 1.021$, *n.s.*; outdoors: $F_{1, 31} = 1.423$, *n.s.*) (*Table 8.7*). This remained the case when data were subdivided into slugs that entered the trap only once and those that went on later to re-enter the trap. Slugs remained under the trap for 3-5 hours on average on the first entry.

Table 8.7: Mean time (± S.E.) spent under refuge trap on the first entry for small (< 100 mg) and large (> 500 mg) Deroceras reticulatum *in indoor and outdoor experiments.*

Experimental Setting	*Slug size*	*N*	*Mean time (mins) under trap on first entry (± S.E.)*
Indoors	Small	19	238.71 ± 40.09
	Large	22	306.28 ± 51.58
Outdoors	Small	16	199.86 ± 38.29
	Large	17	262.23 ± 35.72

8.3.2.2 Multiple trap entries

Counts of slugs entering the trap more than once were resolved into two categories; those re-entering once and those re-entering two or more times; any further categories resulted in numbers too small for analysis. There were no significant differences according to size with similar numbers in each category (Chi-squared: indoors: N = 34, df = 1, $\chi^2_c = 1.000$, *n.s.*; outdoors: N = 24, df = 1, $\chi^2_c = 1.000$, *n.s.*) (*Fig. 8.8*).

There were no significant differences between small and large slugs in the mean time elapsed between initially leaving the trap and the first re-entry (ANOVA: indoors: $F_{1, 32} = 0.271$, *n.s.*; outdoors: $F_{1, 22} = 3.452$, *n.s.*). Similarly, the elapsed time between leaving the trap after the first re-entry and re-entering a second time did not differ according to slug size (ANOVA: indoors: $F_{1, 12} = 0.417$, *n.s.*; outdoors: $F_{1, 10} = 0.099$, *n.s.*). The mean elapsed time between re-entries are summarised in *Table 8.8*. The large standard errors reflect the considerable variation between individuals.

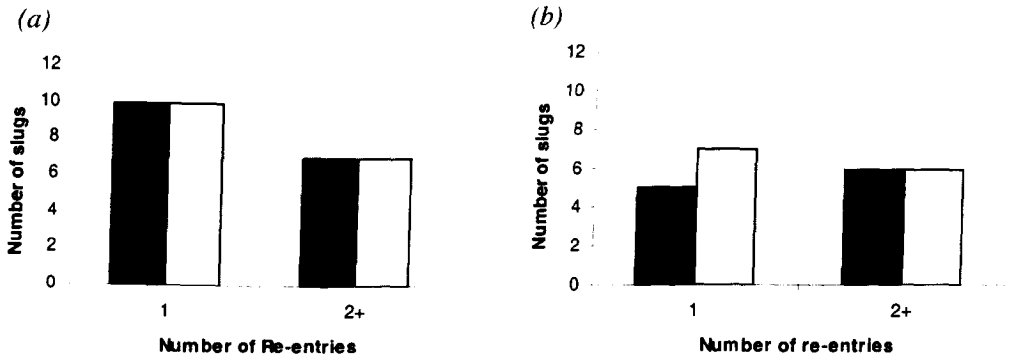


Figure 8.8: Number of small (< 100 mg) and large (> 500mg) Deroceras reticulatum *re-entering refuge once or two or more times during the night (a) indoors and (b) outdoors (black bars = small slugs; white bars = large slugs).*

Table 8.8: Mean elapsed time (± S.E.) between first and second trap re-entries for small (< 100 mg) and large (> 500 mg) Deroceras reticulatum *in indoor and outdoor experiments.*

Experimental setting	*Re-entry*	*Slug size*	*N*	*Mean time (mins) elapsed between refuge trap re-entries (± S.E.)*
Indoor	1	Small	17	85.14 ± 24.10
		Large	17	130.19 ± 65.30
	2	Small	7	50.78 ± 30.04
		Large	7	53.75 ± 24.88
Outdoor	1	Small	11	20.32 ± 6.28
		Large	13	65.53 ± 20.49
	2	Small	6	92.66 ± 58.42
		Large	6	30.59 ± 8.79

8.3.2.3 Activity around dawn

More slugs of both sizes entered the trap during the night than were present at dawn, with a reduction in numbers of approximately two thirds (*Fig. 8.9*). This was significant at $P < 0.05$ in both indoor and outdoor experiments (*Table 8.9*). In indoor experiments slightly more large slugs entered the trap than small slugs whereas this was reversed in outdoor experiments. In both conditions, however, the reduction in numbers observed at dawn did not differ significantly according to size.

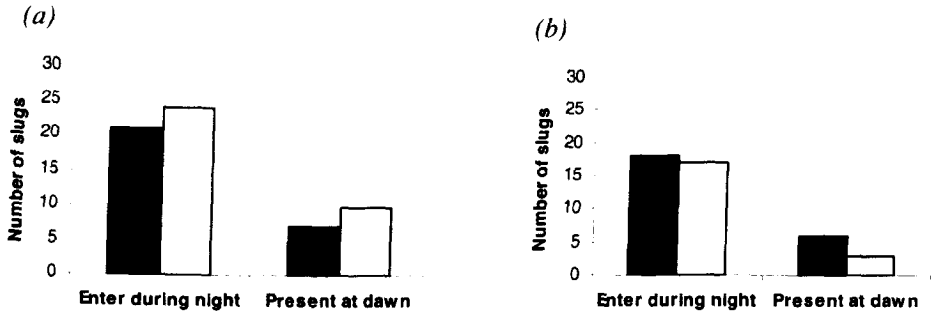


Figure 8.9: Number of small (< 100mg) and large (> 500 mg) Deroceras reticulatum *entering refuge traps during the night and number of each size present at dawn (a) indoors and (b) outdoors (black bars = small slugs; white bars = large slugs).*

Table 8.9: Results of Scheirer-Ray-Hare test to compare numbers of small (< 100 mg) and large (> 500 mg) Deroceras reticulatum *that enter refuge traps with the number present at dawn.*

Experimental Setting	*Factor/Interaction*	SS/MS_{total}	*df*	*P-value*
Indoors	Slug size	0.06	1	*n.s.*
	Number entering trap and present at dawn	4.54	1	*< 0.05*
	Interaction	0.19	1	*n.s.*
Outdoors	Slug size	0.02	1	*n.s.*
	Number entering trap and present at dawn	5.04	1	*< 0.05*
	Interaction	0.00	1	*n.s.*

Between dawn and midday it was observed that most slugs remained under the refuge traps in both indoor and outdoor experiments; one small slug and no large slugs left during this period in indoor experiments and a single individual from each size class left in outdoor experiments. Slug size did not, therefore, significantly affect such activity (Fishers Exact test: indoors: N = 17, P = 0.412, *n.s.*; outdoors, N = 9, P = 1.000, *n.s.*). The three single individuals that left the traps did so 1-2 hours after sunrise.

8.3.3 Field scale comparison of infra-red transparent and standard refuge traps

There were no block effects between assessment nights and, therefore, data were pooled. *Fig. 8.10* shows the counts of different species caught in standard and infra-red transparent refuge traps. Baited traps caught markedly more slugs than unbaited traps. *Arion silvaticus* (Lohmander) was caught exclusively in standard traps, however, patterns are otherwise similar between trap types and analyses are restricted to the two most abundant species, *D. reticulatum* and *A. distinctus.*

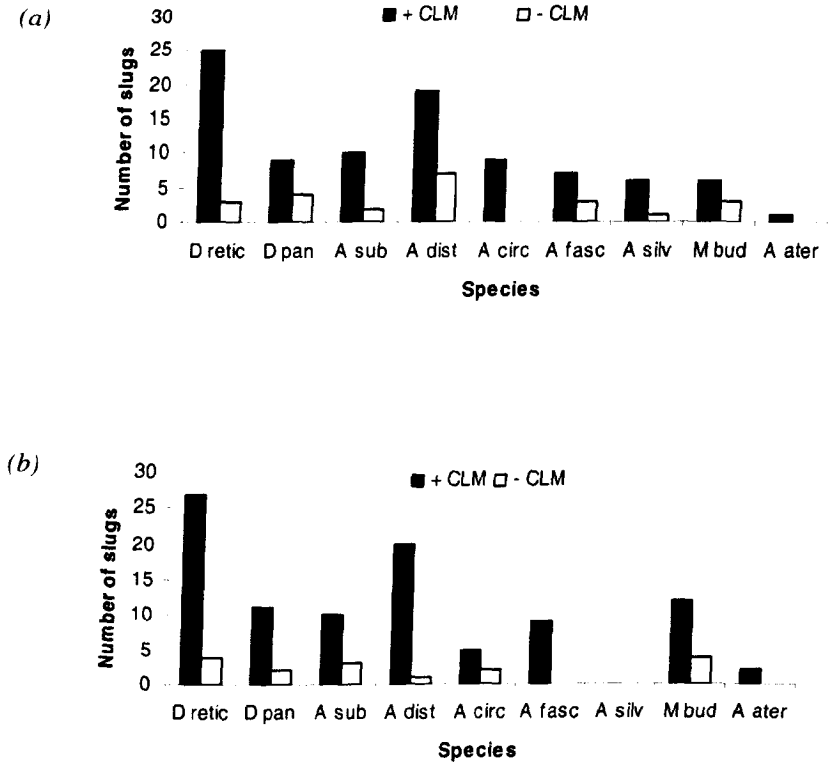


Figure 8.10: Total counts of slug species caught by (a) standard refuge traps and (b) infra-red transparent refuge traps in a grass plot at Close House Field Station over 12 nights (CLM = chicken layers mash bait; D retic = Deroceras reticulatum; *D pan* = Deroceras panormitanum; *A sub* = Arion subfuscus; *A dist* = Arion distinctus; *A circ* = Arion circumscriptus; *A fasc* = Arion fasciatus; *A silv* = Arion silvaticus; *M bud* = Milax budapestensis; *A ater* = Arion ater*).*

For both *D. reticulatum* and *A. distinctus* the infra-red transparent traps caught comparable numbers of slugs to the standard refuge traps according to whether or not they were baited (Chi-squared: *D. reticulatum*: N = 59, df = 1, χ_c^2 = 0.000, *n.s.*; *A. distinctus*: N = 47, df = 1, χ_c^2 = 2.623, *n.s.*). The trap type and presence of bait did not affect the mean weight of *D. reticulatum* caught (*Tables 8.10* and *8.11*).

Table 8.10: Results of two-way analysis of variance (ANOVA) to compare mean weight of Deroceras reticulatum *under infra-red transparent and standard refuge traps, baited and unbaited.*

Factor	*df*	*SS*	*MS*	*F*	*P-value*
Trap type	1	68830.71	68830.71	1.90	*n.s*
Bait	1	72891.73	72891.73	2.01	*n.s*
Interaction	1	16077.54	16077.54	0.44	*n.s*
Error	55	1990932.84	36198.78		

Table 8.11: Mean weights (mg) (± S.E.) of Deroceras reticulatum *under infra-red transparent and standard refuge traps, baited and unbaited.*

Trap type	*Bait*	*N*	*Mean weight (mg) (± S.E.)*
Standard	+	25	353.46 ± 44.66
	-	3	411.64 ± 108.95
Infra-red transparent	+	27	408.54 ± 30.30
	-	4	502.00 ± 89.75

8.4 Discussion

8.4.1 Comparison of refuge traps and DATs

8.4.1.1 Species abundance

In both trap types and both fields the two most abundant species caught were *D. reticulatum* and *A. subfuscus*. The presence of large numbers of *D. reticulatum* is not surprising since it is one of the most serious and widespread slug pests of cereal crops in the UK (Schley & Bees, 2003). *A. subfuscus*, in contrast, is not generally

regarded as a dominant pest of cereals. This may, however, simply reflect regional differences between the study locations and, indeed, earlier work at Heddon Banks Farm also found *A. subfuscus* in noticeable numbers (Young, 1990). This species may, therefore, be an important member of the slug fauna in this particular area.

Although more species in total were caught in the middle field than the bottom field, the numbers of individuals constituting each of these additional species groups was small. It is unlikely that crop related differences in slug distribution would account for this disparity as the crop prior to and during trapping was the same in both fields. Physical differences between the two sites would seem a more probable explanation; the soil in the bottom field is much heavier than the middle field. It is also wetter and prone to pools of standing water as it lies on the flood plain of the River Tyne which may restrict the species it can support.

8.4.1.2 Numbers and sizes of D. reticulatum and A. subfuscus

There was considerable variation in the numbers and mean weights of slugs caught throughout the study period which may explain why the differences between trap types were not significant on some occasions. When there were differences, however, the trends were similar for *D. reticulatum* and *A. subfuscus*.

Relative to DATs, refuge traps tended to catch fewer slugs in early spring and summer, but more in autumn and winter, confirming the results of Byers *et al.* (1989) and Barratt *et al.* (1993). The lower catches in spring may be due to the cool temperatures in north east England at this time of year which prevent slugs becoming more active until later in the season. It is not clear why they performed poorly in summer, but this was exceptionally warm and dry in 2003 which may have driven slugs below the ground to avoid desiccation. These results may, therefore, be atypical. In autumn the often wet and mild conditions encourage much surface activity, increasing the likelihood that slugs will encounter a refuge trap. Neither trap performs particularly well in winter (Hunter, 1968a), but DATs are particularly affected by freezing conditions (Barratt *et al.*, 1993) which may explain the apparent improved catch of refuge traps in this season.

Regardless of the total numbers caught, refuge traps had a lower proportion of small slugs in all seasons relative to DATs, except in summer. These patterns may reflect differences in activity related to the breeding cycle (Glen & Wiltshire, 1986). In spring there is a preponderance of newly hatched slugs which, it is suggested, spend a large proportion of time below ground (Archard *et al.*, 2004) and would not be detected by refuge traps. The summer population is composed principally of juveniles; the sizes of slugs are, therefore, more evenly spread masking differences due to extremes. By autumn the population is maturing and there are many surface active adults, which would encounter the refuge traps while searching for mates and laying eggs. In winter the population is comprised mainly of eggs and juveniles over-wintering below ground.

8.4.2 Slug activity beneath refuge traps

The techniques used in these experiments allowed the behaviour of slugs beneath refuge traps to be studied for the first time. The species used was *D. reticulatum*, a particularly surface active species (South, 1965). It was hypothesised that behavioural differences between small and large slugs on encountering the traps might partly explain why they tend to be biased towards large slugs. These studies, however, found no evidence of size-related differences in the response of small and large *D. reticulatum* for any of the behaviours assessed. This was the case for both indoor and outdoor experiments.

The timings of the initial trap entry varied considerably between individuals in agreement with Grimm (2002), but the mean value corresponded with that reported by Hommay (1998) in his study of activity and sheltering behaviour in this species. The bait used was highly palatable to slugs (Young, 1990). Although olfaction is not thought to play a role in locating pellets or single seeds over distances greater than 3-4 cm (Bailey *et al.*, 1989; Howling, 1991), it may have been of greater significance with the larger quantities of food used in the current study (2.5 g chicken layers mash). Furthermore, its presence in the traps may have influenced the time spent underneath once slugs had entered, particularly as slugs had been starved for 24

hours prior to recordings which has been shown to increase the first meal length (Bailey, 1989).

Most slugs were observed to enter the trap at least once with many re-entering multiple times. This result may be exaggerated due to restricted movement along the margins of the arena. As described in *Chapter 6, section 6.4.1.1*, this is an unavoidable limitation of video assessments of activity. Although studies have indicated that the arena size does not affect the distance moved (Bailey, 1989; Howling, 1990) it might affect the direction of movement; this could be important when there is an object in the centre of the arena, the response to which is the variable of interest.

The number of slugs in the traps at dawn was approximately one third of the total active population. Experiments would need to be repeated under a wider range of conditions and with different species to gauge how reliable this figure is, but such data could help to refine damage risk estimates.

Caution must be exercised in extrapolating laboratory results of behaviour studies to field situations since the former are controlled so as to be repeatable whereas in their natural environment animals are subjected to a great many more variables which may modify their behaviour (Bailey, 1989). For this reason the experiments presented here were performed indoors and outdoors. The necessity of constraining slugs in a fixed arena meant, however, that only semi-field conditions could be achieved; slugs were exposed to naturally fluctuating temperatures and light intensity, but the influence of the arena edges on directional movement and their buffering effects against air currents could not be eliminated. The concordance of indoor and outdoor results indicates that the activity of slugs in the laboratory setup realistically represented behaviour in the field under the conditions tested, but further work is needed to confirm this, for example repeating the experiments in larger arenas and at different times of the year.

The time of year when experiments were conducted (early April 2004) may have particularly influenced the results concerning numbers of slugs leaving the traps

between dawn and midday. It was expected that outdoors, as the air temperature rose following sunrise, conditions under the trap would become increasingly inhospitable for slugs causing them to leave (Judge, 1972; Schrim & Byers, 1980). Although the weather was mild (mean minimum temperature 11.6°C; range 8-21°C) with some sunny days, Tinytalk™ data loggers showed that there was only a 1°C difference between the mean soil and air temperature. Under the conditions of this study, therefore, any heating effect was minimal, but it seems unlikely that this would be the case in the summertime.

The weight difference between small (< 100 mg) and large (> 500 mg) slugs in these experiments was quite wide. This was necessary in order to distinguish between them on the monochrome recordings; natural contraction and elongation of slugs during locomotion alters their appearance on screen and the weights chosen for the two size categories allowed them to be discriminated despite this.

As discussed in *Chapter 6, section 6.4*, food deprivation has been shown to increase the locomotor activity of *D. reticulatum* (Airey, 1987). A short period of starvation prior to studies of foraging and feeding is common practice in order to improve the chances that slugs will be responsive during experiments and was also the case in the current work. Since slugs are known to be capable of tolerating such conditions for several weeks (Lovett & Black, 1920 in Arias & Crowell, 1963), a short starvation period of 24 hours is unlikely to have unduly altered basic physiology over and above simply increasing motivation to feed and there is no evidence to suggest that small and large slugs are differentially affected over such short time intervals.

In summary, the under representation of small slugs by refuge traps relative to DATs does not appear to be due to differences in their behavioural response compared to large slugs upon encountering the trap; they spent similar amounts of time under the traps, entered them similar numbers of times and left before dawn in comparable numbers. The suggestion that small slugs may be overlooked by observers (Archard *et al.*, 2004) may be feasible if farmers are hurriedly checking their own traps, but is unlikely to be the case for research reports which are conducted by biologists with dedicated time for the task and considerable experience (D. Glen, *pers. comm.).* A

failure of small slugs to reach the traps under natural conditions would seem to be a more likely explanation.

8.4.3 Field scale comparison of infra-red transparent and standard refuge traps

This comparison was carried out primarily to ensure that the infra-red transparent traps performed as well as standard traps in the field and this was found to be the case. The infra-red traps are a reliable substitute for opaque plastic saucers and a useful research tool for slug behavioural studies.

The two most abundant species were *D. reticulatum* and *Arion distinctus* (Mabille), both of which are prevalent in the walled garden at Close House (A. Craig *pers. comm.*)

Baited traps caught significantly larger numbers of slugs than unbaited traps. As mentioned previously (*section 8.4.3*), olfaction is not believed to play a role in attraction to food over a distance (Bailey *et al.*, 1989; Howling, 1991). It may be that trap entry was a random process, but once underneath the baited saucers the slugs remained to feed and rest whereas they left unbaited traps again. The presence of these slugs under the baited traps would also have provided mating opportunities to attract and retain others. That the mean weight of *D. reticulatum* did not vary between baited and unbaited traps suggests that the added effect of bait is on total numbers and is not size-related.

8.5 Conclusions and Future Work

In conclusion, it was found that:

1. Refuge traps under represented small *D. reticulatum* and *A. subfuscus* relative to DATs in all seasons except summer.
2. Refuge traps caught fewer *D. reticulatum* and *A. subfuscus* relative to DATs in spring and summer, but caught more in autumn and winter.

3. Behavioural differences towards refuge traps did not differ significantly between small and large *D. reticulatum.*
4. Infra-red transparent refuge traps performed as well as standard opaque refuge traps in field conditions.
5. Many factors influence the efficacy of different methods of population estimation and this must be borne in mind when interpreting the results of comparative studies. It may help if statements regarding traps are qualified with the weather conditions, habitat and the time of year measurements were made.

It would be interesting to see what effect alternative food has on slug behaviour towards refuge traps. Although seedlings would have reduced the clarity of video recordings, it would be possible to use ungerminated seeds in the arena as these would not obscure the view of slugs. It would also be useful to extend the work to different species; it may be that those that are generally less surface active than *D. reticulatum* would behave differently. As described in *section 8.4.3*, it would be helpful to formally assess the effect of the arena edges on directional movement in order to interpret the results of the video experiments more fully.

Chapter 9
General Discussion

9.1 Thesis Context and Objectives

The studies presented in this thesis form part of a four-year Sustainable Arable LINK (SAL) project, involving three collaborating research institutions. The overall aim of the SAL project is to devise a rational risk assessment system for the integrated control of slugs in arable crops. The particular objectives assigned to the University of Newcastle upon Tyne are described in *Chapter 1*. In summary, these addressed specific aspects within three broad areas of slug biology and behaviour. Firstly, lifecycle parameters were studied to provide information about seasonal and temperature effects on hatching, growth and survival along with an investigation of the relationship between body weight and female-phase sexual maturity. Secondly, behavioural experiments were conducted to assess the response of slugs to molluscicide pellets in relation to pellet condition, the method of application and seedbed type. The third area addressed by the thesis concerned the efficacy of refuge traps in estimating surface activity, again through behavioural studies, in addition to field comparisons of different trap types. The thesis focused almost exclusively on *Deroceras reticulatum* (Müller) since it is the most destructive pest of arable crops in the United Kingdom (Schley & Bees, 2003).

9.2 Purposes of this Chapter

This chapter aims to integrate the findings of the thesis as a whole by summarising key results and evaluating the contribution they can make to improving current slug control strategies in arable crops. Detailed discussions of individual experimental results are found in the appropriate chapters. Methodological issues related to particular experimental procedures have also been considered in the relevant individual chapters; however, the general applicability of the laboratory based studies

to field settings will be discussed in *section 9.3.* The implications of the results for different aspects of control are considered in *section 9.4.* This section will, of course, focus specifically on the aspects of the SAL project assigned to the University of Newcastle upon Tyne; the findings will, however, ultimately be combined with the results of the other research partners in order to provide a collective response to the overall objective of the SAL project. *Section 9.5* will discuss the potential impact of the project results for the farming industry in addition to highlighting the new knowledge they bring to our understanding of slug biology. Finally, recommendations for future work will be outlined in *section 9.6.*

9.3 General Applicability of Results

Some of the experimental work comprising this thesis was conducted under field conditions, for example, the comparison of slug population estimates by different trap types (*Chapter 8*). The majority, however, was laboratory based and consequently performed in controlled conditions (e.g. *Chapters 2-4* and *6-7*). This was unavoidable due to the nature of the studies undertaken and the need for repeatability. An inherent limitation arising from this is that, due to the provision of plentiful, good quality food, protection from environmental extremes and a lack of predators the results represent what would be expected under optimal conditions and may overestimate the magnitude of response in natural populations of slugs. Wherever possible, the potential for overestimating effects was minimised by maintaining factors such as temperature and photoperiod at levels as realistic to the natural environment as possible.

Constant temperatures in all experiments were set within a narrow range of 12-15°C, representing a mild spring or cool autumn in the United Kingdom and in studies where temperature was one of the key factors of interest (*Chapters 2-4*), ambient conditions were included as a treatment so that the results at constant temperatures could be contrasted with a more natural situation. Care was taken to ensure that the light regime mirrored the prevailing sunrise and sunset times for the appropriate time

of year (*Chapters 6-8*), unless it had to be fixed over an extended period, in which case long day-length cycles were imposed (*Chapters 2-4*).

Studies of individual behaviour required that slugs be contained within arenas to facilitate continuous identification of a given animal (*Chapters 6-8*). The arenas were of a comparable size to those used by other workers (Howling & Port, 1989; Grimm & Schaumberger, 2002) and as large as possible within the bounds of practicality. It has been shown that arena size does not unduly affect distances travelled (Bailey, 1989; Howling, 1990) and therefore this is not thought to be a major limitation of these experiments. Although the experiments in *Chapters 6* and *7* were conducted at constant temperatures, the assessments of slug behaviour towards refuge traps in *Chapter 8* were performed both indoors and outdoors. The results from outdoor experiments (semi-field situation, with fluctuating temperatures, gradual changes in light intensity and exposure to a variety of weather conditions) confirmed the findings of those conducted indoors, suggesting that the indoor set ups are reasonably representative of the field environment.

Whilst care must, therefore, be taken in extrapolating laboratory results to field settings, it is believed that they nevertheless provide an acceptable basis upon which to evaluate lifecycle parameters and current approaches to slug control, as long as the necessary caveats are borne in mind.

9.4 Implications for Current Approaches to Slug Control

9.4.1 Estimating the need for control

In order to assess whether there is a significant risk of slug damage to a crop, and thus a need for control, it is necessary to estimate the level of surface activity in the population. The most practical means of achieving this is to use refuge traps. It is recommended through the Home Grown Cereals Authority (HGCA) that these are initially used in the period before cultivation for winter wheat and before or after harvest of the preceding crop in the case of winter grown oilseed rape. The traps

usually consist of materials such as hardboard squares or upturned flowerpot saucers (Clements & Murray, 1991; Young *et al.*, 1996). They are baited with chicken layers mash and deployed in a 'W' configuration on a mild night (5-25°C) when the soil surface is visibly moist. They are then examined early the following morning and the numbers of slugs caught per trap are counted. In the case of wheat, a threshold of four or more slugs per trap is considered to represent a possible risk of economically important damage levels (Glen & Wilson, 1995). No threshold has yet been established for oilseed rape, but a figure of one slug per trap has tentatively been suggested to the HGCA by the SAL project committee until further research has been carried out.

Other techniques of estimating slug numbers, such as soil sampling, offer more comprehensive assessments of population structure than refuge traps, including density, size distribution and egg numbers. They are, however, labour intensive and time consuming. They have a valuable place in long-term monitoring programmes and research, but the ease of storing and deploying refuge traps, their transportability and cheapness mean that these are the most practical method on a day-to-day basis for busy farmers and are likely to remain the preferred approach for some time to come.

There has been much effort devoted in the past to comparing population estimates of different sampling techniques with refuge trap catches in an attempt to calibrate one against the other in order that maximum population information can be gained through minimum time and expense. In particular, defined area traps (DATs) have been contrasted with refuge traps to try and relate population density to surface activity estimates (Byers *et al.*, 1989; Clements & Murray, 1991; Barratt *et al.*, 1993; Voss *et al.*, 1998). It is generally reported that the number of small slugs caught in refuge traps is proportionately much lower than the number of large slugs, relative to DATs (e.g. Glen & Wiltshire, 1986; Clements & Murray, 1991) and this was confirmed for the field collected data described in *Chapter 8.*

Reasons for this discrepancy between numbers of small and large slugs caught in refuge traps were unknown, but one possibility is that size related behavioural

differences may be responsible. Video experiments of slug behaviour towards refuge traps did not support this hypothesis; small (< 100mg) and large (> 500 mg) *D. reticulatum* showed similar patterns of activity upon encountering traps (*Chapter 8*). The experiments were carried out on bare soil so that the results would be particularly pertinent to the early assessments of slug activity immediately prior to sowing. Given that behavioural differences were found not to account for the size discrepancy between refuge trap and DAT catches, it must be due to other factors that prevent small slugs from reaching or remaining under refuge traps in field situations. It could be speculated, for example, that carabid beetles, which preferentially prey on small rather than large slugs (Mair & Port, 2001), are more readily attracted to refuge traps than DATs, or that small slugs are more likely to rest during foraging than large slugs, as their metabolic need for food is not as great, and they are thus less likely to encounter the refuge trap. Another possibility is that the refuge presented by traps per se may be less important for small than large slugs as they have a greater number of suitably sized refuges within the soil.

It is common practice for slug population monitoring to continue in crops throughout the susceptible growth stages. In wheat this equates to the period between sowing and first tillering, whereas in oilseed rape it is from germination until the four-true leaf stage. Thus, some cover begins to develop and a highly palatable alternative food source to the chicken layers mash used to bait traps is available. It could be that at this point size related differences in behaviour do become apparent as the food preferences of small and large slugs may vary (*pers. obs.*), but it would be difficult to determine this using video techniques as the young plants would obscure the image of slugs. The results in *Chapter 8* are therefore most relevant to initial assessments.

As a general point, although the reasoning behind attempts to calibrate the outcomes of different methods of slug population estimation is acknowledged, with respect to short-term prediction of damage it is felt that these should not be over-emphasised. Once germination has occurred, the surface active portion of the population is most relevant with respect to damage at establishment (Hommay *et al.*, 1998). It is perhaps more useful, therefore, to know how closely the counts of slugs under refuge traps early in the morning reflect the total numbers that entered during the night. The

studies in *Chapter 8* showed that these numbers differed by a factor of approximately two-thirds, with fewer slugs present in the morning. This finding will help to qualify trap thresholds for damage risk and the experimental procedure itself may be of application in 'fine-tuning' input data for a simple model that relates refuge trap catches and soil moisture in order to indicate whether applications of molluscicide pellets will be beneficial (Young *et al.*, 1993).

9.4.2 Timing of molluscicide pellet application

Once it has been established that there is a risk of slug damage and intervention is required, the timing of molluscicide pellet applications is crucially important to the success of a control programme. They must be applied at an appropriate stage in the growth cycle of the crop; slugs pose the greatest risk to wheat when they feed on seeds whereas in oilseed rape, the seeds are not attacked, but seedlings are highly vulnerable to damage by surface active slugs as the growing point is above ground. Thus, if pellets are applied too late then irreversible yield loss may already have occurred in wheat, and if they are applied too early, slug populations may recover and still be capable of causing significant loss of plant stand in oilseed rape. In addition, applications must coincide with weather conditions that are conducive to slug activity and at a time in the lifecycle when they will have maximum effect; eggs, for example, are not influenced by the active ingredients of molluscicides in a pelleted form (D. Glen, *pers. comm.*).

Despite knowing when molluscicides are likely to be ineffective, it is nevertheless difficult to suggest with precision 'calendar' dates when applications will be most beneficial; this is because slug population activity is so intimately dependent upon weather conditions that peaks in numbers may vary by several weeks between years. Moreover, different species have different life histories which can complicate the decision making process further still. This results in understandable confusion on the part of farmers who are less concerned with slug biology than with minimising damage and may partly explain why those who have experienced severe damage to crops in the past have administered extra molluscicides as an 'insurance' policy (Oakley & Young, 2000). Although it has been shown that there is a 'window of

opportunity' immediately post-harvest when applications can have a significant impact on reducing slug populations the following spring (Port & Port, 1986; Shirley *et al.*, 2001), it is still common practice to use pellets at intervals throughout the growing cycle if the perceived risk of damage is high. There is, therefore, a need for improved guidance with respect to the timing of pellet applications.

The study described in *Chapter 5* may be of considerable value in targeting molluscicide applications to coincide with the critical period before maximum egg laying commences. In this study a probability equation was derived that predicts the likely number of female-phase mature slugs in a population if the weights of a representative sample of individuals are known. If regular monthly monitoring is undertaken, site-specific patterns of the numbers of egg-laying slugs may be analysed by crop consultants to forecast the optimal time for applications. For example, the data reported in *Chapter 5* showed a peak of female-phase mature slugs in May, which were possibly slow growing individuals from the previous generation that managed to over-winter as immature juveniles (*Chapter 3*). An application of molluscicide pellets in March/April could well have markedly decreased the number of slugs in this group before they laid eggs, consequently reducing the size of the next generation. By September, the numbers of female-phase mature slugs was at a minimum, reflecting the rapid mortality following egg laying in autumn (Rollo, 1988). Based on the principle that the best control is achieved when a population is hit at its weakest point (Runham & Hunter, 1970), a further application at around this time would serve to cut the numbers of slugs that would otherwise over-winter and contribute to the peak in spring of the following year.

The suggestion in *Chapter 3*, that there may be 'fast growing' and 'slow growing' individuals in slug populations supports this two-tiered approach to the timing of molluscicide applications. In such a situation, of a given cohort of slugs, some will rapidly contribute to the next generation and then die (the fast growers) whereas others will simply grow slowly and steadily in the background acting as a buffer group that lay eggs much later (the slow growers). Applications of molluscicides clearly need to be timed to target fast growers before they have chance to lay eggs, but a 'follow up offensive' to target any remaining slugs in the slower growing

buffer group would have the advantage of getting 'one-step ahead of the game' for the following year. Obviously, this type of approach would be of most use in ongoing monitoring projects or to crop consultants who can gather such information over a period of time, rather being of direct use to farmers themselves. Furthermore, there would be a need to convince farmers that the rewards of such an approach are delayed; it may take a season before a reduction in numbers will become apparent.

9.4.3 Method of molluscicide pellet application

In addition to appropriate timing of pellet applications, pellet placement must be such that slugs can easily locate them; no matter how effective a product may be with respect to acceptability, palatability and toxicity, if slugs do not readily encounter it then it will not be a successful control measure. The two ways of applying pellets on a field scale are broadcasting and drilling (Glen & Wilson, 1995). The former aims to incapacitate surface active slugs whereas the latter is intended to protect seeds 'at source' by poisoning slugs travelling through air gaps between soil aggregates. Both methods are routinely used by farmers during crop establishment, but there have been few formal assessments of their relative efficiency. The studies in *Chapter 6* redress this gap by characterising the behaviour of individuals towards pellets applied in these two different ways.

Broadcasting pellets was found to be significantly more effective than drilling in arresting the activity of *D. reticulatum*; slugs found pellets sooner, over shorter distances and were more likely to feed on the first one encountered when they were broadcast. Experiments were conducted in arenas without another food source present. As reasoned for the studies constituting *Chapter 8*, this tailors the results to be particularly relevant to the crucial period between sowing and establishment.

In practice, the drilling of pellets is probably less stringent than in the experimental set up described; some may end up along side the drill line on the soil surface if they rebound off soil aggregates and, furthermore, if weather conditions do not favour the closure of the drill slit following sowing, even those pellets that are appropriately placed may remain visible for an extended period of time (Port & Port, 1986). In

such situations, surface active slugs are easily able to access 'drilled' pellets which may increase the efficacy of this method of application. It is argued, however, that since such misplaced drilled pellets are effectively broadcast, money and time could have been saved by doing this in the first place. Indeed, as a result of the work in *Chapter 6*, the HGCA is planning to recommend broadcasting as the application method of choice for oilseed rape; drilling is endorsed for wheat with caution, but only when direct drilling into open, coarse seedbeds.

It was mentioned in *Chapter 6* that slugs are said by some to use furrows in the soil as 'motorways' to locate drilled pellets and seeds (e.g. Allen 1981 in Hogan, 1985; Martin & Kelly, 1986; Bourne *et al.*, 1988). Strictly speaking the experiments assessing behaviour of slugs towards different methods of molluscicide application cannot support or refute this claim since pellets were drilled in hollows loosely covered in soil rather than in furrows. It was shown, however, in *Chapter 7* that undulations in the soil surface on coarse seedbeds with molluscicide pellets present did not alter the feeding behaviour or movement patterns of *D. reticulatum* compared to flat, fine seedbeds, which casts some doubt on these assertions.

Re-application of molluscicides has been precipitated by the assumption that soil splash on pellets following heavy rainfall renders them ineffective (Hass *et al.*, 1999; Simms *et al.*, 2002). Additionally, excessive applications often occur in situations where cultural practices are generally associated with a heightened risk of damage, even if there is no evidence that this has been realised on a specific site. This is a particular trend in minimum tillage systems where pellets are increasingly used prophylactically rather than curatively (Hammond *et al.*, 1996). Such additional applications are expensive and where they confer little benefit in suppressing pest populations they are both economically wasteful and represent an avoidable environmental hazard.

In order to provide a rational basis for such practices, experiments were performed to compare the response of slugs to soil splashed and 'clean' broadcast pellets (*Chapter 6*) along with assessments of surface activity patterns on coarse and fine seedbeds in the presence of molluscicides (*Chapter 7*). It was shown that soil splash does

nothing to diminish pellet efficacy; *D. reticulatum* located and fed on soil splashed pellets as readily as clean pellets. This implies that any competitive effects that might exist between the crop and pellets as a food source will apply regardless of whether they are soil splashed or 'clean', and this lack of discrimination is likely to be independent of crop cover. It is, therefore, highly doubtful that re-application of molluscicides following soil splash has any positive value. Similarly, on loamy soil, slugs encountered pellets on coarse and fine seedbeds in equal measure (*Chapter 7*) and therefore additional applications to fields where minimum tillage results in coarse seedbeds may be wasteful. It is strongly emphasised, however, that this is specific to loamy soils; the established risk of slug problems on heavier coarse seedbeds is well supported and remains important, requiring well-managed approaches to control.

9.4.4 Predicting damage risk in the long-term

Appropriate and prompt measures to control slug populations and contain damage in the immediate forthcoming crop are vital; however, this can result in persistent 'fire-fighting' approaches rather than projected, sustainable strategies to keep slug numbers in check. A reliable prediction system will allow farmers to target slug control measures in a more rational way such that populations can be maintained below critical levels in the long-term and unnecessary prophylactic applications can be greatly reduced. It will also be of considerable benefit to industry by enabling molluscicide manufacturers and distributors to ensure supply meets demand. Consequently, such long-term predictions of damage risk are a key area driving current research efforts in slug biology. Devising a long-term prediction system is, however, no easy task; it requires a thorough understanding of the lifecycle of slugs and accurate data regarding how their physiology and behaviour are influenced by a whole host of complex factors, e.g. temperature, humidity, moisture, competition and cultivations to name but a few. Furthermore, this knowledge needs to be integrated to give an output that offers clear and concise guidance concerning the necessary actions.

As described in *Chapter 2*, the development of population dynamics models has, to date, been hampered by a lack of information on lifecycle parameters. In particular, the estimation of egg banks, juvenile growth rates and patterns of mortality has had to be assumed (e.g. Shirley *et al.*, 2001; Schley & Bees, 2003; Choi *et al.*, 2004). Because these models are not yet robust enough to justify their implementation in the commercial sector, they remain research tools at present. The ultimate aim, however, is to refine them and 'package' them with a user friendly interface whereby a farmer or crop consultant can input relevant local data, for example meteorological information, previous crop rotations, slug counts and the future crop to be sown. The models will then simulate population dynamic processes specifically relating to this set of data in order to provide an output in the form a site-specific predicted risk of damage along with recommendations of when to apply control, if necessary. They could also be used as an 'early warning system' of slug population booms for advisory bodies such as ADAS. All this is a long way off yet, but a number of the studies reported in this thesis have been designed to provide some of the detailed data required to begin verifying the assumptions that had to be made when initially developing existing models, in particular the model of Shirley *et al.* (2001).

The studies described in *Chapter 3* have resulted in the largest and most detailed dataset known regarding the individual growth and survival of *D. reticulatum*. This indicates that not only temperature, but also hatching season has profound implications on the interplay between slug development and lifespan; the effect of these factors at certain constant temperatures is reversed in spring compared to autumn hatching individuals which suggests that prolonged harsh or mild conditions may have different outcomes depending upon the generation. Models must, therefore, take account of such effects.

Interestingly, and contrary to expectation, it was found that the numbers of slugs posing the greatest threat to the size of the next generation, i.e. female-phase egg layers, was relatively small; a binary logistic regression approach indicated that at any one time a maximum of about 20% slugs were female-phase mature (*Chapter 5*). The mean batch size is known from the literature to be approximately 33 eggs (Carrick, 1938). If the mean number of batches per individual could be ascertained

then, in conjunction with reliable estimates of current population size from soil samples, this would provide a simple way to estimate the egg bank, a parameter that has particularly limited modelling approaches in the past. Furthermore, the data in *Chapter 2* indicate that approximately 65-75% of the eggs laid ultimately hatch, regardless of temperature, at least at ranges usually encountered in the United Kingdom. This information, along with age-specific estimates of the chances of survival (*Chapter 3*) could be of value in predicting population mortality over specified time intervals, another assumption that has, by necessity, been made in a number of models to date (e.g. Shirley *et al.*, 2001; Choi *et al.*, 2004).

D. reticulatum reared in isolation were capable of laying viable eggs, albeit in small numbers (*Chapter 4*). It is suggested that self-fertilisation may be a 'backup' mechanism of maintaining the population if numbers become very low, in which case there would still be a baseline cohort of slugs. Furthermore, these individuals showed a capacity for extremely fast growth under a favourable temperature and plentiful food. This implies that if conditions in the natural environment were to improve in the year following a decimation of the previous population, numbers could soon build to previous levels again. More work is required to confirm and extend the work in *Chapter 4*, but models may potentially need to incorporate some kind of algorithm to account for this. Interestingly, these self-fertilised individuals failed to produce viable eggs themselves. This suggests that, if self-fertilisation is a backup mechanism then although numbers may be low, it could be an ideal time to apply control since any eggs laid without mating by this generation probably will not hatch.

9.4.5 Reassessing slug populations

It is very important to continually reassess slug populations; this would be the case even with reliable prediction models. The reason is that it gives an indication of whether previous control strategies have been successful and sufficient; the persistence of pest slug species in spite of concerted efforts to keep numbers at a minimum vouches for their extreme adaptability and resilience. The preceding discussion (*section 9.4.4)* highlighted the potential for different growth rates and

reproductive strategies to maintain a 'buffer' population (*Chapter 3*). Glen *et al.* (1991) suggested that at best molluscicides kill approximately 50% of a population, since many slugs are not surface active and warns that if populations are especially dense then numbers may not be reduced below thresholds of economic injury. In addition, Frain and Newell (1983) stress that the efficacy of slug pellets should be judged by assessing the residual population as individuals may recover from poisoning under certain conditions, e.g. high humidity and warm temperatures, and that juveniles are less susceptible to molluscicides that adults. The corollary of this in view of the size variation shown in *Chapter 3* is that there will always be some less susceptible individuals in the population. Sampling to assess the relative numbers of different sized slugs may help to indicate whether the residual population poses and immediate risk for crop damage and whether pellet applications would be likely to have an effect at that stage.

The recommendations arising from this thesis are summarised in *Table 9.1*.

9.5 Impact and Benefits of Research Findings

The impact and benefits of the research presented in this thesis are two-fold. Firstly, as should be apparent from *section 9.4*, the studies undertaken have supplied data that will help to alleviate some of the current confusion concerning optimal approaches to slug control, both directly and indirectly. Secondly, experiments have contributed to an increased understanding of the biology of *D. reticulatum*, in particular its growth, maturity and survival. This has raised a number of interesting academic questions for future research (*section 9.6*) and has practical applications not only in agricultural settings, but also other systems where slugs are pests, e.g. field vegetables and nurseries producing ornamental plants.

The thesis results will be overlaid with those of the other research collaborators involved with the SAL project, bringing together all the relevant data required to progress the development of a rational risk assessment system for the control of slugs in arable crops, as explained in *section 9.1*. This will include meteorological and

agronomic factors, detailed information on crop vulnerability and analyses concerning the influence of the relationship between sowing dates and molluscicide applications on control success.

Table 9.1: Recommendations based on studies undertaken in the thesis.

	Recommendations arising from the studies undertaken in this thesis
1	Estimates of slug populations using refuge traps may be improved by applying a multiplication factor of two-thirds to account for slugs leaving the traps before catches are counted at dawn
2	A two-tiered approach to the timing of control measures, based on site-specific estimates of the percentage of mature female-phase slugs in the population, may markedly reduce populations by targeting individuals before the spring and autumn peak in egg laying
3	Broadcasting pellets is more effective than drilling in the period between sowing and establishment
4	Reapplication of new pellets to supplement soil splashed pellets following heavy rainfall is unnecessary
5	Increased applications of pellets to coarse seedbeds on loamy soils is unlikely to offer any additional benefit in controlling slugs compared to fine seedbeds
6	Lifecycle data has great potential to help refine existing population dynamics models, offering the prospect of reliable estimates of risk
7	Reassessment of slug numbers following primary molluscicide applications are important in assessing control success, defining the residual population and forecasting the future need for further action
8	Guidelines must be published to distil research findings of the thesis and SAL project as a whole in such a way that recommendations may be easily implemented

Any applied research is limited in its usefulness if it is not available to the end-users in a suitable format and, ultimately, the extent to which research findings from the SAL project will benefit the farming community depends upon effective dissemination of information. To date, the project has been introduced at agricultural events, such as 'Cereals 2004' and The Great Yorkshire Show, where the response from arable farmers has been encouraging; they felt the objectives were relevant to

their personal experience, which highlights the importance of involving farming representatives with the project from the start. Now that the SAL project is nearing its completion plans to launch the results in the farming press are underway. This is timetabled for September 2005. Along with publishing guidelines which will be distributed through the relevant advisory bodies, a web-based warning system has also been proposed as an industry wide approach to translating results into practice and may help to demystify some of the conflicts between perceptions versus the reality of risk.

Cost is often the bottom line in determining whether new initiatives are adopted and farming in the 21st century is as much a business as a way of life. It is difficult to make forecasts of the total financial savings that could be achieved through implementation of guidelines from the SAL project because figures concerning the proportion of current use that is unnecessary are inherently vague, often coming down to 'back of the envelope' calculations based on word of mouth reports. Individual farmers would be in a good position to work out what the guidelines would mean in monetary terms to them personally, however, and a survey may be of help in translating this to estimates for the arable farming sector in general.

9.6 Future Work

Specific ways in which the findings of individual studies comprising this thesis could be developed further have been mentioned in the relevant chapters. These will be briefly summarised.

The growth and survival studies in *Chapter 3* could be developed by increasing the range of temperatures investigated, including further constant temperatures and fluctuating regimes. The hypotheses put forward concerning self-fertilisation in a predominantly cross-fertilising species (*Chapter 4*) need to be explored further through breeding programmes and genetic approaches.

As highlighted in *Chapter 2*, an estimate of the baseline chances of a single egg hatching would help to develop further the results concerning the relationship between hatching and batch size. Additionally, there is little reported in the literature that attempts to define the 'developmental zero' temperature for *D. reticulatum* and this, along with day-degree assessments of hatching requirements would be a valuable way to extend the work in *Chapter 2,* providing useful input data for models.

The reliability of the equation derived in *Chapter 5* to relate body weight and female-phase maturity needs to be subjected to further testing; in particular it is important to ascertain whether the results are confirmed at other sites.

Regarding behavioural assessments of activity in response to molluscicides (*Chapters 6* and *8*), an obvious 'next step' is to examine patterns of activity in the presence of alternative food such as crop plants; this would help to relate control strategies to overall damage levels, translating biological data into something a little more tangible to farmers. For oilseed rape seedlings this could involve leaf area index measurements in mini field plots to which pellets have been applied; for wheat, seeds could be used in further video assessments since these would not interfere with the visibility of slugs and could be designated as 'zones' using the analysis software.

There are also a number of general areas that could profit from more research in the future. In particular, there is a lack of up to date information concerning the persistence of molluscicide pellets in the field under a range of conditions, i.e. weather effects on pellet decay rates. This is an area where an absence of clear guidance relevant to a particular field situation can lead to excessive applications, paralleling the situation described in *section 9.4.3* for soil splashed pellets. A definitive investigation would help to judge the need for applications in a more informed manner.

As described in *section 9.5*, an up-to-date economic appraisal detailing the cost of slugs as pests in arable crops is urgently needed. This could be based on a national survey mediated through the National Farmers Union (NFU) and not only would it highlight the scale of the problem to a wider audience, but it would help to make the

case for the provision of further research funds. In particular, assessments of the net value of damage are required along with the costs of molluscicide pellets, application machinery and labour as well as time diverted from other tasks on the farm. There should be an undertaking to update the figures of such an appraisal on at least a biennial basis in line with the interval between national pesticide usage surveys commissioned by DEFRA.

Finally, the growing public demand for sustainable agriculture must be acknowledged. It is impractical to suggest that arable farming could manage without molluscicides at present, but more targeted usage is an attainable goal and reliable estimates of long-term damage risk will become pivotal in achieving this. It is the hope that the studies in this thesis and the SAL project in general will provide a firm basis upon which to build more rational and responsible control strategies in the future.

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Appendix A (overleaf)

Factsheet for the Sustainable Arable LINK project

Sustainable Arable LINK Programme Factsheet

Integrated Control of Slugs in Arable Crops

LINK
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Background

Slugs often cause serious damage to winter cereals and oilseed rape after sowing and during crop establishment. They are particularly troublesome in mild, wet years and their numbers have increased as a result of widespread adoption of environmentally driven agronomic practices such as reduced tillage and incorporation of crop residues. Control is often expensive and relies on molluscicidal products formulated as baits (pellets), with multiple applications often being made in years of severe damage. During the autumns and winters of 2000 and 2001, plant losses due to slugs were often severe resulting in widespread patchy cereal and oilseed rape crops.
There is a generally agreed need to improve the efficiency and targeting of control methods to avoid environmental risks and to reduce costs to farmers. However, molluscicidal products based on new chemistry are unlikely to be available commercially for some time.

▸ **A field slug (*Deroceras reticulatum*) feeding on a wheat seed**

▸ **Oilseed Rape seedling damaged by slugs, slug mucus is visible**

Objective

The Project aims to devise a rational risk assessment system for the integrated control of slugs in arable crops, which will be appropriate for incorporation into integrated crop management (ICM) guidelines. It places emphasis on investigations to optimise the prediction of damaging populations and usage of molluscicide pellets, together with cultural practices appropriate for cereals and oilseed rape.

The intention is to alter radically the ways in which the arable industry approaches slug control, so improving the ability of farmers and consultants to make rational decisions.

This new Project complements a HortLink Project on integrated control of slugs in high-value horticultural crops (particularly salads and Brussels sprouts).

Work plan

The Project will explore the influences of key factors such as soil, weather and agronomic conditions, including the timing and method of pellet application, on the activity of slugs and efficacy of slug control. Relationships between slug populations and conditions in the previous crop will be quantified to evaluate their use as a damage indicator in winter cereals and oilseed rape. This part of the Project will develop new understanding of slug population dynamics, building on a simulation model, which incorporates, for the first time, all the major factors thought to affect slug population growth. Model predictions will be tested against slug dynamics in different arable fields and the results used to refine the model. The ability to predict slug damage will be rigorously tested in studies in arable fields. A reliable predictor of crop damage and the need for and timing of molluscicide pellets is sought, based on these relationships.

Slugs (mainly *Deroceras reticulatum*) resting beneath a trap consisting of an upturned plastic flowerpot saucer

Outcome

The Project will provide a set of improved guidelines for users and, potentially, a web-based warning system for farmers and their advisers operated through the industry. A series of demonstration events will be held and the agricultural press kept closely informed with a regular output of articles on the aims and findings of the Project.

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The Partners

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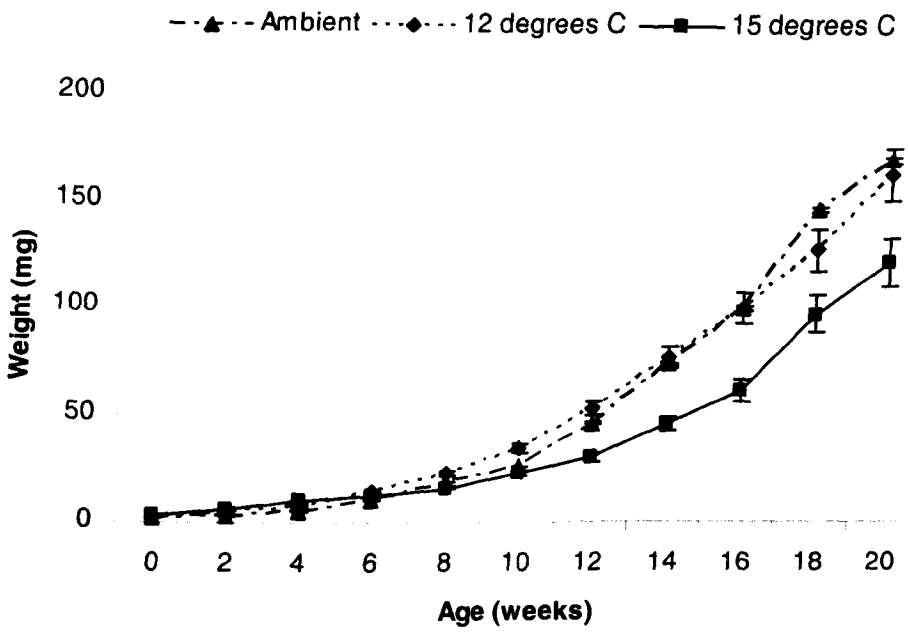
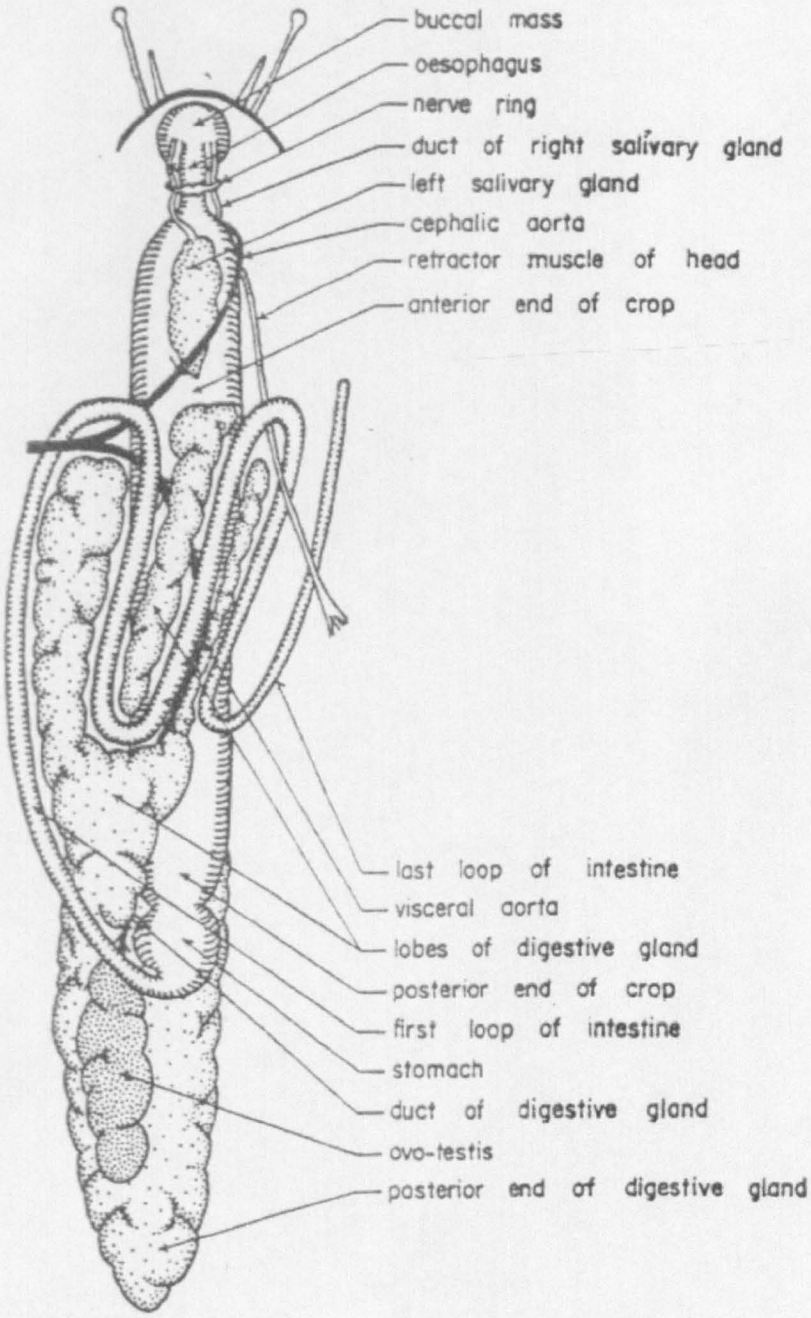
Appendix B



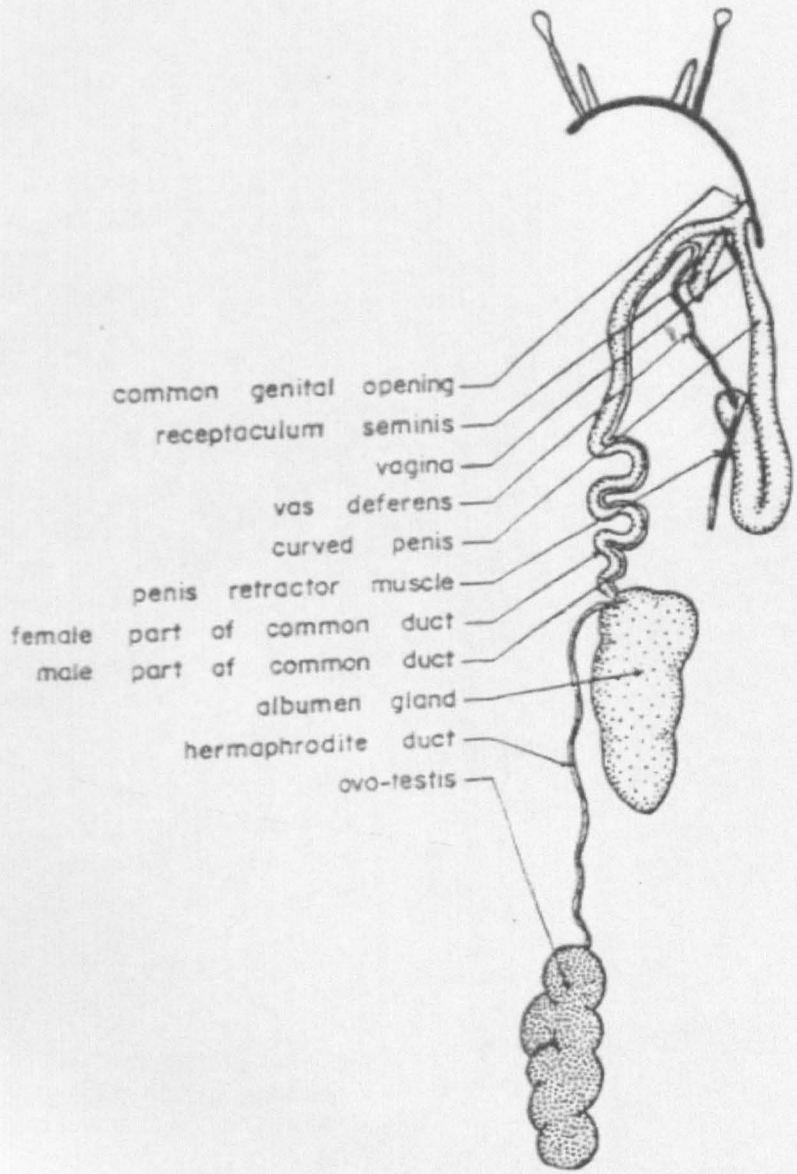
A duplicate of Fig. 3.3 (Chapter 3, section 3.3.3) with an expanded scale to illustrate the differences in the mean weight ± S.E. (mg) of D. reticulatum hatching in spring and reared at ambient temperature, 12°C or 15°C (weeks 0-20).

Appendix C



Diagrammatic representation of the relative position of the digestive and reproductive system in Limax maximus (L.) (after Bullough, 1970). The arrangement is similar in Deroceras reticulatum (Müller).

Appendix D



Limax maximus (L.) reproductive system (after Bullough, 1970)
(Although the details of the male organs differ in Deroceras reticulatum (Müller), the general layout is similar and this diagram indicates clearly the relative positions on the ovotestis, hermaphrodite duct and albumen gland).