Aspects of Feeding in the Grey Field Slug (*Deroceras reticulatum*)

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ABSTRACT

Gastropod damage to crop plants has a significant economic impact on the United Kingdom's agriculture and horticulture industries, with the Grey Field Slug (*Deroceras reticulatum*) being particularly culpable. The main form of crop protection employed by farmers are pellets containing the active ingredient, metaldehyde. During rainfall events or with poor application, metaldehyde can leach into the water system, thus preventing or limiting it from entering the water system is a high priority. Greater understanding of the interaction between slugs and slug pellets could reveal an area of vulnerability or potential manipulation to be targeted by molluscicides. Improved slug pellet formulations could reduce risks to water.

The main objective was to discover if novel formulations improved molluscicide efficiency (increased feeding and mortality) in comparison to commercially available molluscicides. Bioacoustics experimental work enabled comparison of feeding patterns between pellet types by recording the number and length of bites. Bioassay experiments aimed to compare mortality differences between pellet types over 3 to 5 days. Secondary questions aimed to discover if novel formulations would slow or reduce leaching of metaldehyde out of the pellet and to explore the commercial viability of the novel formulations. Soil column type experiments were used to compare leaching of metaldehyde between formulations. Mixed methods were used to discuss the viability of a novel pellet, with a focus on previous molluscicide sale and use in the UK. Overall, the novel formulations tested did not increase mortality or reduce leaching when compared to currently commercially available pellets. Due to changes in legislation during the project, work shifted from a metaldehyde focus to ferric phosphate towards the end of the project. The improved methodologies and discussion will be useful to the development of future molluscicides, as metaldehyde is still in use in over 30 countries.

for my beloved late grandparents, Maylin and Nissanka de Silva, my aachie and seeya

thank you for all you have done

With all my love

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METALDEHYDE DISCLOSURE

“A ban on the outdoor use of metaldehyde, a pesticide used to control slugs in a range of crops and in gardens, is to be introduced across Great Britain from spring 2020, the Environment Secretary announced today (19 December). . . .The outdoor use of metaldehyde will be phased out over 18 months to give growers time to adjust to other methods of slug control. It will be legal to sell metaldehyde products for outdoor use for the next six months, with use of the products then allowed for a further 12 months. . . .” (Gov.uk, 19 December 2018)

From the project onset in May 2017 until December 2018, all decisions made by the sponsoring organisation, the supervisory team and author were made with the idea that metaldehyde would continue to be available in the UK. Significant changes to the project outputs were made from December 2018 until July 2019. In July 2019 the ban on metaldehyde slug pellets was overturned and declared unlawful by the High Court in London after the ban was legally challenged by Chiltern Farm Chemicals. From July 2019 until completion of work for this project, metaldehyde remained on the market. In September 2020, it was confirmed that “outdoor use of metaldehyde, a pesticide used to control slugs on farms and in gardens, is set to be banned in Great Britain from the end of March 2022 in order to better protect wildlife and the environment… Metaldehyde will be phased out by 31 March 2022 to give growers and gardeners appropriate time to switch to alternative slug control measures… [Instead] pesticides containing ferric phosphate can provide effective control without carrying the same risks to wildlife as metaldehyde slug pellets” (Gov.uk, 18 September 2020).

Although the use of metaldehyde in the UK has been withdrawn, metaldehyde is a global molluscicide, currently in use within 21 EU nations, Asia, Australasia as well as South and North America.

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Definitions

Acceptability	likelihood of the slug feeding after initial physical contact with the pellet (Howling 1991)
Antifeedant	substance that deters further consumption after the initial feeding response
Arrestant	a substance that physiologically prevents movement away from the pellet
Attractant	a substance that increases the chances of the slug moving towards the pellet, while spatially separated
Attractiveness	the effect of the pellet on the slug when detectable but spatially separated (Howling 1991)
Bioassay	experimental method to determine the effect of a substance on an animal, typically including allowing the animal to feed on a substance and measuring consumption, mortality and / or behaviour
Bite amplitude	loudness or the size of the vibrations
Bite count	number of bites per trial or per defined time frame
Bite length	time taken for a complete bite
Bite position	time of bite within the length of the trial
Consumption	the amount of food consumed by the slug
Deterrent	a substance that decreases the chances of the slug moving towards or staying in contact with the source, once contact has been made
Feeding session	an uninterrupted bout of feeding with breaks of less than 60 seconds
FERA	(Formerly the) Food and Environment Research Agency
Incitant	a substance that initiates initial feeding response (tasting)
Lonza	industrial partner
Lucideon	industrial partner
Palatability	effect of an active ingredient or formulation on consumption (Howling 1991)
Reacceptance	feeding on the pellet 60s or more after a feeding session is completed
Repellent	a substance that decreases the chances of the slug moving towards the pellet, while spatially separated
Stimulant	a substance that promotes the feeding response and increased consumption after feeding initiation
Suppressant	a substance that causes physiological feeding inhibition
UKWIR	United Kingdom Water Industry Research

Abbreviations

AHDB	Agriculture and Horticulture Development Board
AI	Active Ingredient
EDTA	Ethylenediaminetetraacetic acid
iCRT	Inorganic Controlled Release Technology
RAD	recommended agricultural doses
°C	Degree Celsius
UK	United Kingdom
µm	Micro metre(s)
µg	Microgram(s)
M	Meter(s)
Mm	Millimeters
cm	Centimeter(s)
IPM	Integrated Pest Management
H	Hour(s)
EU	European Union
Met	Metaldehyde

Chapter 1: General Introduction

1.1 Project Motivation and Objectives

Deroceras reticulatum (Muller) is the most important mollusc pest to the UK agricultural and horticultural industries (Ramsden et al, 2017). The most common way to mitigate slug damage is through the application of metaldehyde containing pellets to the growing area. Metaldehyde pellets aim to kill pest molluscs or paralyze them, making them more vulnerable to predators and the elements. Poor application or rainfall events causes metaldehyde to leach into the water system and it is near impossible to remove once incorporated. The European Union's regulatory drinking water standard for an individual pesticide is 0.1 μg L^{-1} and 0.5 μg L^{-1} for total pesticides ('Drinking water legislation - European Commission', n.d.). Metaldehyde has repeatedly been the main cause for failure to achieve drinking water standards (Jönsson et al., 2014). The work undertaken for this industrial PhD has been kindly funded by United Kingdom Water Industry Research (UKWIR). One of five UKWIRs goals is to achieve 100% compliance with drinking water standards (at point of use) by 2050.

The main concerns that arose due to metaldehyde pellets use are:

1. Metaldehyde leaching, which may result in non-compliance with standards
2. Metaldehyde pellets may not be effective, enabling further slug damage to crops and consequential financial loss in agricultural and horticultural industries

Novel metaldehyde formulations were developed by an independent company, Lucideon. The new formulations aimed to release metaldehyde slowly, the benefits of which would be two-fold:

1. The quantity of metaldehyde entering the water system at any given point would be reduced, allowing water companies to maintain set standards
2. More slugs would consume a lethal amount, resulting in a higher death rate and consequently less damage to crops

The initial overall aim of the study was to provide Lucideon with feedback on the novel metaldehyde formulations for product optimisation. However, due to the results of the feeding bioassays and changes in legislation, the project developed to incorporate further work on an alternative slug pellet, containing ferric phosphate.

1.2 General

Deroceras reticulatum, more commonly known as the Grey Field Slug, belongs to the family Limacidae, commonly known as Keelback slugs, and has several close relatives in *Deroceras leave* (Müller), *Deroceras panormitanum* (Lessona and Pollonera) and *Deroceras agreste* (Linnaeus). Keelback slugs are indigenous to Europe but, due to synanthropic association with humans and agricultural produce, are present on all continents except for polar regions. Within Britain, *D. reticulatum* has been documented on most land types, including but not limited to, farmland, gardens, grasslands, hedgerows (Willis et al., 2006; Wilson, 2017). Adults contract when handled but measure from 3.5 to 5cm when extended. *Deroceras reticulatum* are generally a grey-cream colour, but this can vary from bluish-black to cream-white (Figure 1.1). A comprehensive guide to identification is provided by Rowson et al. (2014).

Figure 1.1 *Deroceras reticulatum* on soil next to a singular metaldehyde pellet (Gordon Port)

Activity and life cycle of *D. reticulatum* is greatly influenced by environmental conditions, due to not being able to regulate temperature as an ectotherm [1] (Young & Port, 1991; Hommay et

[1] This section will refer to conditions in the UK or in other temperate climates, unless specified.

al., 1998; Kim et al., 2009a). Mating between adults occurs usually after trail following and takes place on damp ground during the evenings or at night-time (South, 1992). The period between mating and oviposition ranges from 8-10 days (Runham & Hunter, 1970). Oviposition (egg laying) generally happens in two periods of the year (spring and autumn) when conditions are most favourable (Bett, 1960; Dmitrieva, 1969; Hunter & Symonds, 1971). However, the species will reproduce at any time of the year in peak conditions, resulting in a variety of life stages at any time of the year (Hunter & Symonds, 1971; Port & Port, 1986). A level of 53% soil moisture and temperature of 18°C were reported as the most favourable for oviposition (Willis et al., 2008). Changes in oviposition behaviours related to photoperiod have been observed in other mollusc species, but not in *D. reticulatum* to date (Rollo, 1983; Hommay et al., 2001; Kozłowski & Sionek, 2009). As eggs, *D. reticulatum* are vulnerable to desiccation, where larger eggs and larger batches of eggs may be more protected due to increased moisture uptake and greater protection from desiccation (Runham & Hunter, 1970). For discussion of the use of molluscicides on *D. reticulatum* eggs see Iglesias, Castillejo, Ester, et al. (2002); Iglesias, Castillejo, & Ester, (2002). *Deroceras reticulatum* is a simultaneous out-crossing hermaphrodite. Development is protandric, meaning that juveniles are usually males and mature adults are generally female, although there can be considerable overlap (Runham, 1985; Schley & Bees, 2002). The life span of slugs is around one year, although there is naturally observed mortality at all life stages (South, 1982, 1992). The life span of laboratory raised slugs was observed to be shorter than observed in natural conditions, ranging from 6-10 months (South, 1982; Zotin, 2007). For further discussion of cultured slugs see Chapter 1.7.

1.3 Physiology

The physiology of slugs is well studied and documented in the literature, mostly from taxonomic view. The physiology of the digestive system is similar between species (Figure 1.2). Food fragments are broken off by the radula, pass down the buccal cavity and through the oesophagus. Food then moves to the oesophageal crop or, in some species, directly into the stomach or gastric pouch and past the ducts of the digestive gland. Fine food particles move into the glands while larger particles are condensed into faecal strings consisting of four to five faecal pellets that leave the slug body via the anus (Barker, 2001). *Deroceras reticulatum* is particularly well studied, due to its status as a crop pest. This section will refer only to *D. reticulatum* unless otherwise specified. General reviews of the physiology and associated functions are found in Barker (2001) and Lobo-da-Cunha (2019).

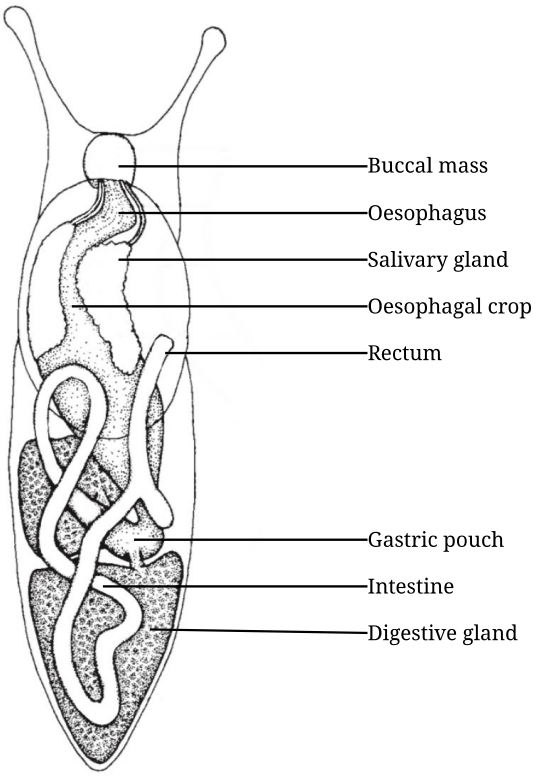

Figure 1.2 Digestive system of *Deroceras reticulatum,* adapted from Barker (2001)

1.3.1 Buccal mass

The buccal mass or foregut refers to the mouth parts of the slug and is responsible for coordinated feeding and mastication. Dentition in gastropods is well documented within the literature; historically it was used for taxonomic purposes, but little was known about the function and mechanisms of the buccal mass until more recently (Mackenstedt & Märkel, 2001; Ponder et al., 2008). The entrance of the mouth leads to the buccal cavity. At the bottom of the buccal cavity are the main organs of the buccal mass - the radula, odontophore and jaw muscles. The odontophore is a cartilaginous organ, in shape comparable to a tongue, that supports the radula which consists of a membrane with multiple rows of teeth (Figure 1.2). The radula which is a flexible membrane that supports the teeth (denticles) is a distinctive feature of molluscs and differs between species (Barker, 2001).

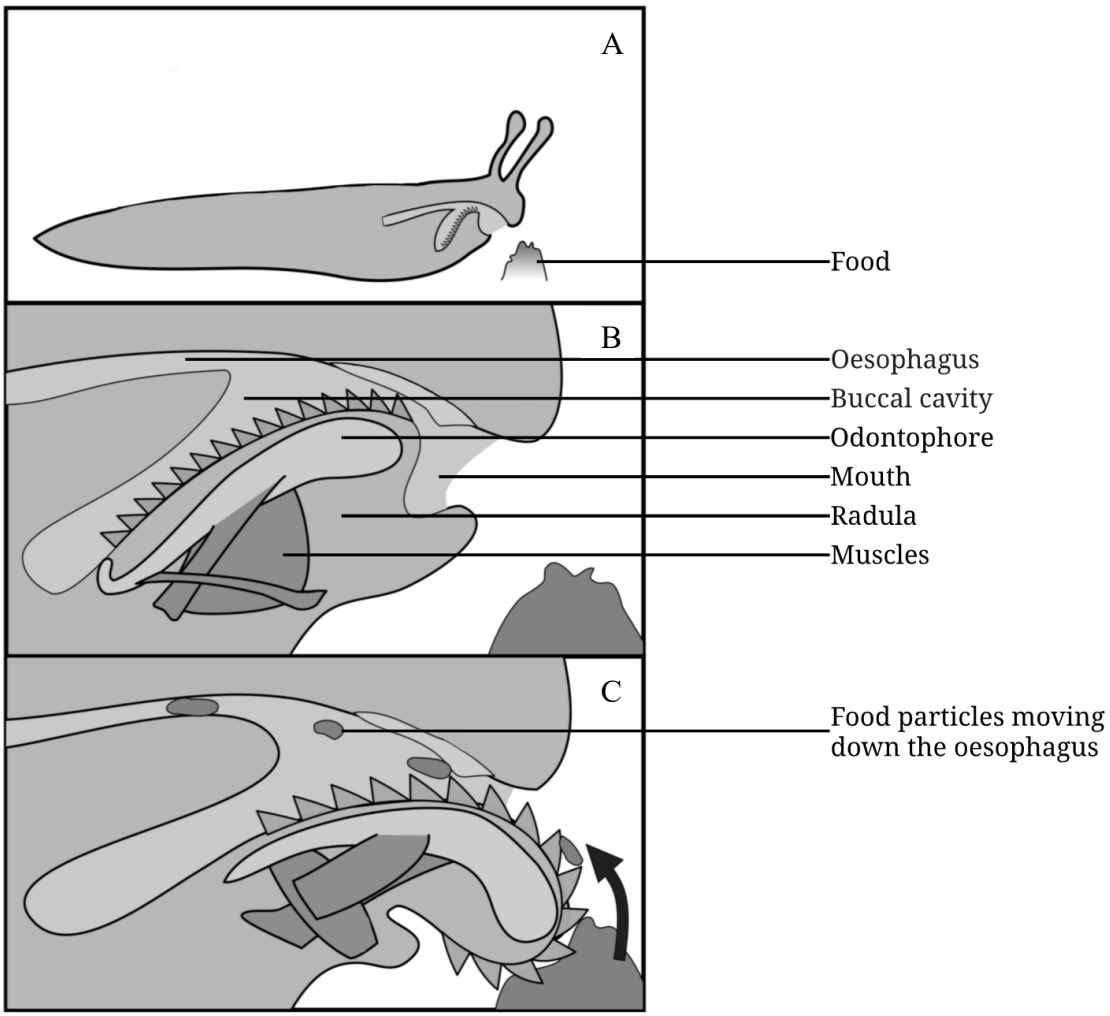

Figure 1.2 Location of A) buccal mass B) teethed radula and associated structures (odontophore, muscles) towards a food substrate C) Movement of food particles into the buccal cavity and oesophagus. Adapted from an image by Debivort (2006).

The odontophore is pushed forward by the protraction of associated muscles, out of the mouth, so that the teeth-covered radula makes contact with food. Teeth at the forefront of the mouth, in contact with food, are disintegrated while feeding, but new teeth at the rear of the mouth are produced at an even rate. With every rasp (bite), new teeth are pushed forwards, while worn teeth move back towards the buccal cavity where they are swallowed and eventually discarded in faeces. The radula membrane where the worn teeth were is reabsorbed. Specialised jaw muscles are responsible for the repeated movement of the radula forward towards and against the feeding substrate and return into the buccal cavity (Mackenstedt & Märkel, 2001). The retraction of the radula back into the cavity moves food particles into the buccal cavity where they mix with salivary secretion. A pair of salivary glands can be found at the back of the buccal cavity on either side of the oesophagus (Walker, 1970b). These secrete saliva which lubricates food particles, allowing food to pass from the buccal cavity into the oesophagus more easily. There has been some suggestion that the saliva may contain some digestive enzymes and may

play a small role in extracellular digestion (Walker, 1970a). For a more detailed description of the radula in molluscs see Chapter 4 of Barker (2001), where for more on the buccal mass in *D. reticulatum* see Curtis & Cowden (1977), Moens & Rassel (1985) and Wright & Huddart (1999).

Metaldehyde and the buccal mass

Several authors have suggested that poor consumption of metaldehyde was a result of paralysis of the feeding apparatus (Wedgwood & Bailey, 1988; Mills et al., 1990). The number and rate of rasps or bites, as well as length of the meal, when consuming a metaldehyde pellet, was noted as markedly reduced when compared to a pellet without metaldehyde. Additionally, consumption of metaldehyde deters reacceptance of a pellet without metaldehyde (Wedgwood & Bailey, 1986, 1988; Bailey & Wedgwood, 1991). For further discussion see Chapter 3.

1.3.2 Oesophagus and crop

Food passes from the buccal cavity to the oesophagus. The oesophagus is a thin tube that begins from the back of the buccal cavity and leads to the gastric pouch. In some species, like *D. reticulatum,* the latter part of the oesophagus forms the crop or oesophageal crop (Bourne et al., 1991). This is more clearly defined in some species and there are many structural and functional similarities between the oesophagus and crop. As a result, there may be confusion in the literature differentiating the anterior of the oesophagus from the modified posterior. Food is transported along the oesophagus mostly by muscular contractions that can be likened to peristalsis. The oesophagus epithelium is ridged and lined with mucus producing and ciliated epithelium cells which may aid the movement of food down the alimentary canal. The presence of peroxisomes (enzyme containing organelles associated with metabolic reactions) reported in oesophagus of *D. reticulatum* suggest a minor metabolic role, while in other species, peroxisomes were only reported in the crop. Food is passed almost immediately from the buccal cavity, through the oesophagus and into the oesophageal crop (Walker, 1972).

The crop epithelium consists of mostly modified storage cells with sparse mucus cells and is the first major digestive site for soluble materials. Food particles are retained in the crop generally for 20 to 40 minutes, although it may take several hours depending on the food ingested and the size of the meal. If particularly fine, particles may be retained in the crop for

an extended period (Bourne et al., 1991). In starved animals, endocytosis took place within five minutes and glucose, galactose and glycine were also observed to be taken up in the crop epithelium (Walker, 1969). The crop of *D. reticulatum* appears to be visually uniform but it has been suggested that the crop may have two functional parts, hindcrop and forecrop (Triebskorn & Florschütz, 1993). This initially was established in the species *Arion ater* by Roach, (1968). Further work would be required to determine if this was the case in *D. reticulatum*, although differential storage of food containing thorium sulphate in *D. reticulatum* observed by Triebskorn & Florschütz, (1993) suggests this may be so. However, other studies that focus on the crop make no mention of this differentiation (Dobson & Bailey, 1982).

Metaldehyde and the crop

The crop would be the initial area subjected to prolonged contact with molluscicides (Dobson & Bailey, 1982). Metaldehyde may cause crop paralysis and weakened peristalsis (Wright & Williams, 1980; Bourne et al., 1991; Bowen & Antoine, 1995). The initial effects of metaldehyde may result in prolonged retention of the toxin when compared to non-toxic foods, as was observed with the carbamate molluscicide Cloethocarb, (Triebskorn & Florschütz, 1993). Metaldehyde was observed to cause severe damage to crop cells in multiple investigations (Triebskorn, 1989; Bourne et al., 1991). Damage to the surface of the crop cells was only apparent between 2 and 24 hours after ingestion of 4% metaldehyde and methiocarb (Bourne et al. 1991). The exact length of time to cause damage, and whether damage continues after the molluscicide particles leave the crop, is unclear. Bourne et al. (1991) suggested that increasing the uptake of molluscicides in the crop, through the addition of pinocytotic agents, may improve the efficiency of the molluscicides. There is no information in the public domain suggesting that this has been tested.

1.3.3 Stomach and digestive glands

The mechanisms by which food is transported from the stomach are not clearly understood. Small and soluble particles may be moved by cilia to the hepatic duct, cilia lining the ducts then transport this material directly to the digestive glands. However, as the cilia were observed to move in the direction of the stomach there is some uncertainty if cilia were responsible for movement or if for general sorting (Walker, 1972). It may be that contractions of the stomach

musculature pushes suitable material into the digestive glands instead (Graham, 1972). Walker (1970) noted that passage of material from the stomach to the digestive glands could only be followed using material of particle size 0.1 - 04 μm. Particles of this size were only phagocytosed in the digestive cells of the digestive glands. The stomach is sac shaped, with three highly ciliated interior folds - a pair of longitudinal folds, the typhlosoles and the triangular accessory fold. The walls of gastropods tend to have a specialised gastric shield, sorting areas, caecum, typhlosoles and style sac (Lobo-da-Cunha 2019). *Deroceras reticulatum* have true typhlosoles; the two ridges form a waste-carrying valley from the digestive gland to the intestine. A crystalline style, formed from material secreted by the style sac, is uncommon in terrestrial gastropods and has not been described in *D. reticulatum* previously. Other species, *Arion subfuscus* (Draparnaud, 1805), *Milax budapestensis* (Hazay, 1881), *Limax maximus* (Linnaeus, 1758) and *Limacus flavus* (Linnaeus, 1758) possess stomach folds similarly situated to *D. reticulatum*, so digestion studies on these species may be more relevant (Walker, 1972). The anatomy and histological features of the stomach in *D. reticulatum* have been described in detail by Walker (1972), Triebskorn (1989) and Triebskorn & Florschütz (1993). Further reading on general molluscs includes Chapter 5 of Barker (2001) and Lobo-da-Cunha (2019)

Metaldehyde and the stomach

Metaldehyde prevents mucus cells, dominant in the stomach and intestine, from producing mucus after an initial intense period of activation. Severe cellular damage was observed to most structures and the quality of mucus was changed after poisoning (Triebskorn, 1989; Triebskorn & Ebert, 1989). Severity of damage is slightly limited in warmer, humid conditions, which may explain recovery (Triebskorn et al., 1998; Campbell et al., 2021). For more details of ultrastructural changes see Triebskorn & Ebert, (1989) and Triebskorn (1989).

1.3.4 Excretion

As there is no clear separation between the stomach and intestine, this work will consider the intestine to start from the typhlosoles. The main functional role of the intestine is as the site where faecal matter is compacted, moisture is extracted and mucus added to the faeces as they pass down the intestine (Barker, 2001). Early works suggested that there were two types of faeces, a darker pellet from the intestine and a lighter coloured pellet from the mid gut

(Jezewska, 1969). Pallant (1970) proposed that in *D. reticulatum* there may be a third type, a white pellet from the midgut caecum, which was suggested to be uric acid. The darker type of pellet is usually covered in mucus (75-90%) but can also be standalone pellet fragments or mucus. The excreta from the mid-gut is a brown liquid with lighter coloured granules and occasionally both types of pellets appear at the same time. The order of appearance was observed as the pellet from the intestine, followed by the lighter pellets and white pellet (Pallant, 1970).

1.3.5 pH of digestive tract

Little is known about the pH values of gastropods digestive tracts. Walker et al. (1996) is the principal study which determined the pH of the digestive system in *D. reticulatum*. Significant differences were observed between fed and starved slugs, in the crop, digestive gland and salivary gland. In starved slugs, all regions had values between 6.6 and 7.0, but pH decreased between 0.42 – 0.76 in slugs that had fed (Walker et al., 1996). Secretion of digestive enzymes in fed slugs may explain the differences in pH between fed and starved slugs, although it is possible that the type of food material may have influenced the resultant pH. Values between 5.8 – 7 were observed in *Arion intermedius* (Normand, 1852) and *Tandonia budapestensis* (Hazay, 1880) (Walker et al., 1998). The pH of *Cornu aspersum* (Müller, 1774), *Helix pomatia* (Linnaeus, 1758) and *Elona quimperiana* (Blainville, 1821) is referred to in Charrier & Brune (2003). Initial investigations used pH papers to analyse invertebrate gut homogenates, but this may not accurately reflect the conditions present *in vivo* as homogenates do not occur in natural conditions. *In vivo* investigation using micro-electrodes allows measurement with minimal damage to surrounding tissue which enables more spatially accurate results (Walker, 1996). A review on the use of microsensors for the study of invertebrate digestive systems is provided by Schramm (2005).

1.4 Slug Management

Molluscicides refer to pesticides used to kill molluscs; in this thesis, unless specified, molluscicides refer to those used on terrestrial slugs and snails. Professional growers should not rely on a single control method; incorporating multiple controls into an Integrated Pest Management (IPM) scheme is a requirement for professional users through Directive 2009/128/EC.

1.4.1 Mechanical, physical, and cultural control

Home growers and amateur use

Mechanical control methods physically stop slugs accessing food. The use of mechanical methods alone tends to be most popular amongst home gardeners who are willing to invest proportionally more time, effort and funds into crop protection than commercial growers. Many home growers prefer mechanical or biological control, to pellets. For more on biological control refer section 1.4.4. Methods used by home growers include physical removal of slugs (by trapping or manual searching) and barrier methods including copper wires, mulch, eggshells and grit. Little published scientific literature is available on the popularity and success of control methods used by home growers. Further work on the control of gastropods in an amateur setting, currently pending publication, can be found at on "Gastropod barriers" at RHS.org.uk[2].

Cultural control

Cultural control refers to exploitation of the crop growing system that discourages pest proliferation. Examples of cultural control include changes to sowing time, repeat cultivations, specific crop rotations and tilling practices (Port & Port, 1986; Howling, 1990; Kennedy et al., 2013). Best practice to limit slug numbers varies with soil type, crop type and environmental conditions but considerations to other crop pests need to be accounted for. Glen (2000) provides further information on cultural controls that both increase and decrease slug damage to cereal crops (wheat, barley and oats). Overall, the reliance on cultural control has diminished in parallel to the improvements in insecticides and molluscicide (Glen, 2000; Nash et al., 2014).

1.4.2 Chemical controls

Chemical controls use toxicants that function by poisoning the slug upon contact, when ingested by the slug, or both. The term active ingredient (AI) refers to the chemical or compound in a pesticide that is responsible for repelling or killing the pest; all other ingredients are referred to as inert ingredients. It is a statutory requirement in the UK for the active ingredient to be

[2] Available at: https://www.rhs.org.uk/science/plant-health-in-gardens/entomology/rhs-projects-on-plant-pests/gastropod-barriers-experiment

described on the label, with the relevant concentrations permitted for use under different scenarios. There is no obligation to report inert ingredients, and most molluscicides sold in the UK do not have the inert ingredients reported (HSE, n.d.).

This thesis is mainly concerned with the active ingredient's metaldehyde and ferric phosphate, as these were the most used molluscicides in the UK at the time of writing. In the UK, metaldehyde has been the main active ingredient used to protect crops from slugs from the mid 1990's (Fera Science Ltd. (Fera), n.d.). There are multiple delivery methods available for metaldehyde in which it is, but the main form is in pellet form. Pellets may also be referred to interchangeably as baits in the literature, however, the use of 'bait' may confuse the readers as not all baits are attractive to slugs. Pellets were applied to 17% of all crops making this method the most used agricultural method of control (Glen & Moens, 2009). Other chemicals, methiocarb and thiodicarb, previously were used to control slugs but are no longer in use in the UK. After March 2022 it is expected that ferric phosphate will be the most popular active ingredient, due to the withdrawal of metaldehyde pellets for outdoor use (Department for Environment & Affairs, 2020).

Slug pellets are made of a cereal base to encourage slug feeding, dye to discourage the ingestion of the pellet by non-target species and compounds (stabilisers, binders, fungicides) to extend the longevity of the pellet. Some pellets may include compounds that aim to attract slugs towards the pellet or prolong feeding on the pellet (Barker, 2002). Pellets available to home growers may differ slightly from those available to professional growers; those available to the latter may have a higher concentration of active ingredients (see Chapter 5). Little information is available in the public domain about the formulation of pellets and their efficiency. It is thought that much experimental work is done by manufacturers but is not available due to commercial confidentiality.

Pellets rely on the slug ingesting the active ingredient to function, although there can be some adverse reaction by the slug based on contact made with the pellet. Exclusively contact molluscicides are, however, considered less effective than pellets and would require large amounts of active ingredient. The mucus coating of the slug presents a barrier to contact molluscicides and at low concentrations required by law, most active ingredients cannot penetrate effectively. Contact toxins could be applied as a spray, a powder, or in granular form, but under the limitations of application rates per hectare per year would not be effective.

Metaldehyde

The molluscicidal properties of metaldehyde were accidently discovered 1934 and were first mentioned in published work in 1936. Metaldehyde containing pellets became the most popular slug pellet within five years and has been in constant use since its discovery (South, 1992). Consumption of metaldehyde pellets causes, amongst other effects, excessive mucus production and paralysis (resulting in an inability to find a suitable environment to recover), which are thought to be the main causes of death (Wright & Williams, 1980; Glen & Orsman, 1986). Other symptoms of metaldehyde poisoning in slugs include alternate bursts of immobilisation and convulsions that can last up to several hours and slugs taking on a shrivelled appearance. Physiological changes upon the consumption of metaldehyde are described in detail by Triebskorn (1989), Triebskorn & Ebert (1989), Triebskorn & Florschütz (1993) and Triebskorn et al (1998).

Reduced feeding on metaldehyde pellets may result in sub-lethal poisoning and subsequent recovery if the slug is in the favourable warm and humid conditions (Campbell et al., 2021). It is thought that as metaldehyde is consumed, paralysis hinders the consumption of further pellet. The regular consumption of sub-lethal amounts of metaldehyde as an explanation of high recovery rates has been documented well in the literature (Wright & Williams, 1980; Briggs & Henderson, 1987; Bourne et al., 1988, Wedgwood & Bailey, 1988; Bailey & Wedgwood, 1991; Campbell et al., 2021). In pellets with only metaldehyde and pellets with metaldehyde in combination with methiocarb, increased metaldehyde concentration decreased the amount of pellet consumed and the likelihood of further feeding, even after recovery (Glen & Orsman, 1986; Bourne et al., 1990; Bailey & Wedgwood, 1991; Henderson et al., 1992). Combining metaldehyde with other active ingredients could produce a more effective pellet with a lower concentration of actives but is not a probable solution for the UK market due to legal constraints on the use of metaldehyde and other actives (Bourne et al., 1990). For more on the manufacture and sale of metaldehyde pellets in the UK, see Chapter 5.

Carbamates

Carbamate pesticides cover a range of chemicals that incorporate the carbamate ester functional group, $ROCONR_2$. Prominent carbamate molluscicides include methiocarb and thiodicarb, both of which have been withdrawn from use in the UK (Barker, 2002). Methiocarb and thiocarb

were never the dominant molluscicides and were commonly used in combination pellets. In molluscs carbamates act as nerve poisons by the inhibition of cholinesterase, which prevents the breakdown of neurotransmitters (Bourne et al., 1990, 1991; Essawy et al., 2009). Field evaluations show thiodicarb to be as effective as methiocarb, though laboratory trials suggest that thiodicarb may give better control in wet conditions, while methiocarb may be better in dry conditions (Ferguson et al., 1995; Gaulliard & Laverriere, 1989). Slugs poisoned by methiocarb, unlike metaldehyde, do not produce excess mucus, but similarly have alternate bursts of immobilisation with convulsions and / or paralysis, taking on a bloated appearance shortly before or after death (Bourne et al., 1988).

1.4.3 Metal compounds

Metal compounds, particularly copper, iron and aluminium, were perceived to have molluscicidal properties and have been used for gastropod control since the 1900s (Ryder & Bowen, 1977; Henderson & Martin, 1990; Henderson et al., 1990; Bullock et al., 1992; Henderson et al., 1992). Of the metal compounds explored for this use, iron (III) phosphate, or ferric phosphate ($FePO_3$), is the most widely used and is the only metal-based molluscicide available in pellet form in the UK (Fera Science Ltd. (Fera), n.d.).

Ferric phosphate

Ferric phosphate is a relatively new molluscicide, released as Ferramol (Neudorff GmbH, Germany) that became available in the UK in June 2005 (Horgan, 2006). Ferric phosphate, ranging in concentration from 1 – 3%, is listed as the AI, but requires a chelating agent such as Ethylenediaminetetraacetic acid (EDTA), or similar, to function as a molluscicide. The chelating agent allows the ferric phosphate product to be absorbed more by the mollusc than if it was not present which increases the molluscicidal properties of the product. Ferric phosphate pellets are described as "…an inert, edible mollusc pellet with two active ingredient precursors. Individually the active ingredient precursors are not toxic to the molluscs. It is only when the entire composition, including active ingredient precursors, is ingested by molluscs that molluscicidal activity is achieved"; the first active ingredient refers to a "simple iron compound" and the second as one of "a hydroxyl derivative of edetic acid, or the salts of this acid" (Puritch et al., 1995). EDTA is reported to show little risk to mammals and the

environment, due to occurring naturally, but several papers suggesting dangers to earthworms have been published (Buhl et al., 2013; Edwards et al., 2009; Langan & Shaw, 2006).
Ferric phosphate interferes with calcium metabolism in slugs, particularly in the crop and digestive gland (Atkinson et al., 2006; Horgan, 2006; Certis, 2015). It does not cause immediate paralysis, nor does it interfere with mucus production. This allows slugs to survive for a longer post exposure period of 3 – 7 days before death (if an adequate amount is consumed) when compared to metaldehyde pellets. The available literature on ferric phosphate as a molluscicide is limited compared to metaldehyde. For more on the use and sale of ferric phosphate pellets in the UK, see Chapter 5.

1.4.4 Biological control

Biological control refers to the introduction or encouraged proliferation of a pests' natural enemy. The nematode *Phasmarhabditis hermaphrodita* (Schneider, 1859) has been marketed as a commercially available biological control agent since 1994 under the trade name Nemaslug®, and has been established as a successful control of molluscs in closed environments with potential for larger agricultural scale use (Wilson, Glen, Hughes, et al., 1994; Wilson, Glen, Wiltshire, et al., 1994; Wilson, Glen, George, et al., 1994; Rae et al., 2007).The nematodes *Steinernema feltiae* and *Heterorhabditis* species have also been considered for their potential as slug biocontrols, but did not show potential (Wilson et al. 1994). Although widely used by hobbyists/gardeners, the high costs of nematode use for slug control, due to high application rates, temperature sensitivity which increases storage costs, short shelf life, and low efficacy against some slug families, means that complete dependence on biological control is not a realistic option for farmers operating at full field scales (Cutler & Rae, 2020; Glen et al., 2000; Grewal et al., 2001).

Wilkinson (2011) investigated the bacteria associated with *D. reticulatum*, to determine if any were essential to the survival of the slug, and thus a potential target for biological control. However, little evidence that a bacterial symbiont exists was found. Thus far, a successful agricultural scale biological control for *D. reticulatum* has not been found. See Barua et al. (2021) for review of biological control strategies for slugs.

Other natural enemies

Many birds and small mammals will at least occasionally feed on *D. reticulatum*, but none are specialised slug feeders. *Deroceras reticulatum* and many other slug species will extrude mucus when they are attacked or handled and this may deter some opportunistic predators (Pakarinen, 1994; Mair & Port, 2001; Barker, 2004; Mair & Port, 2002; Gould et al., 2019). Predation of *D. reticulatum* by carabid beetle species is well documented in the literature (Ayre, 2001; Kromp, 1999; Thomas et al., 2008; Bursztyka et al., 2016; El-Danasoury & Iglesias-Piñeiro, 2018). Some species, such as *Pterostichus niger* (Schaller, 1783), are generalist predators and will feed on slugs opportunistically. Species such as *Cychrus caraboides* and *Carabus violace* could conversely be described as specialist slug feeders, as they have shown adaptations to enable then to kill molluscs (*Arion fasciatus* and *Deroceras reticulatum)* (Pakarinen, 1994; Ayre, 1995). Environmental conditions can potentially affect predatory activity, as could relative predator and prey size (Ayre, 2001; McKemey et al., 2001). These specialist species are also relatively low in abundance in the UK and the contribution of more common generalist species to slug predation cannot be totally relied upon for effective agricultural slug control, although there is potential (Asteraki, 1993; Oberholzer et al., 2003). For more on vertebrate mollusc predators see Allen (2009) and for more on natural predators of molluscs *per se* see (Barker, 2004)

1.4.5 Efficiency of different molluscicides

The efficiency of different molluscicides is of great concern to users and pellet manufacturers (Table 1.1).

Table 1.1 Studies comparing metaldehyde to other molluscicides, formulations, slug species and summaries of results presented[3]

Molluscicide	Species	Results summary	Reference
Metaldehyde Methiocarb	*D. reticulatum A. distinctus*	Slugs fed less on pellets containing molluscicide, but almost always fed on the first pellet encountered. Metaldehyde poisoned slugs were immobilised rapidly. *Deroceras reticulatum* took more bites, moved more and had more meals.	Bailey & Wedgwood, 1991
Novel metaldehyde Metaldehyde Ferric phosphate	*D. reticulatum*	Novel pellets were not consumed more than commercial pellet types. Commercial pellet types did not differ in consumption.	de Silva et al., 2021
Metaldehyde Methiocarb Aluminium sulphate	*D. reticulatum*	In laboratory trials, methiocarb was observed to cause significant mortality, recovery was observed with metaldehyde pellets. Recovered slugs fed less than un-poisoned slugs for both chemical molluscicides. Neither chemical pellet reduced trapped slugs in field trials.	Glen & Orsman, 1986
P. hermaphroditais Metaldehyde	*D. reticulatum*	Metaldehyde caused greater and more rapid slug mortality than the nematodes, the application with nematodes had an equal or lesser number of leaves damaged and leaf area eaten than metaldehyde.	Grewal et al., 2001

[3] Not exclusive or exhaustive list

P. hermaphroditais Ferric phosphate Ioxynil octanoate Metaldehyde	*Field conditions, species not controlled*	No difference between treatments at 3 weeks. After 5 weeks, plants treated with metaldehyde and *P. hermaphroditais* were less damaged.	Jiglesias et al., 2001
Metaldehyde Acetaldehyde	*L. stagnalis*	Symptoms after feeding on both pellets were similar, a decrease in feeding was observed with an increasing metaldehyde concentration.	Mills et al., 1990
Ferric phosphate Metaldehyde	*D reticulatum A Lusitanicus*	Metaldehyde was more effective in preventing slug damage and reduced numbers of all slug species present. Iron phosphate reduced numbers of *A. Lusitanicus* but not *D. reticulatum,* reduced leaf loss, increased the number of marketable produce.	Speiser & Kistler, 2002
Metaldehyde	*A. hortensis* *D. reticulatum,* *D. caruanae*	As concentration of metaldehyde increased, meal length and bite number decreased.	Wedgwood & Bailey, 1988
Metaldehyde Methiocarb	*D. reticulatum*	Mortality was higher for metaldehyde dominant combination pellets than for methiocarb dominant combination pellets and for each AI alone. A 2:1 meta1dehyde:methiocarb ratio was the most effective	Bourne et al., 1990

1.4.6 Molluscicides and non-target species

Birds, farm and domestic animals

Limited published work is available on the interaction between birds with molluscicide pellets or metaldehyde contaminated slugs; similarly, for wild mammals. Methiocarb, a molluscicide that was historically used as a bird repellent, was withdrawn from use in the UK in 2014 due to the well documented detrimental effect on grain feeding species (Tobin & DeHaven, 1984; Hardy et al., 1993; Dolbeer et al., 1994). Several incidents of metaldehyde poisoning in domestic birds are documented in public access literature but can be attributed to malicious intent or accidental access (Andreasen, 1993). Similar instances of poisoning are documented in domestic and farm animals (Sutherland, 1983; Campbell, 2008; Mills, 2008; Daniel et al., 2009; Bates et al., 2012; De Roma et al., 2017; Botelho et al., 2020; Teichmann-Knorrn et al., 2020). As metaldehyde was withdrawn from use due to 'the unacceptable risk metaldehyde pellets to birds and mammals…' it is thought that much of the evidence that exists to support non-target effects of these groups remains unpublished (Department for Environment & Affairs, 2020).

Invertebrates

Earthworms are recognised as critical indicators of soil quality and essential to nutrient cycling, soil formation and health (Syers et al., 1979; Aira & Domínguez, 2011). They were shown to deplete the proportion of molluscicide in the field available for slug control up to 17% (Edwards et al., 2009; Jovana et al., 2014). However, similar work presented contradicting results, where highly active earthworms had no effect on molluscicidal activity (Gavin et al., 2012; Dörler et al., 2019). Metaldehyde pellets (4%) did not affect activity, growth or mortality of earthworms, even when applied at doses greater than four and eight times the recommended agricultural dose (Langan & Shaw, 2006; Edwards et al., 2009; Jovana et al., 2014). However, ferric phosphate pellets containing chelating agents may be more toxic to earthworms (Langan & Shaw, 2006; Edwards et al., 2009). Although iron phosphate alone did not impact earthworm health measures, EDTA and ethylenediamine-N, N'-disuccinic acid (EDDS), another chelating agent, were found to be less palatable and caused weight loss (Edwards et al. 2009).

Consumption of ferric phosphate pellets *per se* has been found to induce increased earthworm mortality, weight loss, reduced growth, surface activity and feeding (Langan and Shaw 2006).

As carabid beetles predate on slugs, they may feed on slugs that are poisoned completely or sub-lethally by metaldehyde. *Pterostichus madidus* was observed in laboratory conditions to feed preferentially on slugs that had fed on a molluscicide (be they dead or alive), possibly due to the excessive mucus production resulting from ingesting the molluscicide (Langan et al., 2001). This preference was not observed for *Pterostichus melanarius* exposed to slugs that had fed on methiocarb pellets (Langan et al., 2004).

1.4.7 Integrated Pest Management

Integrated control or integrated pest management (IPM) should utilise all available techniques to maintain pest population levels below thresholds so that minimum economic injury is incurred (Smith, 1967). Most current strategies for protecting crops from slug damage involve combinations of both cultural and chemical control methods, for example see Glen (2002) and Glen et al. (1992). However, no single approach is completely effective. Due to differences in environmental conditions and slug populations a method that may work in a drier region (which may vary with factor such as soil type, weather, time of year) may not work in the same region when the conditions create are more humid (Glen et al., 1989; Wareing & Bailey, 1989).

Integrated pest management strategies generally suggest that slug control should be deployed when the slug population exceeds a critical threshold as a curative measure, not as a pre-emptive (preventative) measure (Hammond, 2004; Forbes, M. Back, et al., 2020; Scaccini et al., 2020). However, growers tend to be reluctant to adopt this approach due to the risks of financial loss and the complexity of predicting highly localised slug populations and the environmental conditions that facilitate slug activity. In the UK, IPM is required for professional users and is recommended for home growers (‘Get Pelletwise!’, 2021; ‘RHS project: Integrated gastropod management / RHS Gardening’, 2018).

Several gaps in knowledge are identified and discussed in sections 1.1 to 1.4. The aims listed below and in each subsequent chapter were developed to provide better understanding on these aspects.

1.5 Thesis Outline

The overall aim of this project was:

1. Investigate aspects slug physiological and behavioural response to, and commercial potential of, novel metaldehyde formulations

This overall aim was supported by addressing objectives in each chapter as follows:

Chapter 2

1. Develop a standardised bioassay methodology to test the acceptability and palatability of any pellet or formulation to *D. reticulatum* or any other slug species
2. Provide data on slug consumption of, and associated survival after ingesting, novel metaldehyde powder 1, novel control powder 1, novel metaldehyde powder 2, novel non-toxic control pellet, novel metaldehyde pellet
3. Compare novel formulations to current commercially available products

Chapter 3

1. Develop a standardised laboratory-based bioacoustics methodology to test the consumption of any pellet or formulation to *D. reticulatum* or any other slug species
2. Provide data on slug acceptability and consumption of pellets in relation to the length of bites, number of bites, and general patterns of feeding
3. Compare novel pellet formulations to current commercially available products
4. Investigate whether the environment in laboratory trials had an impact on the results of experiments, also exploring if it would be possible to use this methodology under field conditions

Chapter 4

1. Compare leaching of novel pellet formulations to current commercially available products

Chapter 5

1. Investigate trends in the use of metaldehyde on arable crops in the UK from 2015-2016.
2. Discuss developmental costs of a new pellet, focusing on novel metaldehyde formulations
3. Discuss overall thesis findings in concluding remarks

Chapter 2: Acceptability and Palatability of Experimental and Commercial Pellets

2.1 Introduction

Chemoreception plays a role in a variety of behavioural processes in molluscs, including finding food, response to food, reproduction and homing behaviour (Gelperin, 1974; Croll, 1983; Livshits & Fishelson, 1983; Chelazzi, 1990; Dodds et al., 1999; Baker et al., 2012). However, the events that follow from a slug finding food to consumption are not completely understood. Most literature agrees that under pesticide and predator-free conditions *D. reticulatum* forage randomly (Bailey, 1989; Bailey & Wedgwood, 1991; Howling, 1991). It is not thought that this species has the physiological features that could enable them to seek out food from a long distance, and there is no evidence to date that slugs are able to do this – refer section 2.1.2 for further discussion. Nevertheless, slugs may use chemical or olfactory cues left by predators to avoid them and, at a short distance, find food sources (Pickett & Stephenson, 1980; Bailey & Wedgwood, 1991; Dodds et al., 1997, 1999; Birkett et al., 2004). Once a slug has come across a potential food substance, it is suggested that slugs take a few bites to determine if the substance is a food source of value (Bailey & Wedgwood, 1991). If suitable, the slug may continue feeding.

The production of secondary metabolites (secondary plant compounds) by plants is well documented (Erb & Kliebenstein, 2020). These play a role in defence against herbivory (Bourgaud et al., 2001; Makkar et al., 2007). These compounds may initiate a positive or negative feeding response (Barone & Frank, 1999; Aguiar & Wink, 2005). As many molluscicides are dependent on consumption of molluscicide for function, compounds affecting consumption in slugs are suspected to be well investigated by manufacturers. However, due to commercial interests and confidentiality, manufacturers publish little information. Many laboratory-developed and naturally occurring compounds have been screened against slugs to gauge feeding response (Clark et al., 1997; Devlin, 1997; Cordoba et al., 2018).

2.1.1 Chemical and olfactory cues

Chemical cues triggering behavioural responses in marine molluscs are well documented. While the physiology of marine and terrestrial molluscs may be similar in some respects, due to differences in the medium by which the chemical cues are transmitted only terrestrial gastropods will be discussed in this chapter. For further information on feeding preferences in marine molluscs see Sakata (1989), and see Faulkner (2019) for chemical defence. Most terrestrial gastropods possess two pairs of tentacles that are associated with feeding and orientation. In *D. reticulatum,* while not completely understood, it is suggested that both tentacles play a role in reception of chemical cues, where the posterior tentacles are the main organ responsible for olfaction (Chase, 1982, 1986; Chase & Tolloczko, 1993; Dodds et al., 1997). For further information on general sensory organs in molluscs see Chapter 3, The biology of terrestrial molluscs (Barker, 2001) and for olfaction in *D. reticulatum* see Garraway (1992).

Terrestrial slugs have been observed to consume less, move more, move faster and move away from areas where predatory beetles were previously present (McKemey et al., 2004; Armsworth et al., 2005; Thomas et al., 2008; Bursztyka et al., 2016; Ferrante et al., 2017). Slugs have been observed to detect cues in the extracts of predatory beetles and respond with anti-predatory behaviour, but it is not fully understood how they detect and respond, nor if the behaviour is generalised to all predatory beetles, or only those species used experimentally. Airborne volatiles from the *Pterostichus melanarius* beetle have been observed to cause activity in the olfactory nerve, suggesting olfaction may play a part in predator detection (Dodds et al., 1997).

Olfactory based investigations can be used discover or test the suitability of new compounds that could be used to improve the consumption of molluscicides (Ballhorn & Kautz, 2013; Cordoba et al., 2018). Most use two-choice olfactometers that consist of a stem and two arms in a Y or T shape. Each arm usually carries a different odour, normally the test odour and control (clean) air. The animal is placed in the tube before the bifurcation and can select which arm to move down. Cordoba et al (2018) present a high throughput method for screening compounds. However, olfactory decisions made in experimental settings, where the cues are artificially high, may not reflect field conditions and behaviour (Ballhorn & Kautz, 2013). Comparing the results of olfactory experiments with the results of bioassay and field trials may yield more conclusive information (Dodds et al., 1999; Birkett et al., 2004). For a review on the usefulness

of olfactory investigations see Ballhorn and Kautz (2013) and for further detail on olfactory cues used for feeding in terrestrial gastropods see Kiss (2017).

2.1.2 Attractants

The attractiveness of the food source determines if the animal approaches the food source and if an initial bite is taken. Many molluscicide pellets claim to contain attractants that increase the chances of the slug moving towards the pellet. An **attractant** (that can cause attractiveness), in this thesis, is defined as a substance that would encourage the slug to move towards the food source, more so than a food source that did not contain that attractant (Cordoba et al., 2018). There is little evidence on defining a distance at which slugs may be able to detect food sources (Tiwari & Singh, 2004; Dahirel et al., 2015; Veasey et al., 2021). The brown garden snail, (*Cornu aspersum* (Müller, 1774)), showed olfactory perception at distances between 20 and 40cm from an odour source, suggesting that the range of olfactory perception may vary considerably between species (Dahirel et al., 2015). Repellent properties would decrease the attractiveness of a food substance (Hollingsworth et al., 2002, 2003; Schüder et al., 2003)

2.1.3 Repellents and deterrents

Seed coatings, copper tape and other physiochemical barrier methods are often described as having repellent properties to slugs (Schüder et al., 2003; Ingo Schüder et al., 2004; Capinera, 2018). True **repellent** properties would cause the slug to move away from the source before physical contact was made. Substances causing the slug to move away after physical contact has been made are referred to, in this thesis, as **deterrents**. Repellent and deterrent properties have been reported in a variety of materials, including garlic, caffeine, hydrated lime and selected essential oils (Hollingsworth et al., 2002; Schüder et al., 2003; Capinera, 2018). It is possible that historical contact with repellents and deterrents may alter future feeding preferences in molluscs (Gelperin, 1975; Kasai et al., 2006). However, it has not been established if artificially induced learning of aversion is comparable to mollusc behaviour in the field. Additionally, it is important to consider the effects of dose-dependency; compounds at lower concentrations may elicit an attractant response but could, with repeated expose or higher doses, elicit repellent or deterrent responses (Garraway, 1992). Repellent and deterrent properties have also been reported in commonly used chemical controls, including metaldehyde

(Bourne et al., 1990; Hollingsworth et al., 2002; Schüder et al., 2003). Chemicals that may be toxic to slugs, and so be an effective molluscicide if consumed, but have strong repellent or deterrent properties (that cannot be overcome) would not be useful as a molluscicide. A pellet with repellent or deterrent properties could still be effective if an efficacious and fast-acting arrestant is present.

2.1.4 Arrestant, antifeedant, and suppressants

An **arrestant** is a substance that physiologically prevents movement away from the pellet, usually by paralysing the mollusc. Metaldehyde is a well-documented arrestant, with slugs that have consumed sufficient metaldehyde observed to be paralysed in contact with or close to the pellet (Bourne et al., 1990). In a pellet that requires continued consumption, if arrestment properties have an early onset, sublethal poisoning may occur and the slug may subsequently recover (Campbell et al., 2021). However, arrestant properties may be beneficial if a sufficiently poisoned slug that is paralysed in contact with the pellet continues to receive the AI due to contact toxicity (Henderson & Martin, 1990; Abobakr et al., 2021). Food that contains an arrestant could be misinterpreted as encouraging increased feeding instead of immobility. Refer to Chapter 3 for discussion of the reliability of using meal length (determined by time at food source) as a proxy for the amount consumed. A **suppressant** is a substance that causes physiological feeding inhibition in slugs. These are not well documented in the literature, possibly due to the difficulties in distinguishing repellent, deterrent or antifeedant properties (Frank & Friedli, 1999). See Tierney (2020) for a review on possible appetite suppressants in invertebrates, including molluscs.

Barrier control methods commonly include **antifeedant** properties, that deter further consumption after the initial feeding response (Dodds et al., 1999; Schüder et al., 2003; Birkett et al., 2004; Farias et al., 2020; Zolovs et al., 2020). Seed coatings containing cinnamamide are known to promote antifeedant behaviour from slugs (Watkins et al., 1996; Simms et al., 2002; Schüder et al., 2003 and Schüder et al., 2004). Several naturally occurring compounds (such as volatiles from coriander and parsley) have been identified through screening as having antifeedant properties, but none are widely used or depended upon as slug control products, especially when compared to chemical molluscicides (Airey et al., 1989; Garraway, 1992; Clark et al., 1997; Birkett et al., 2004; Farias et al., 2020; Zolovs et al., 2020). Arrestants, suppressants and antifeedants must be consumed to function and do not affect the slug from a distance.

2.1.5 Incitants, stimulants

Once the slug makes physical contact with the pellet, any substance that initiates initial feeding response (tasting) is defined as an **incitant** (Sakata, 1989; Egonmwan, 1992). In the literature, concerning *D. reticulatum,* there is no clear distinction made between incitant and attractant substances or properties. A single substance could act as an attractant from a distance and initiate a feeding response when in contact. However, a substance that is an attractant from a short distance, could also have repellent properties when in close contact with a pellet – this is particularly common with substances in higher concentrations (Cragg & Vincent, 1952; Wedgwood & Bailey, 1988; Campbell et al., 2021). Once a slug has begun feeding, a **stimulant** may promote the feeding response and increase consumption (more than a food without it) after feeding initiation (Senseman, 1977; Godan & Others, 1983; Howling, 1991). As above, the same substance may act as a stimulant, an incitant and, simultaneously, an attractant. Starch has been suggested as a stimulant for the banana slug, *Ariolimax californicus,* due to longer feeding being observed on diets containing starch (Senseman, 1977). Table 2.1 shows further examples of foods and substances investigated for feeding response.

Table 2.1. Studies investigating behavioural or feeding response in slug species to various compounds as reported in published papers

Species	Potential compounds tested	Author
Lymnaea acuminata	Glucose, sucrose, maltose, starch, citrulline, tryptophan, proline, serine	Agrahari & Singh, 2010
Theba pisana, Cernuella virgata, Cochlicella acuta	Lettuce, carrot, celery, d-serine, alginic acid, glucose powder, strawberry, pineapple, shitake mushroom, rye meal, rice bran oil, olive oil, peanut oil, avocado oil, canola oil, corn flour, barley seeds, mung bean seed, durum wheat flour, molluscicide pellets (metaldehyde), stale beer	Baker et al., 2012
Deroceras reticulatum	Extracts of volatiles from C*onium maculatum, Coriandrum sativum, Petroselinum crispum* (mill)	Birkett et al., 2004
Euconolus fulvus, Nesovitrea occidenalis, Zonitoides arboreus, Vitrina alaskana, Discus cronkhite,Vertigo gouldi	Faeces of bighorn sheep (fresh moist, fresh sun dried, weathered faeces)	Boag, 1983
Deroceras reticulatum	Cuticular extracts from: *Carabus auratus, Carabus hispanus, Carabus nemoralis, Carabus coriaceus, Musca domestica*	Bursztyka et al., 2013
Cernuella virgata, Helix aspersa, Cepaea nemoralis, Arion Ater	Faeces (solid or dissolved in plant extract) from: domestic sheep, domestic goats, domestic cows, domestic horses	Cabaret & Vendroux, 1986
Deroceras reticulatum	methanol extracts, anise, chinese cabbage, liquorice basil, mexican tea plant, oregano, peppermint, rosemary, sweet basil, tarragon, hexane extracts, hemlock extract, geraniol (1.5%), (+)-fenchone (1.5%), (+)-fenchone (0.5%), (-)-fenchone (1.5%), (-)-fenchone (0.5%)	Clark et al., 1997
Deroceras reticulatum	Chopped aubergine, chopped decomposing citrus fruit, chopped tomato, glyphosate treated plants, grass seedlings, plantain lily, tetramin fish food, cucumber seed oil, papaya flavoured oil, sun luck toasted sesame oil, cat food distillate, Guinness stout, marmite, propylene glycol 25%, slug saloon bait, cucumber juice, chopped cucumber, chopped carrot	Cordoba et al., 2011

Xerolenta obvia	Fresh chopped cucumber	Cordoba et al., 2020
Cornu aspersum	Nettle leaves, repulsive plant leaves, blend of nettle leaves and repulsive plant leaves, repulsive plant leaves: common borage, garden chervil, nastrutium devil in the bush, pot marigold, alyssum, french marigold, zinnia, yarrow, giant fennel, hyssop, white clover, red amaranth	Dahirel et al., 2015
Deroceras reticulatum	*Petroselinum crispum, Pastinaca sativa, Coriandrum sativum, Chaerophyllum temulentum, Anthriscus cerefolium, Conium maculatum, Sium latifolium, Smyrnium olusatrum, Crithmum maritimum, Myrrhis odorata, Sison amomum, Bupleurum fruticosum, Angelica sylvestris, Berula erecta, Apium graveolens, Falcaria vulgaris, Aegopodium podagraria, Seseli libanotis, Peucedanum officinale, Anthriscus sylvestris (cow parsley), Oenanthe lachenalia, Heracleum sphondylium, Daucus carota, Pimpinella major, Hydrocotyle vulgaris, Scandix pecten-veneris, Ligusticum scoticum, Carum carvi physospermum cornubiense, Torilis japonica, Atheusa cynapium, Silaum silaus, Selinum carvifolia*	Dodds et al., 1999
Arion lusitanicus	Bulldock 25 ec, Karate-Zeon 050 cs, Decis 2.5 ec, Sumi-Alpha 050 ec, Fastac 100 ec, Talstar 100 ec, Nomolt 150 ec, Mospilan 20 sp, Admiral 100 ec, Diazol 500 ew	Piechowicz & Stawarczyk, 2012
Lissachatina fulica	Synthetic compounds of papaya oil (22)	Roda et al., 2019
Achatina fulica	shredded carrots, shredded cucumber, lettuce	Croll & Chase, 1977
Deroceras reticulatum	Beer (fresh), beer (stale), grape juice (unfermented), drosophila fermented bait, wine (dry grape), wine (blackberry), ethyl alcohol, methyl alcohol, vinegar 10%, dimalt 5%, metaldehyde 4% in corn cobs with beer, metaldehyde 4% in corn cobs with water, water	Smith & Boswell, 1970
Lymnaea acuminata	Glucose, fructose, sucrose, maltose, starch	Tiwari & Singh, 2004
Deroceras reticulatum	Cinnamamide, 3,5-dimethoxycinnamic acid	Watkins et al., 1996

Arion vulgaris	liquid extracts of C*ladonia rangiferina, Cladonia stellaris, Pseudevernia furfuracea*	Zolovs et al., 2020

2.1.6 Slug collection and maintenance

The overall aim of the thesis was to provide Lucideon with feedback on novel metaldehyde formulations for product optimisation (see sections 1.1 and 5.1). The main function of the novel formulations was to slow the release of metaldehyde into water courses during rain events to prevent fast leaching (that results in large amounts of metaldehyde in the water), but the encapsulation material was also sensitive to 'harsh' environments, including acute ion concentrations and pH values.

The novel formulations aimed to:

1. Prevent slugs from detecting the repellent properties of metaldehyde, resulting in early meal termination
2. Prevent slugs from succumbing to early paralysis, also resulting in early meal termination
3. Allow slugs to consume enough novel pellet to be killed or seriously paralysed

These aims were supported by the objectives of this chapter below:

1. Develop a standardised bioassay methodology to test the acceptability and palatability of any pellet or formulation to *D. reticulatum* or any other slug species

2. Provide data on slug consumption of, and associated survival after ingesting, novel metaldehyde powder 1, novel control powder 1, novel metaldehyde powder 2, novel non-toxic control pellet, novel metaldehyde pellet

3. Compare novel formulations to current commercially available products

Multiple small-scale experiments were carried out to achieve objective 1, resulting in the methodology described below. The experiments did not take place simultaneously, but consecutively, allowing time between for Lucideon to develop new formulations. For this reason, each experiment is presented with an independent Results and Discussion section, with a unified discussion at the end of the chapter.

2.2 Materials and Methods

2.2.1 Slug collection and maintenance

Slugs were collected manually from chicken feed baited traps, from a variety of field sites around Newcastle upon Tyne (United Kingdom), approximately 36 hours before experimental feeding was anticipated. Slugs were stored in plastic containers prior to use, lined with damp tissue paper to maintain a high humidity level, in a refrigerator (3–5 °C). The time spent in storage varied from 0-48 hours. Slugs were able to feed freely on carrots from the time of collection until 24 hours before the feeding trial. Preliminary experiments[4] did not suggest that storage before bioassay affected consumption.

Twenty-four hours before the feeding trial, each slug was transferred to an identifiable nine cm Petri dishes lined with damp filter paper. Slugs were allocated so that each treatment had a similar distribution of sizes using visual judgment. Stacked Petri dishes were sealed in plastic sleeves to maintain humidity levels. Petri dishes were housed in a controlled temperature room at 15°C with a day:night cycle of 12:12 for 24 hours. Powder formulations were mixed 0-12 hours before feeding and were refrigerated until use (3–5 °C)[5]. Pre-formed pellets were allowed to take up moisture from a damp filter paper at 15°C for 24 hours prior to use. This pellet pre-treatment was undertaken as pre-formed pellets are hard and unpalatable to slugs if they are not allowed to take up moisture; pellets applied in the field take up moisture from the soil.

Animals were weighed individually immediately before the feeding trial. A single food pellet was added to the centre of each dish. During the feeding trial Petri dishes were kept in a controlled temperature room under the conditions described above. The condition (dead/ alive) of the slug and a visual estimate of pellet consumption on a scale from 1-10 at predetermined hours were recorded. If two experimenters were present, the consumption scale was mutually agreed[6].

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[4] used methodology as described in 2.2.1 with the variation of time between collection and bioassay (storage). These did not find a difference in consumption. Pellets used were commercial metaldehyde pellet and non-toxic control pellet (see table 2.2 for pellet composition).

[5] Powder formulations were mixed with measured amounts of water or oil as discussed with manufacturers

[6] This work was part of a larger project and discussed in other published work, any collaborative work is noted

In the following chapter, the mention of “24”, “96”, “144” or any other standalone hour will refer to the time at which recording was undertaken. The mention of “0-24”, “24-96”, “96-144” or any other combination of hours refers to the time passed between mentioned hours.

2.2.2 Formulations

Novel control powders (novel metaldehyde powder 1, novel metaldehyde powder 2) used encapsulation technology - Inorganic Controlled Release Technology (iCRT) where metaldehyde was incorporated into a silica matrix as a powder ('Inorganic Controlled Release Technology - iCRT', n.d.). Novel control powder 1 was the same chemical base formulation as novel metaldehyde powder 1, but did not include the metaldehyde, nor go through the chemical process to incorporate the metaldehyde. Novel metaldehyde powder 2 used the same chemical formulation, but a different technique to incorporate the metaldehyde. Novel metaldehyde pellets and non-toxic control pellets used a silica matrix to coat pellets and an Axcela ® metaldehyde and Axcela ® cereal pellets respectively (Table 2.2). Axcela ® metaldehyde pellets are commercially available baits containing META™ metaldehyde manufactured by Lonza. Axcela ® cereal were produced by Lonza for this project as a control of Axcela ® metaldehyde. Axcela ® metaldehyde and Axcela ® cereal pellets contain the same ingredients except that Axcela ® cereal pellets do not contain META™ metaldehyde.

Table 2.2 Text reference, formulation name, novel powder percentage (w/w)%, AI w/w%, hours recorded at, number of observers for pellets used in bioassay experiments A, B, C, D, E, F and G

Experiment	Text reference	Formulation	Novel powder w/w %	AI w/w %	Hours	Observers
A	Novel control powder 1 Novel metaldehyde powder 1 Laboratory metaldehyde 1% Laboratory control	iCRT control 1 iCRT metaldehyde 1 --- ---	20.8 20.8 0 0	0 1 1 0	24, 96	2
B	Novel metaldehyde powder 2 5% Novel metaldehyde powder 2 20% Laboratory metaldehyde 0.28% Novel metaldehyde powder 1 20%	iCRT control 2 5% iCRT metaldehyde 2 20% --- Silica matrix metaldehyde 1 20%	5 20 0 20	0.07 0.28 0.28 0	24, 96	2
C	Commercial metaldehyde pellet Novel metaldehyde pellet Non-toxic control pellet	Axcela ® metaldehyde iCRT coated Axcela cereal Axcela ® cereal	0 N/A N/A	3 3 0	24, 96	2
D	Novel control pellet Laboratory control	iCRT coated Axcela cereal ---	N/A N/A	0 0	24, 96	1
E	Commercial ferric phosphate pellet Chicken feed	Sluxx HP ® ---	N/A N/A	2.97 0	24, 96, 144	1
F	Commercial ferric phosphate pellet Non-toxic control	Sluxx HP ® carrot	N/A N/A	2.97 0	24, 96, 144	1
G	Commercial ferric phosphate pellet Commercial metaldehyde pellet Non-toxic control	Sluxx HP ® Axcela ® metaldehyde carrot	N/A N/A N/A	2.97 3	24, 96, 144	1

2.2.3 Statistical analysis

All statistical analysis was conducted using IBM SPSS Statistics 26.0.

Weight of slugs

To determine if there was a difference between the mean weights of slugs between treatment groups one-way ANOVAs were used. Outliers were measured by the inspection of a boxplot. Groups were checked for normality and homogeneity of variances using the Shapiro-Wilk ($p > 0.05$) and Levene's Test ($p > 0.05$). If normality and homogeneity of variances were not established, Kruskal-Wallis tests were performed instead. Data is presented as mean ± standard deviation. For one-way ANOVA analyses that showed statistical significance a Tukey HSD Test was used to identify significant differences between pairs of means. For Kruskal-Wallis tests, pairwise comparisons were performed using Dunn's (1964) procedure with a Bonferroni correction for multiple comparisons. Adjusted p-values are presented. Slugs that died between 0 - 24 hours were removed from the analysis of 24 – 96 hours and 96 – 144 hours. Slugs that died in 24 – 96 hours were removed from 96 – 144 hours analyses.

Consumption

Mann-Whitney U tests were conducted to determine if there were differences in consumption between two treatment groups, if there were three or more treatment groups Kruskal-Wallis tests were conducted. For Kruskal-Wallis tests that showed significance, pairwise comparisons were performed using Dunn's (1964) procedure with a Bonferroni correction for multiple comparisons. Adjusted p-values are presented. Distributions of consumption was assessed by visual inspection of a boxplot. Slugs that died between 0 - 24 hours were removed from the analysis of 24 – 96 hours and 96 – 144 hours. Slugs that died in 24 – 96 hours were removed from 96 – 144 hours analyses.

Survival

Chi-square tests of homogeneity or Fisher's exact test (2 x c) were used to investigate survival. Chi-square tests of homogeneity were used when all cells of the 2 x c table had an expected count greater than or equal to five. If not, Fisher's exact test (2 x c) was used. For analyses that showed statistical significance, pairwise comparisons were conducted using multiple Fisher's exact tests (2 x 2) with a Bonferroni correction.

2.3 Experiment 2A: Is there a Difference in Consumption and Survival Between Novel Metaldehyde Powder 1 and Novel Control Powder 1?

2.3.1 Overview

Professional metaldehyde pellets available in the UK generally contain 3-4% AI (see Chapter 5). Metaldehyde formulations in 2A were made up to contain 1% active ingredient. If slugs were unable to detect or less deterred by the lower concentration of metaldehyde (compared to commercially available pellets), they would consume more pellet (and more metaldehyde) than usually consumed with currently available pellets. In a field setting this would mean that less metaldehyde would be applied to the field and be available to leach into the water system. The methods used in 2A are as described in section 2.2.1, 2.2.2 and 2.2.3.

2.3.2 Results

Distribution of slug weight

There were statistically significant differences between groups ($F_{(3,129)}= 5.121$, $p < 0.01$), the weight distribution of slugs in novel metaldehyde powder 1 and novel control powder 1 were different to those in the laboratory control (Figure 2.1). There were significant differences between novel control powder 1 and laboratory control, as well as novel metaldehyde powder 1 and laboratory control.

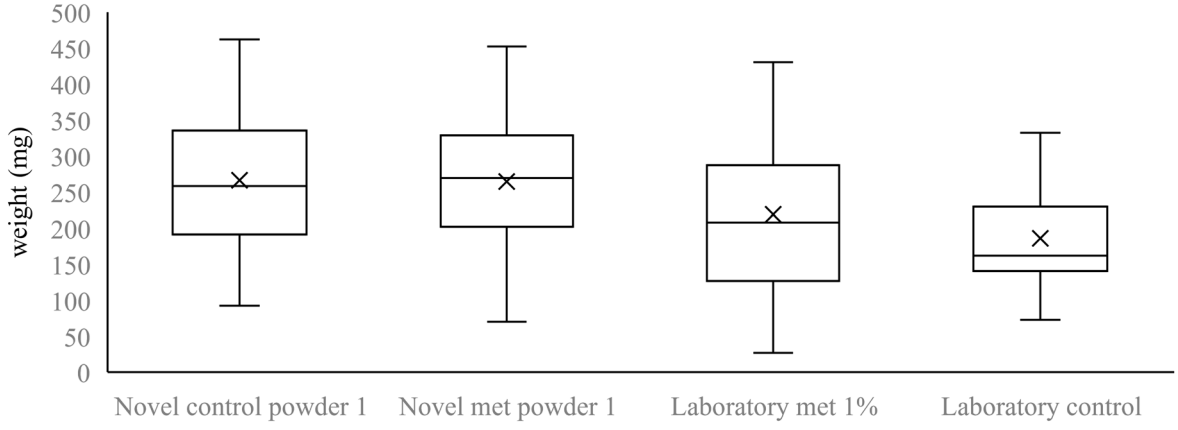

Figure 2.1 Distribution of slug weight (mg) in treatments before feeding; novel control powder 1 (n = 33), novel metaldehyde powder 1 (n = 33), laboratory metaldehyde 1% (n = 32) and laboratory control (n = 31). The box indicates the upper and lower quartiles, the whiskers indicate the range of data outside the upper and lower quartiles. The black cross within the box indicates the mean. Circles and crosses outside of the box show outlying values.

Differences in consumption between pellet types (0 - 96 hours)

Consumption was statistically significantly different between the different treatment groups, $\chi^2(3) = 67.421$, $p < 0.001$ (Figure 2.2). There were significant differences between the pellet types; novel control powder 1 and novel metaldehyde powder 1, novel control powder 1 and laboratory metaldehyde 1%, novel metaldehyde powder 1 and laboratory control, laboratory metaldehyde 1% and laboratory control.

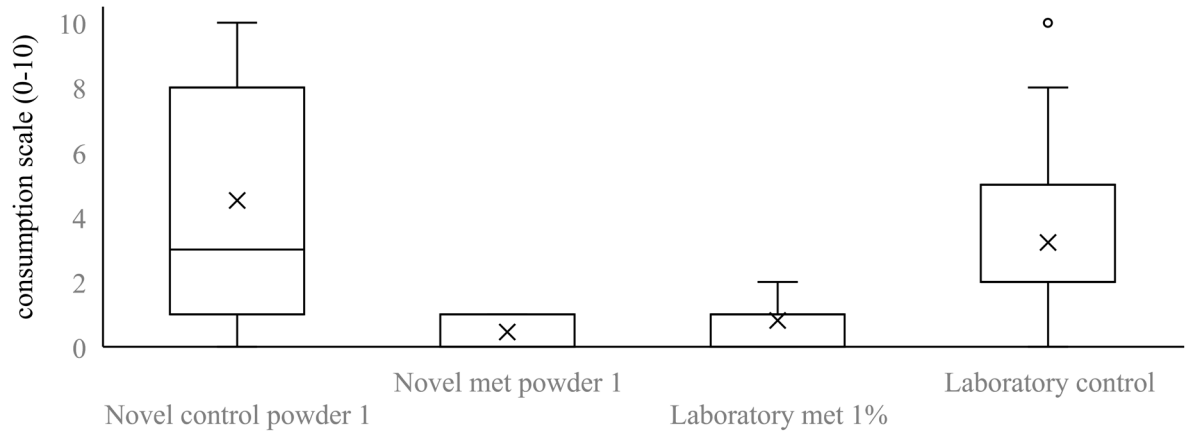

Figure 2.2 Consumption on scale of 0 – 10 (0 = none consumed, 10 = all consumed) of novel non-toxic powder 1 (n = 33), novel metaldehyde powder 1 (n = 33), laboratory metaldehyde 1% (n = 32) and laboratory control (n = 31) after 96 hours. The box indicates the upper and lower quartiles, the whiskers indicate the range of data outside the upper and lower quartiles. The black cross within the box indicates the mean. Circles and crosses outside of the box show outlying values.

Differences in survival between pellet types (0 - 96 hours)

There was a difference in the survival percentage of slugs between pellet types at 0 – 96h, as assessed by Fisher's exact test, $p > 0.05$ (Figure 2.3). There was a significant difference between novel control powder 1 and laboratory metaldehyde 1%, novel control powder 1 and laboratory control, novel metaldehyde powder 1 and laboratory metaldehyde 1%, novel metaldehyde powder 1 and laboratory control as well as laboratory metaldehyde 1% and laboratory control.

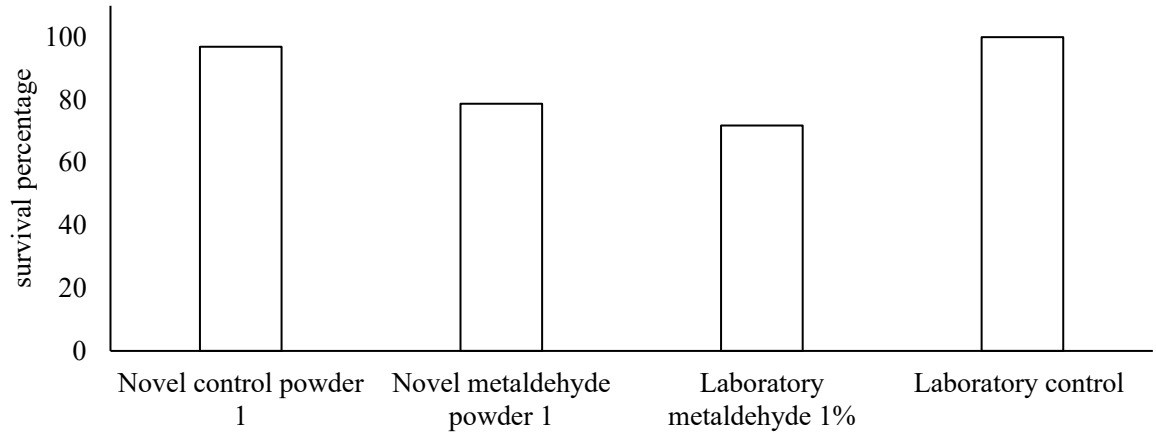

Figure 2.3 Survival percentage novel non-toxic powder 1 (n = 33), novel metaldehyde powder 1 (n = 33), metaldehyde 1% (n = 32) and laboratory control (n = 31) after 96 hours in bioassay trial.

2.3.3 Summary

Consumption

Slugs in treatment groups with metaldehyde consumed less food than those in groups without metaldehyde. There was little difference in the consumption and mortality between the two metaldehyde-based treatments. Slugs were able to detect the metaldehyde in the novel metaldehyde powder 1, despite using novel technology, and showed reduced feeding. As there was no difference between the novel non-toxic powder 1 and the laboratory control, it could be assumed that slugs find the novel product, before the incorporation of metaldehyde, acceptable. Reduced feeding on the novel metaldehyde powder 1, may be due adverse chemical cues from the metaldehyde resulting in sub-lethal consumption, or a product resulting from the interaction of metaldehyde with the novel product.

Survival rate

No statistical difference was observed between survival rates in novel metaldehyde powder 1 and laboratory metaldehyde 1%, which suggests it may not be competitively better as a molluscicide; further supported by there being no difference in survival between the novel control powder 1 and novel metaldehyde powder 1. Mortalities in the novel control powder

pellet 1 may be attributed to random causes and were not different from random mortality observed in preliminary experiments.

Weight

While statistically significant differences were observed between the mean weights of groups, it is thought that this had little effect on the resulting consumption and mortality. If it is suggested that larger slugs, as seen in both novel groups, consumed more than smaller slugs - this was not supported by the results of the laboratory control where smaller slugs consumed more overall.

2.3.4 Conclusion

No benefit to slug control using novel technology was observed in this experiment.

2.4 Experiment 2B: Is there a Difference in Consumption and Survival between Different Concentrations of Novel Metaldehyde Powder 2?

2.4.1 Overview

Due to the limited consumption of novel metaldehyde powder 1 in experiment 2A, a variation of the novel powder was produced and used in experiment 2B at the request of the funder. Although not ideal, novel control powder 1 acted as a control due to similarity in formulation and to minimise costs. A control for novel metaldehyde powder 2 could have been produced for further work if the results of experiment 2B were encouraging. The methods used in 2B are as described in section 2.2.1, 2.2.2 and 2.2.3. Details on pellet composition can be referred to in Table 2.2.

2.4.2 Results

Distribution of slug weight

There was no statistically significant differences between groups, $F_{(3,120)}= 0.910$, $p = 0.439$ (Figure 2.4).

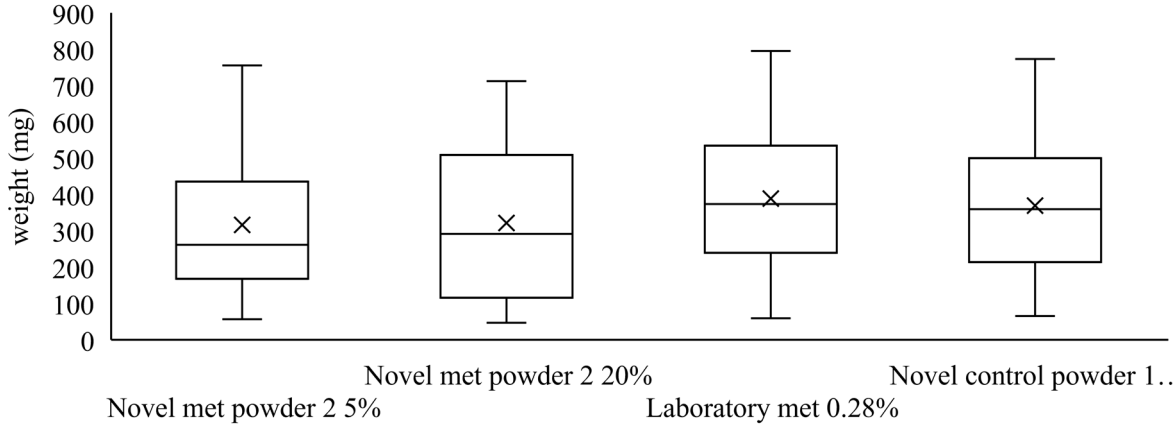

Figure 2.4 Distribution of slug weight (mg) for each treatment before feeding, novel metaldehyde powder 2 5% (n = 30), novel metaldehyde powder 2 20% (n = 30), metaldehyde 0.28% (n = 30), novel control powder 1 (n = 30). The box indicates the upper and lower quartiles, the whiskers indicate the range of data outside the upper and lower quartiles. The black cross within the box indicates the mean. Circles and crosses outside of the box show outlying values.

Differences in consumption between pellet types (0 - 24 hours)

Distribution of consumption levels were similar for all groups, as assessed by visual inspection of a boxplot. Consumption was not significantly different between the different pellet groups, $\chi^2(3) = 6.790$, $p = 0.079$ (Figure 2.5)

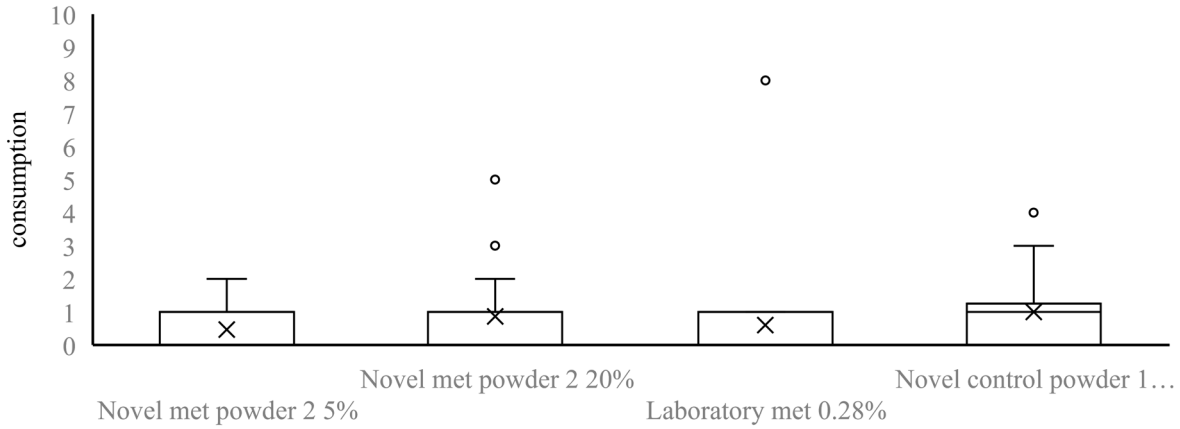

Figure 2.5 Consumption on scale of 0 – 10 (0 = none consumed, 10 = all consumed) of each treatment before feeding, novel metaldehyde powder 2 5% (n = 30), novel metaldehyde powder 2 20% (n = 30), metaldehyde 0.28% (n = 30), novel control powder 1(n = 30) at 24 hours. The box indicates the upper and lower quartiles, the whiskers indicate the range of data outside the upper and lower quartiles. The black cross within the box indicates the mean. Circles and crosses outside of the box show outlying values.

Differences in consumption between pellet types (24 - 96 hours)

Distribution of consumption levels were similar for all groups, as assessed by visual inspection of a boxplot. Consumption was significantly different between the different pellet groups, $\chi^2(3) = 11.5$, $p < 0.05$ (Figure 2.6). Differences were found between novel metaldehyde powder 2 20% and novel control powder 1 20%.

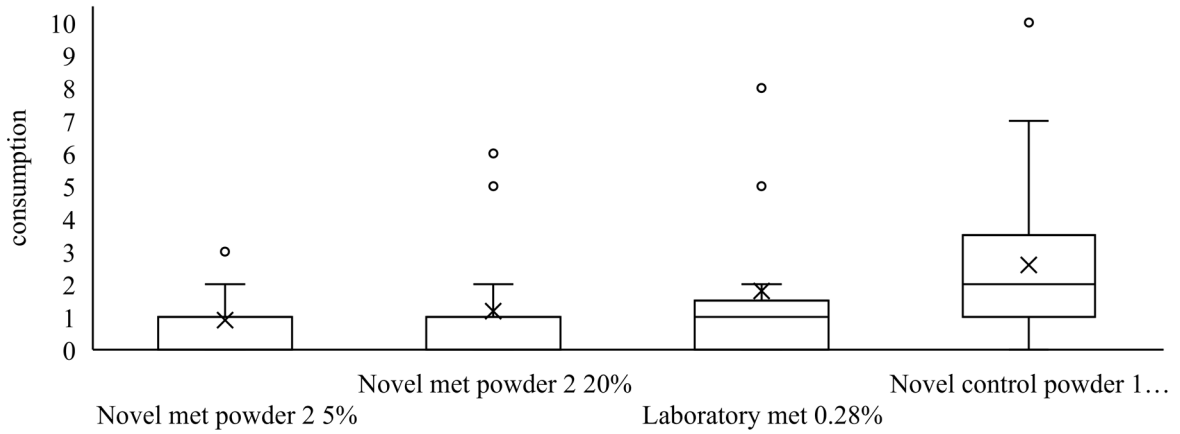

Figure 2.6 Consumption on scale of 0 – 10 (0 = none consumed, 10 = all consumed) of each treatment before feeding, novel metaldehyde powder 2 5% (n = 30), novel metaldehyde powder 2 20% (n = 28), metaldehyde 0.28% (n = 25), novel control powder 1(n = 29) at 96 hours. The box indicates the upper and lower quartiles, the whiskers indicate the range of data outside the upper and lower quartiles. The black cross within the box indicates the mean. Circles and crosses outside of the box show outlying values.

Differences in survival between pellet types (0 - 24)

There was no difference in the survival percentage of slugs between pellet types at 0 – 24 hours, as assessed by Fisher's exact test, $p = 0.079$, (Figure 2.7).

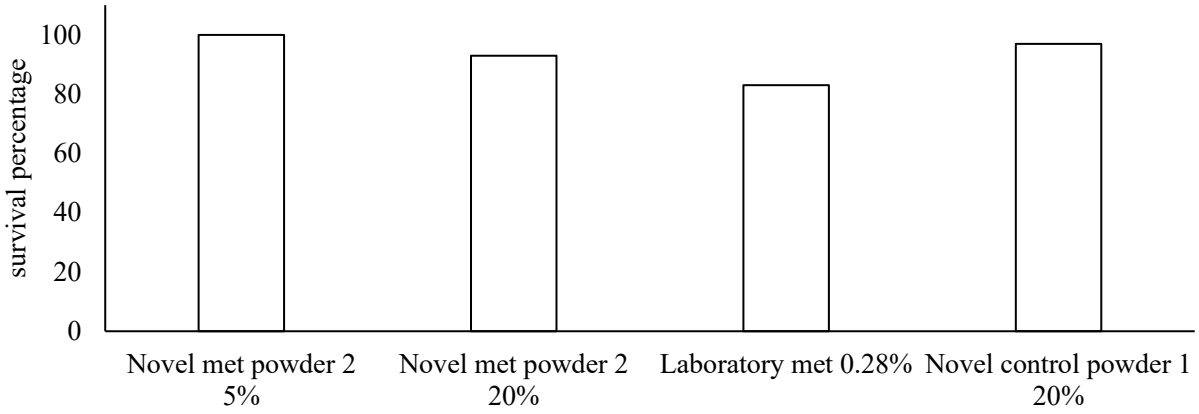

Figure 2.7 Survival percentage novel metaldehyde powder 2 5% (n = 30), novel metaldehyde powder 2 20% (n = 30), metaldehyde 0.28% (n = 30), novel control powder 1(n = 30) after 24 hours

Differences in survival between pellet types (24 – 96 hours)

There was no difference in the survival percentage of slugs between pellet types at 24 – 96 hours, as assessed by Fisher's exact test, $p = 0.473$, (Figure 2.8).

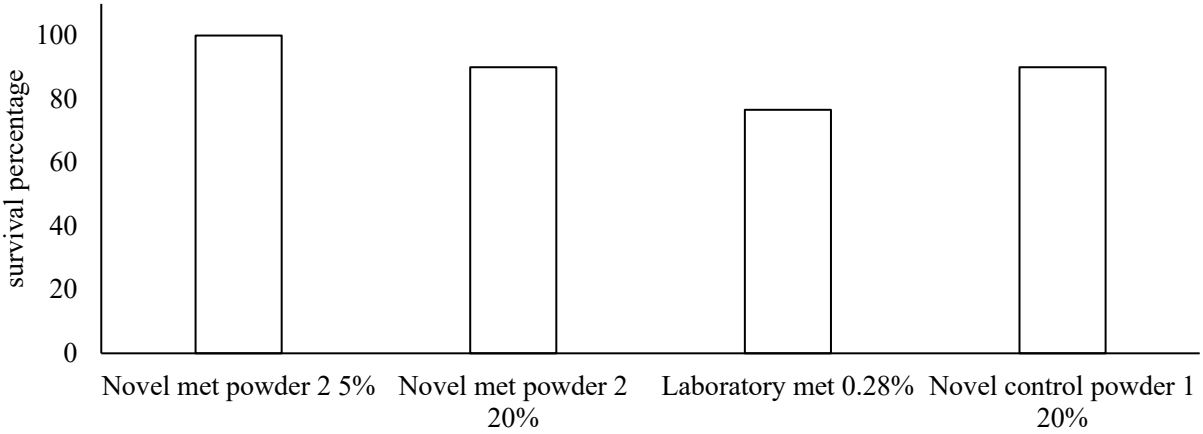

Figure 2.8 Survival percentage novel metaldehyde powder 2 5% (n = 30), novel metaldehyde powder 2 20% (n = 28), metaldehyde 0.28% (n = 25), novel metaldehyde powder 1(n = 29) after 96 hours

2.4.3 Summary

Consumption

There was no significant difference between consumption in pellet groups at 24 hours, but there was a significant difference in consumption between groups at 96 hours. This suggests that in laboratory conditions slugs can take up to 96 hours in close contact with a pellet to consume different pellet types. The consumption level between all toxic pellets was similar and mostly low on the consumption scale. Slugs may have been discouraged from feeding due to combination of the metaldehyde in the formulations, the product resulting from the interaction between novel formulation and metaldehyde, or components of the novel metaldehyde powder 2. If metaldehyde were the only or overriding factor affecting consumption, then it could be argued that consumption may have been greater in the novel metaldehyde powder 2 5% than in the other toxic pellets, due to the difference in the concentration of metaldehyde. This suggests that slugs were deterred by the novel metaldehyde powder, or the product resulting from the interaction between the powder and metaldehyde. However, it is also possible that a threshold amount of metaldehyde in this form, smaller than what was found in the novel metaldehyde powder 2 5%, is all that is needed to discourage consumption.

More novel control powder 1 was consumed than novel metaldehyde 2 powder 20%. However, slugs in experiment 2B consumed less novel metaldehyde powder 1 than slugs in experiment 2A if comparing the overall consumption levels at the end of the experiments. It should be noted that novel metaldehyde powder 1 in 2A was at a concentration of 20.8%, but at a concentration of 20% for 2B. It was not anticipated that this should be the reason behind the difference in consumption. Preliminary experiments on novel metaldehyde powder 1 also showed more feeding than was observed with 2B[7].

Survival

There was no significant difference in mortality between the experimental groups over time. This suggests that novel metaldehyde powder 2 is not useful for molluscicidal purposes.

[7] using the same methodology as described in sections 2.2.1 and the same pellets as detailed in experiments 2A and 2B showed similar results (n=5). See table 2.2 for details on pellet composition.

2.4.4 Conclusion

As consumption and mortality with the novel metaldehyde powder 2 formulation was lower than with novel metaldehyde powder 1 formulation, it was decided not to proceed with further trials or further powder formulations of this type.

2.5 Experiment 2C: Is there a Difference in Consumption and Survival between Commercial and Novel Metaldehyde Pellets?

2.5.1 Overview

Slugs did not find the novel materials in 2A and 2B palatable. The novel formulations in the experiments in 2A and 2B were chemically identical, but with metaldehyde incorporated differently. Slugs did find laboratory metaldehyde formulations palatable. Preliminary experiments[8] had shown more useful molluscicidal properties with commercially available pellets. The novel metaldehyde pellet is a commercial metaldehyde pellet that has been coated with a novel formulation (a slight variation to those in 2A and 2B). In 2A and 2B, consumption of the novel control powder 1 was the greatest, so it was anticipated that slugs would not be deterred from feeding by the novel coating due to the similarities in the raw materials used to create all the novel products. It was also anticipated that there would be little chemical interaction between the novel coating and the commercial metaldehyde pellet – limiting the possibility that a by-product would deter the slugs from feeding. The methods used in 2C are as described in section 2.2.1, 2.2.2 and 2.2.3. Details on pellet composition can be referred to in Table 2.2.

2.5.2 Results

Distribution of slug weight

There were no statistically significant differences between groups, $F_{(2,87)}= 0.565$, $p = 0.570$, (Figure 2.9).

[8] using the same methodology as described in sections 2.2.1 with commercial metaldehyde pellet and commercial ferric phosphate (n=5). See table 2.2 for details on pellet composition.

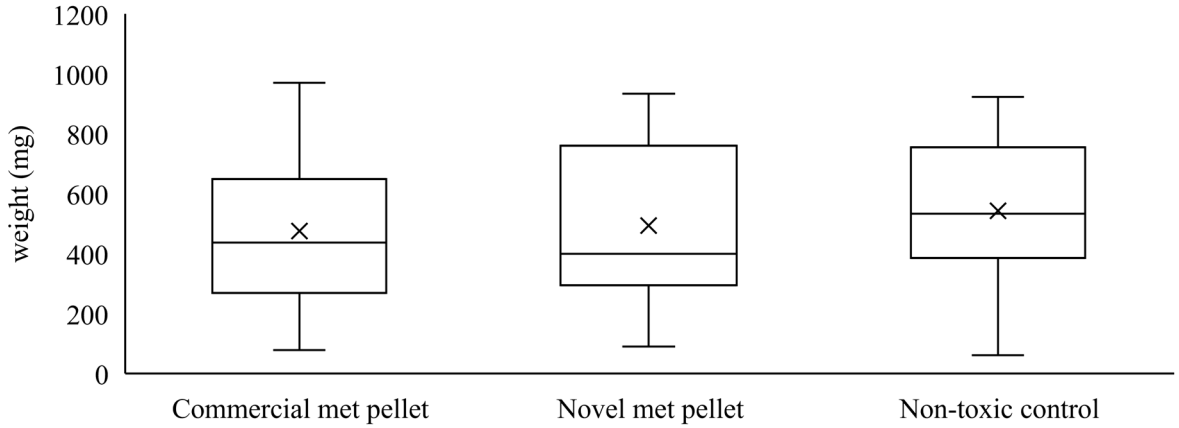

Figure 2.9 Distribution of slug weight (mg) in treatments before feeding; commercial metaldehyde pellet (n= 30), novel metaldehyde pellet (n = 30), non-toxic control (n= 30). The box indicates the upper and lower quartiles, the whiskers indicate the range of data outside the upper and lower quartiles. The black cross within the box indicates the mean. Circles and crosses outside of the box show outlying values.

Differences in consumption between pellet types (0 - 24 hours)

Distribution of consumption levels were similar for all groups, as assessed by visual inspection of a boxplot. Consumption was statistically significantly different between the different pellet groups, $\chi^2(2) = 71.714$, $p < 0.001$ (Figure 2.10). There were differences between commercial metaldehyde pellet and non-toxic control, as well as between the novel metaldehyde pellet and non-toxic control.

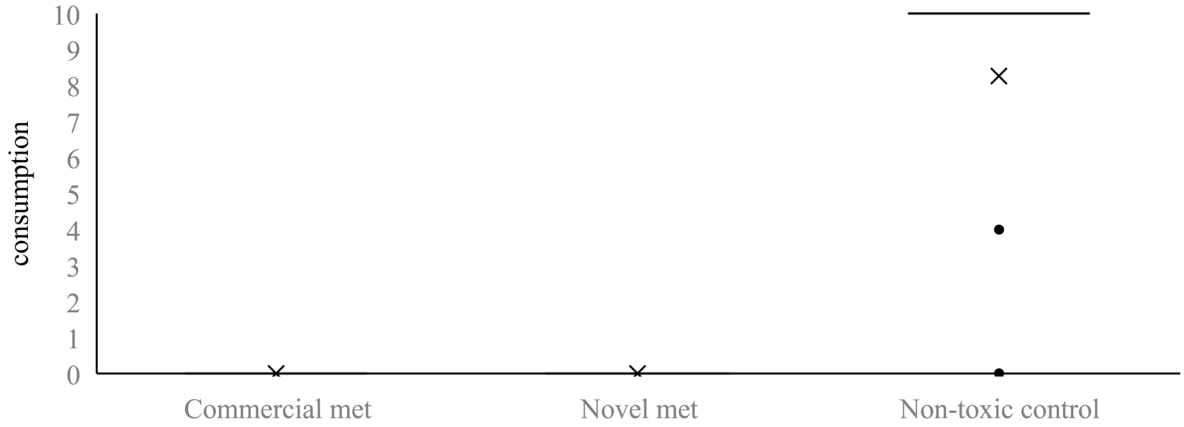

Figure 2.10 Consumption on scale of 0 – 10 (0 = none consumed, 10 = all consumed) of commercial metaldehyde pellet (n= 30), novel metaldehyde pellet (n =30), non-toxic control (n= 30) after 24 hours. The box indicates the upper and lower quartiles, the whiskers indicate the range of data outside the upper and lower quartiles. The black cross within the box indicates the mean. Circles and crosses outside of the box show outlying values.

Differences in consumption between pellet types (24 - 96 hours)

Distribution of consumption levels were similar for all groups, as assessed by visual inspection of a boxplot. Consumption was statistically significantly different between the different pellet groups, $\chi^2(2) = 62.861$, $p < 0.001$ (Figure 2.11). There were differences between commercial metaldehyde pellet and non-toxic control, as well as novel metaldehyde pellet and non-toxic control.

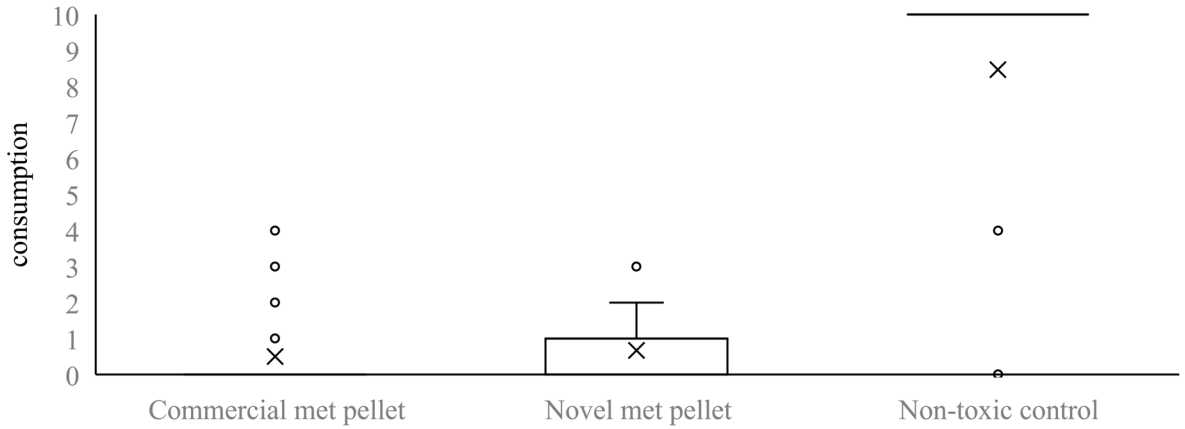

Figure 2.11 Consumption on scale of 0 – 10 (0 = none consumed, 10 = all consumed) of commercial metaldehyde pellet (n= 30), novel metaldehyde pellet (n =30), non-toxic control (n= 30) after 24 hours. The box indicates the upper and lower quartiles, the whiskers indicate the range of data outside the upper and lower quartiles. The black cross within the box indicates the mean. Circles and crosses outside of the box show outlying values.

Differences in survival (0 - 24)

There were no mortality events recorded at 24 hours for any pellet formulations.

Differences in survival (24 - 96)

There was no significant difference in survival, assessed by Fisher's exact test, $p = 0.135$.

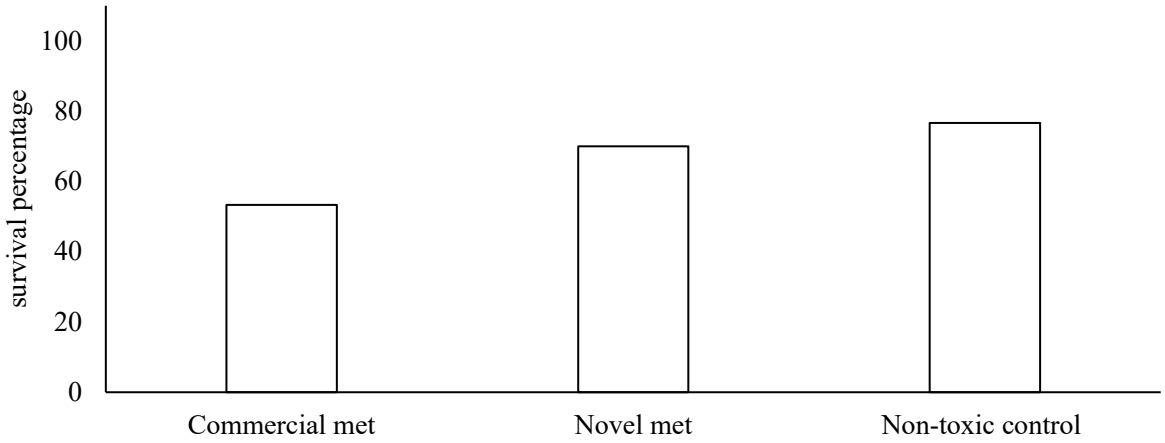

Figure 2.12 Survival percentage in pellet formulations, commercial metaldehyde pellet (n = 30), novel metaldehyde pellet (n=30), non-toxic control pellet (n=30) after 24 hours

2.5.3 Summary

Consumption

There was no difference between toxic pellet types at 0 – 24 hours, nor at 24 – 96 hours, but there was an increase in consumption of both pellets from 24 – 96 hours compared to 0 – 24 hours. The novel coating did not increase consumption any more than a commercially available pellet, suggesting it may not be useful as a molluscicide. After 24 hours 80% of the non-toxic control pellets were completely consumed, increasing to 84% after 96 hours. Based on preliminary experiments, it was not anticipated that feeding would be limited by pellet availability[9]. Future work should increase the amount of pellet so that consumption could be measured more accurately.

Survival

There was no significant difference between the pellet groups over 0 - 24 hours, nor over 24 - 96 hours. However, survival was under 80% for all pellet types, including non-toxic types. This was not observed in previous or preliminary experiments. This may be attributed to

[9] Preliminary experiments using the same methodology as described in sections 2.2.1 provided an excess of non-toxic control pellet and the amount consumed was used to determine the amount of pellet provided in the final methodology. See table 2.2 for details on pellet composition.

environmental conditions at the time of slug collection and is discussed further in section 2.9, General discussion.

2.5.4 Conclusion

The novel metaldehyde pellet did not improve consumption or mortality when compared to the commercial metaldehyde pellet

2.6 Experiment 2D: Is there a Difference in Consumption and Survival between Novel Control Pellets and Control Pellets

2.6.1 Overview

From experiment 2C it was not possible to determine if the slugs were deterred from consuming the novel metaldehyde pellets due to the presence of metaldehyde, the novel coating, or an interaction between the novel coating and the metaldehyde within the interior (commercial metaldehyde) of the pellet. This in mind, it was decided to test the effect of novel coating alone. If the slugs were deterred from consuming the novel control pellet, it would suggest that the materials in the novel formulation were inciting repellent or deterrent cues to the slugs. The methods used in 2D are as described in section 2.2.1, 2.2.2 and 2.2.3. Details on pellet composition can be referred to in Table 2.2.

2.6.2 Results

Distribution of slug weight

There was a statistically significant difference between the groups ($F_{(1,58)}= 9.620$, $p < 0.005$), Figure 2.14.

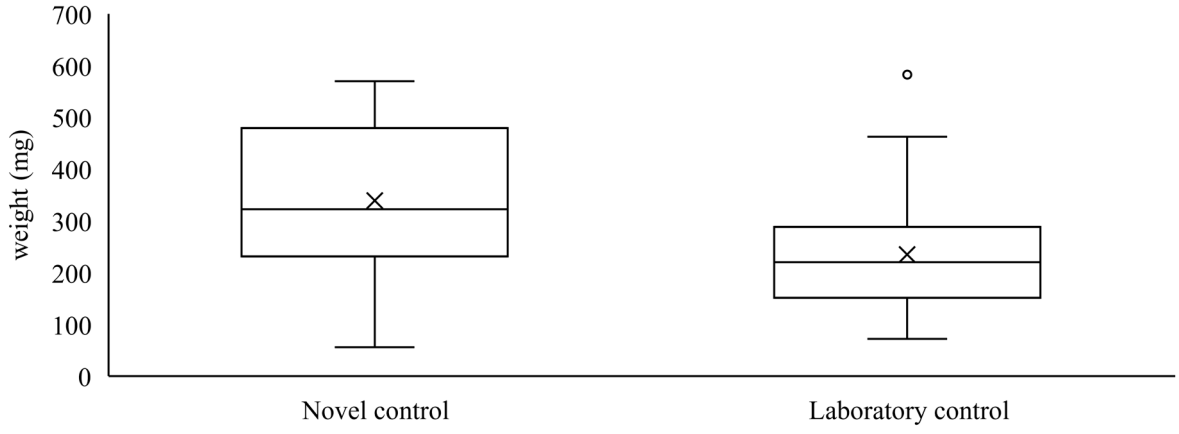

Figure 2.13 Distribution of slug weight (mg) in treatments before feeding; novel control pellets (n=30) and laboratory control pellets (n=30). The box indicates the upper and lower quartiles, the whiskers indicate the range

of data outside the upper and lower quartiles. The black cross within the box indicates the mean. Circles and crosses outside of the box show outlying values.

Differences in consumption between pellet types (0 - 24 hours)

Consumption was statistically significantly different between the different treatment groups, $U = 15.00$, $p < 0.001$, Figure 2.15.

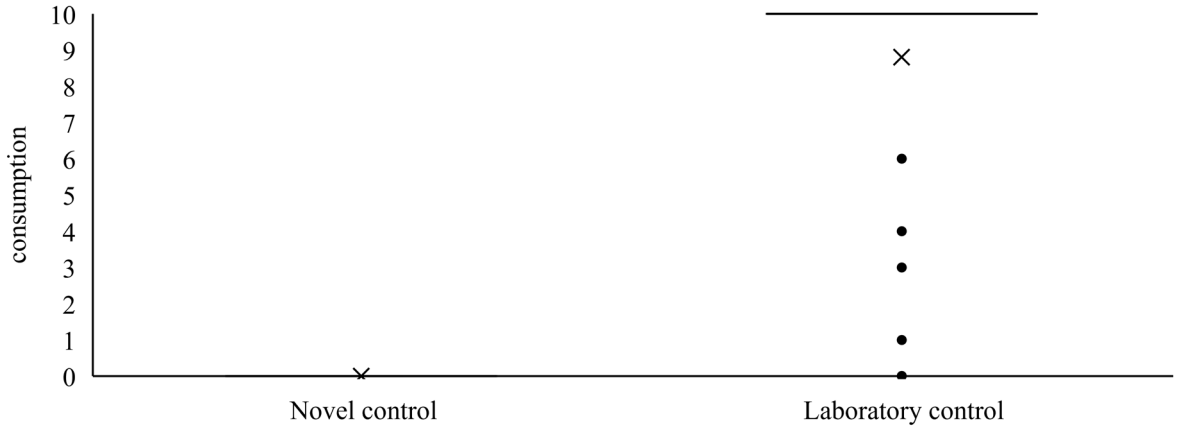

Figure 2.14 Consumption on scale of 0 – 10 (0 = none consumed, 10 = all consumed) of novel control pellets (n=30) and laboratory control pellets (n=30) after 24 hours. The box indicates the upper and lower quartiles, the whiskers indicate the range of data outside the upper and lower quartiles. The black cross within the box indicates the mean. Circles and crosses outside of the box show outlying values.

Differences in consumption between pellet types (24 - 96 hours)

Consumption was statistically significantly different between the different treatment groups, $U = 15.50$, $z = -7.564$, $p < 0.001$, Figure 2.16.

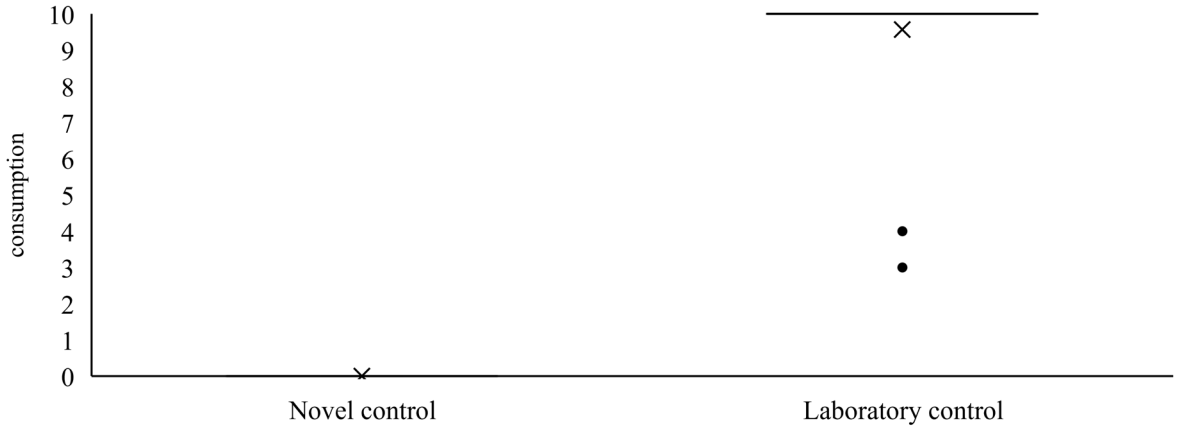

Figure 2.15 Consumption on scale of 0 – 10 (0 = none consumed, 10 = all consumed) of novel control pellets (n=30) and laboratory control pellets (n=30) after 96 hours. The box indicates the upper and lower quartiles, the whiskers indicate the range of data outside the upper and lower quartiles. The black cross within the box indicates the mean. Circles and crosses outside of the box show outlying values.

Survival

There was no recorded mortality from 0 – 96 hours for either treatment.

2.6.3 Summary

Consumption

Control pellets were consumed significantly more than the novel control pellets. As many slugs had consumed the entire control pellet during 0 – 24 hours, there was little difference in consumption from 24 – 96 hours. Future work should provide an excess of food to determine consumption more accurately. The low consumption of the novel pellet suggests that slugs may be discouraged by the novel coating, or the slugs may not be able to perceive the pellet as a food substance.

Survival

There were no recorded deaths for either pellet type from 0 –96 hours. Apart from random slug deaths, it was not anticipated that either pellet would cause mortality because neither contained metaldehyde or any other active ingredient.

2.6.4 Conclusion

The results of experiment 2D suggest that the novel coating does not encourage consumption and possibly acts as a repellent.

2. 7 Experiment 2E: Does Ferric Phosphate Affect Consumption and Mortality?

2.7.1 Overview

Due to the withdrawal of metaldehyde for outside use, shortly after completing experiment 2D, the project refocused work on the chemical slug control product, ferric phosphate. Approximately 3 grams of chicken mash were used per feeding trial, all other parameters were the same. Consumption was not measured due to difficulties visually gauging changes in irregularly shaped chicken feed. Trials were run for 144h as the literature suggests that ferric phosphate pellets take a longer time to cause slug mortality than metaldehyde. The methods used in 2E are as described in section 2.2.1, 2.2.2 and 2.2.3. Details on pellet composition can be referred to in Table 2.2.

2.7.2 Results

Distribution of slug weight

There was no statistically significant difference between the groups ($F_{(1,28)} = 0.37$, $p = 0.85$), Figure 2.17

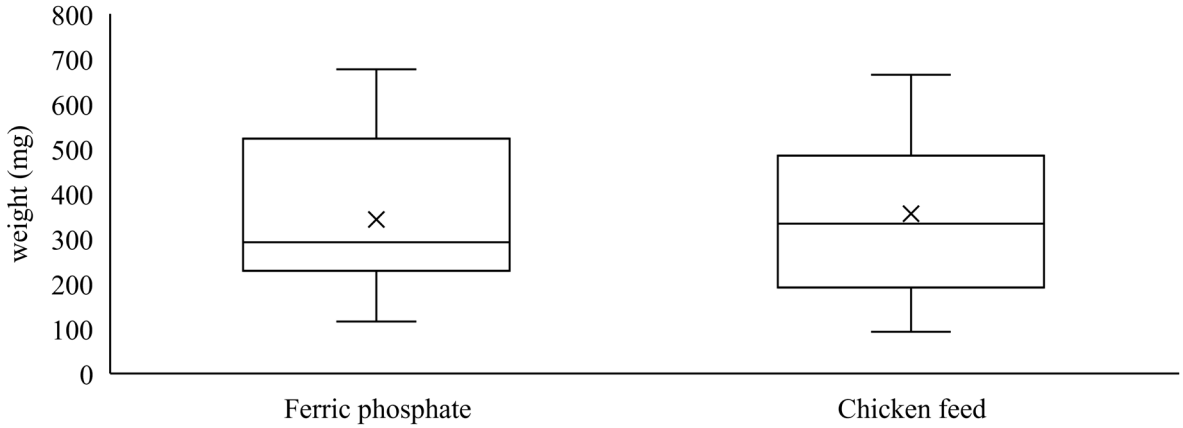

Figure 2.16 Distribution of slug weight (mg) in treatments before feeding; ferric phosphate feeding (n = 15) and chicken feed (n = 15). The box indicates the upper and lower quartiles, the whiskers indicate the range of data

outside the upper and lower quartiles. The black cross within the box indicates the mean. Circles and crosses outside of the box show outlying values.

Differences in mortality (0 –24)

There was no statistically significant difference between mortality between formulations, as assessed by Fisher's exact test, $p = 0.169$.

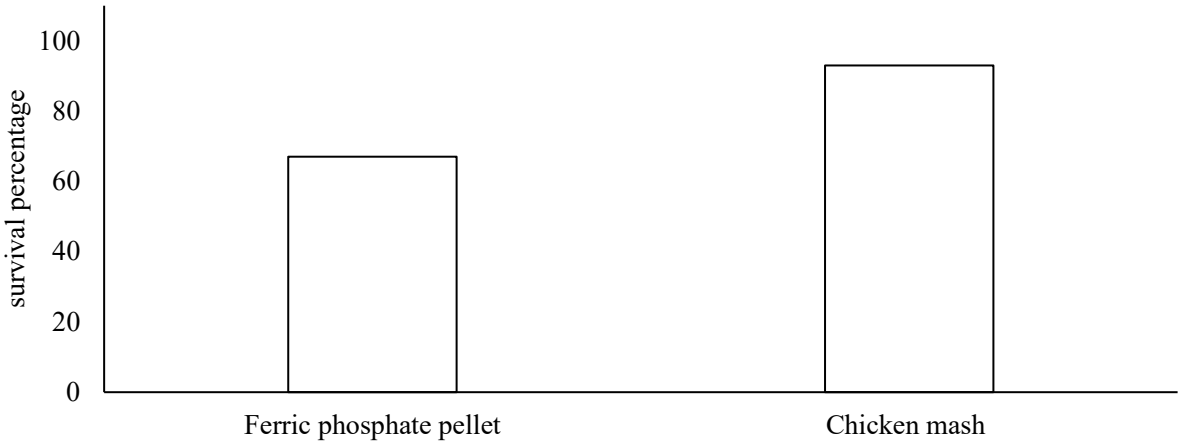

Figure 2.17 Survival percentage ferric phosphate (n =15) and chicken feed (n=15) after 24 hours

Differences in mortality (24 – 96)

There was no statistically significant difference between mortality in pellet formulations, as assessed by Fisher's exact test, $p = 1.00$.

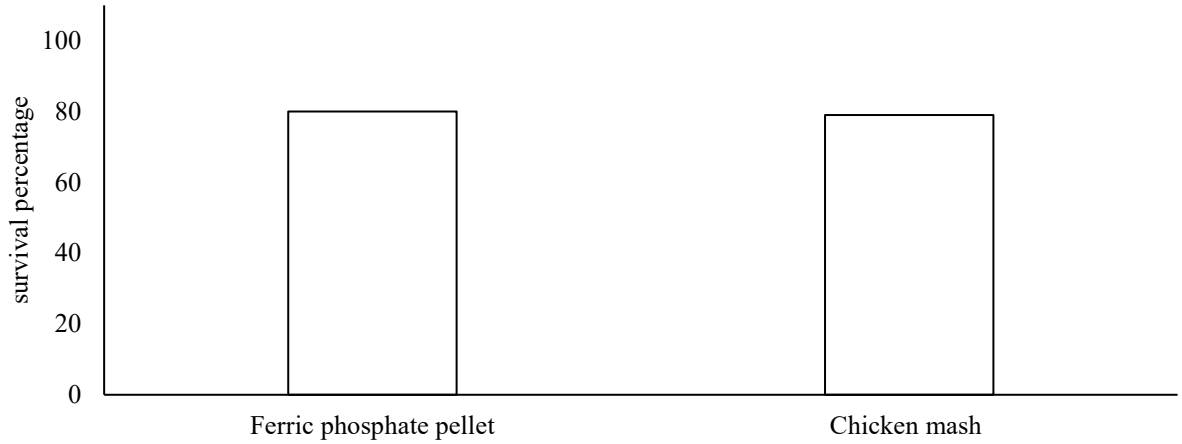

Figure 2.18 Survival percentage ferric phosphate (n =10) and chicken feed (n=14) after 96 hours

Differences in mortality (96 –144 hours)

There was a statistically significant difference between mortality in pellet formulations, as assessed by Fisher's exact test, $p = 0.041$.

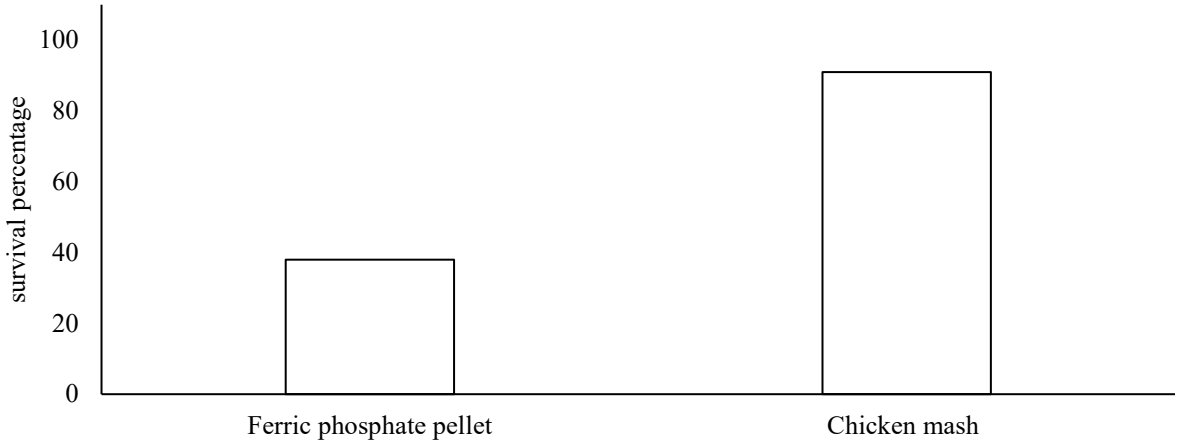

Figure 2.19 Survival percentage ferric phosphate (n =10) and chicken feed (n=14) after 144 hours

2.7.3 Summary

Survival

There was no difference in survival between 0 - 96 hours but there was a difference at 96-144 hours. Ferric phosphate has been suggested to take a longer time than other active ingredients to cause mortality, which may explain why a significant difference between the groups was only observed from 96 – 144 hours. It was not anticipated that chicken feed would cause mortality because it did not contain any other active ingredient; the deaths observed in slugs feeding on mash are therefore likely to have been due to natural random mortality. Another possibility is that the chicken mash absorbed more water from the filter paper, leaving the petri dish dryer than when other pellets are used, causing death due to dehydration.

2. 8 Experiment 2F: Is there a Difference in Consumption and Survival between Commercial Ferric Phosphate Pellet and a Non-toxic Control?

2.8.1 Overview

To compare consumption, commercial ferric phosphate pellets were compared to a non-toxic control (carrots). The methods used in 2F are as described in section 2.2.1, 2.2.2 and 2.2.3. Details on pellet composition can be referred to in Table 2.2.

2.8.2 Results

Distribution of slug weight

There were no differences in weight between treatment groups, $F_{(1,28)} = 0.475$, $p = 0.496$ (Figure 2.20).

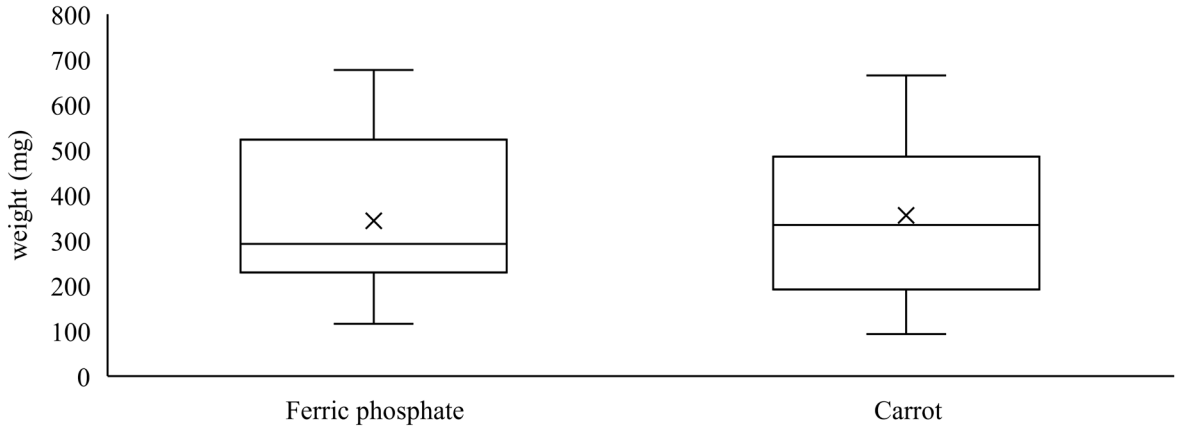

Figure 2.20 Distribution of slug weight (mg) in treatments before feeding; ferric phosphate (n=15) and laboratory control pellets (n=15). The box indicates the upper and lower quartiles, the whiskers indicate the range of data outside the upper and lower quartiles. The black cross within the box indicates the mean. Circles and crosses outside of the box show outlying values.

Differences in consumption (0 – 24 hours)

There was no difference in consumption between treatment groups, $U = 142.5$, $p = 0.217$ (Figure 2.21).

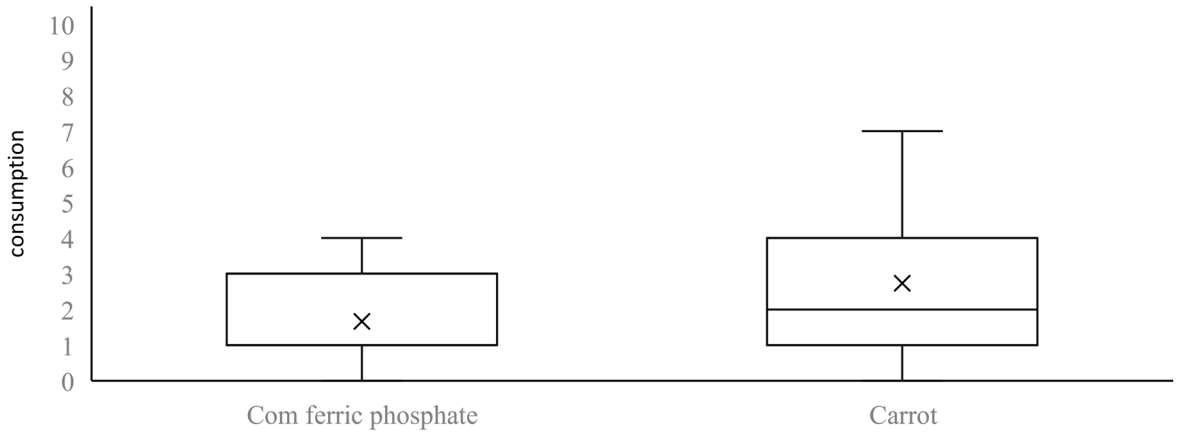

Figure 2.21 Consumption on scale of 0 – 10 (0 = none consumed, 10 = all consumed) of ferric phosphate (n=15) and carrots (n=15) after 24 hours. The box indicates the upper and lower quartiles, the whiskers indicate the range of data outside the upper and lower quartiles. The black cross within the box indicates the mean. Circles and crosses outside of the box show outlying values.

Differences in consumption (24 – 96 hours)

There was a difference in consumption between treatment groups, $U = 176.5$, $p > 0.001$ (Figure 2.22).

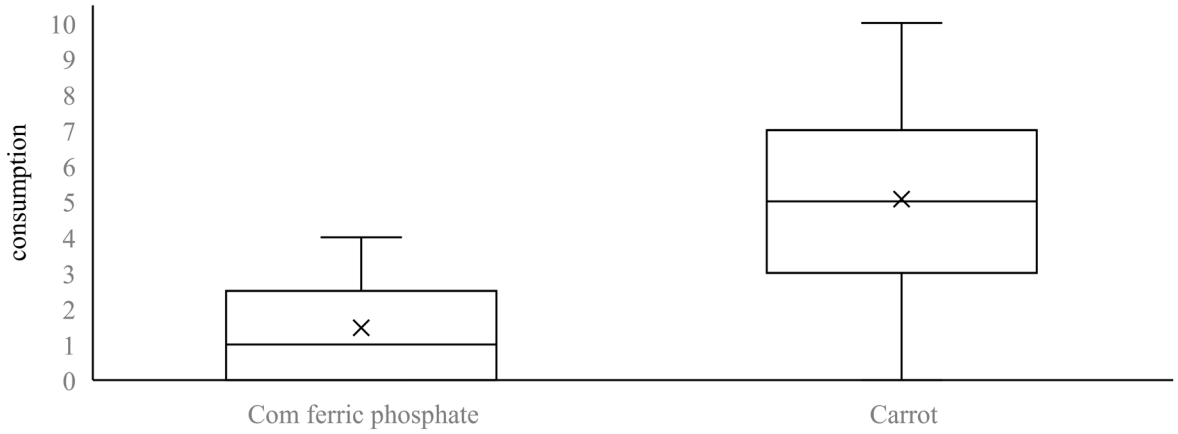

Figure 2.22 Consumption on scale of 1 - 10 of ferric phosphate (n=13) and laboratory control pellets (n=15) after 96 hours. The box indicates the upper and lower quartiles, the whiskers indicate the range of data outside the upper and lower quartiles. The black cross within the box indicates the mean. Circles and crosses outside of the box show outlying values.

Differences in consumption (96 – 144 hours)

There was a difference in consumption between treatment groups, $U = 150.5$, $p > 0.001$ (Figure 2.21).

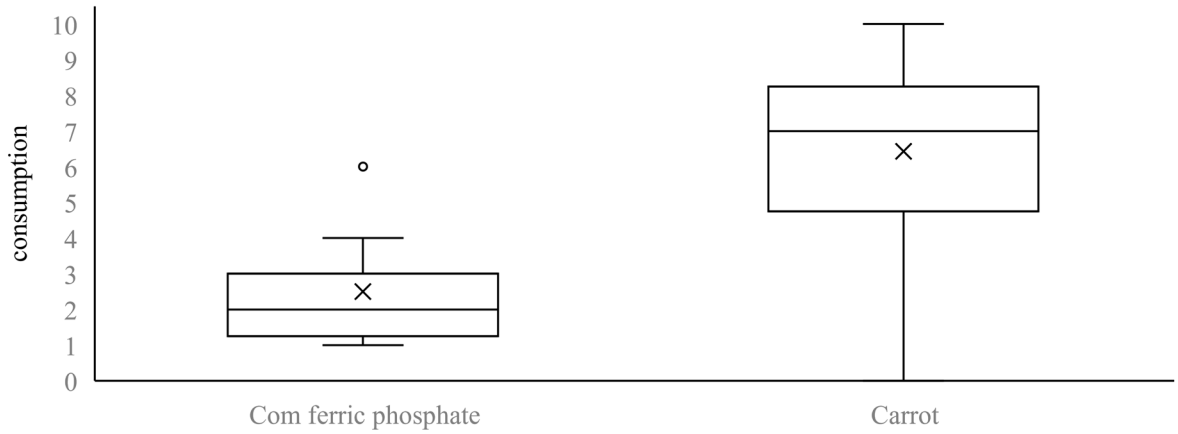

Figure 2.23 Consumption on scale of 0 – 10 (0 = none consumed, 10 = all consumed) of ferric phosphate (n=15) and laboratory control pellets (n=15) after 144 hours. The box indicates the upper and lower quartiles, the whiskers indicate the range of data outside the upper and lower quartiles. The black cross within the box indicates the mean. Circles and crosses outside of the box show outlying values.

Differences in survival (0 – 24 hours)

There was no difference in the survival percentage of slugs between pellet types at 0h – 24h, as assessed by Fisher's exact test, $p = 0.483$ (Figure 2.24).

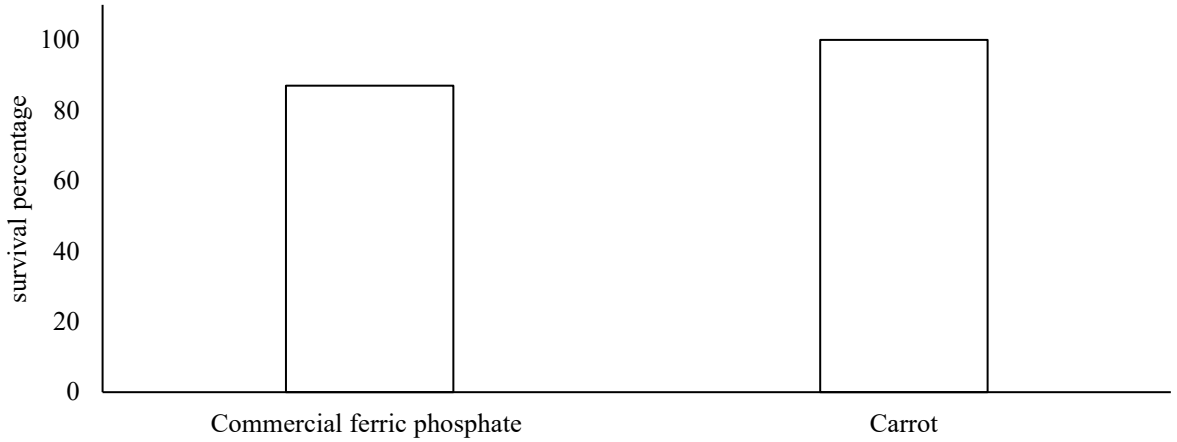

Figure 2.24 Survival percentage in pellet formulations, commercial ferric phosphate (n=15) and laboratory control pellets (n=15) after 24 hours

Differences in survival (24 – 96 hours)

There was no difference in the survival percentage of slugs between pellet types at 24 – 96 hours, as assessed by Fisher's exact test, $p = 0.583$ (Figure 2.25).

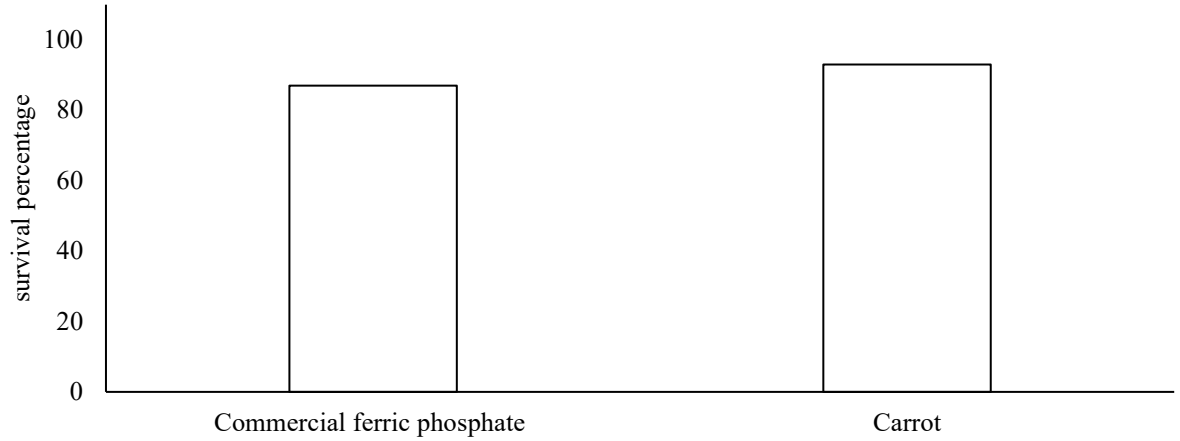

Figure 2.25 Survival percentage in pellet formulations, commercial ferric phosphate (n=13) and laboratory control pellets (n=15) after 96 hours

Differences in survival (96 – 144 hours)

There was no difference in the survival percentage of slugs between pellet types at 96h – 144h, as assessed by Fisher's exact test, $p = 0.288$ (Figure 2.26).

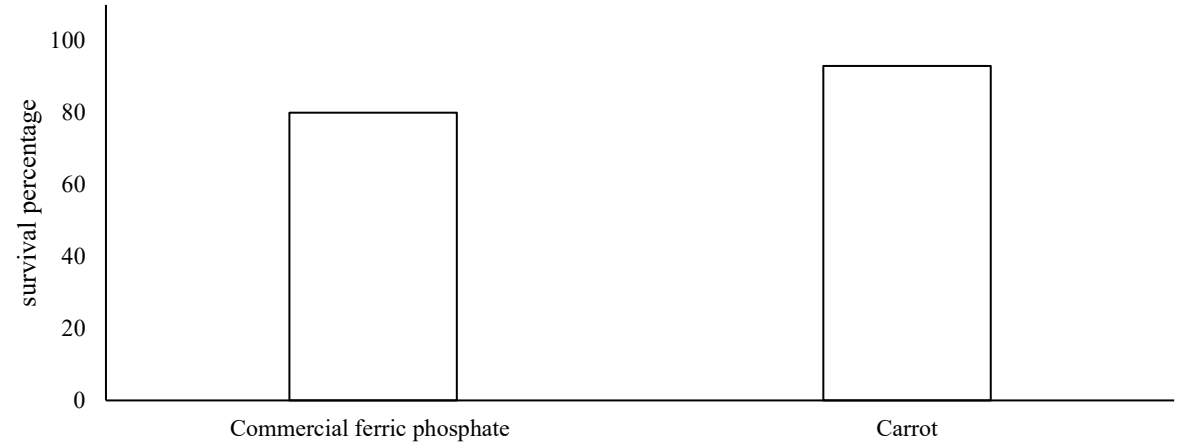

Figure 2.26 Survival percentage in pellet formulations, commercial ferric phosphate (n=11) and laboratory control pellets (n=14) after 144 hours

2.8.3 Summary

Consumption

There was no difference in consumption between pellet types initially (before 24 hours) but there was increased consumption of carrot compared to commercial ferric phosphate after 24 hours.

Survival

There was no difference in survival between commercial ferric phosphate pellets and a non-toxic control food (carrot).

2.8.4 Conclusion

No difference in efficiency was observed between commercial ferric phosphate pellets and the non-toxic control.

2. 9 Experiment 2G: is there a Difference in Consumption and Survival between Commercial Ferric Phosphate and Metaldehyde?

2.9.1 Overview

Due to the withdrawal of metaldehyde (see metaldehyde disclosure, start of thesis), it is anticipated that ferric phosphate will soon become the major slug control product used in the UK. Commercial ferric phosphate and commercial metaldehyde pellets were compared to investigate if there is a difference in efficiency. The methods used in 2G are as described in section 2.2.1, 2.2.2 and 2.2.3. Details on pellet composition can be referred to in Table 2.2.

2.9.2 Results

Distribution of slug weight

There were no differences in weight between treatment groups, $F_{(2,87)} = 0.555$, $p = 0.576$ (Figure 2.27).

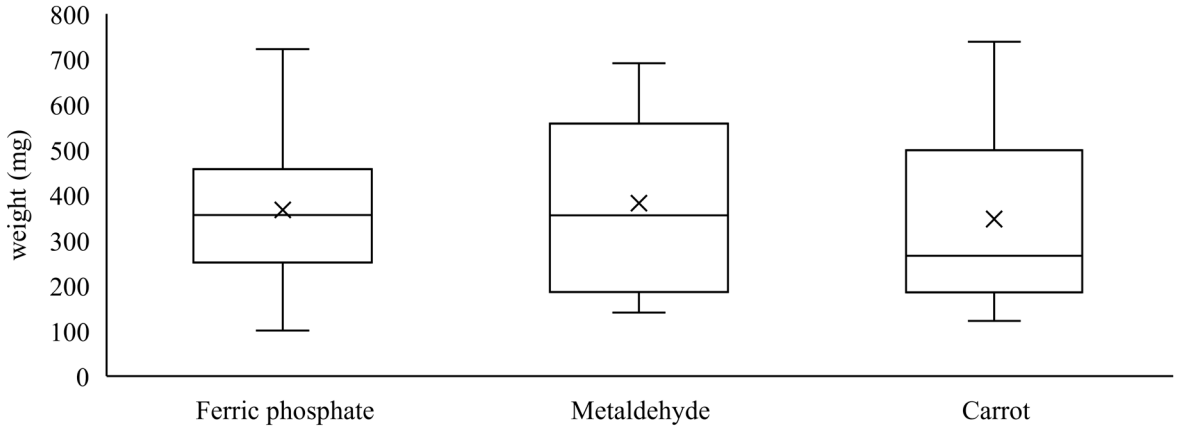

Figure 2.27 Distribution of slug weight (mg) in treatments before feeding; commercial ferric phosphate (n=30), commercial metaldehyde (n=30) and carrots (n=30). The box indicates the upper and lower quartiles, the whiskers indicate the range of data outside the upper and lower quartiles. The black cross within the box indicates the mean. Circles and crosses outside of the box show outlying values.

Differences in consumption (0 – 24 hours)

Distribution of consumption levels were not similar for all groups, as assessed by visual inspection of a boxplot. Consumption was significantly different between the different pellet groups, $\chi^2(2) = 22.8, p < 0.001$ (Figure 2.28). Differences were found between commercial ferric phosphate and carrot as well as between commercial metaldehyde and carrot.

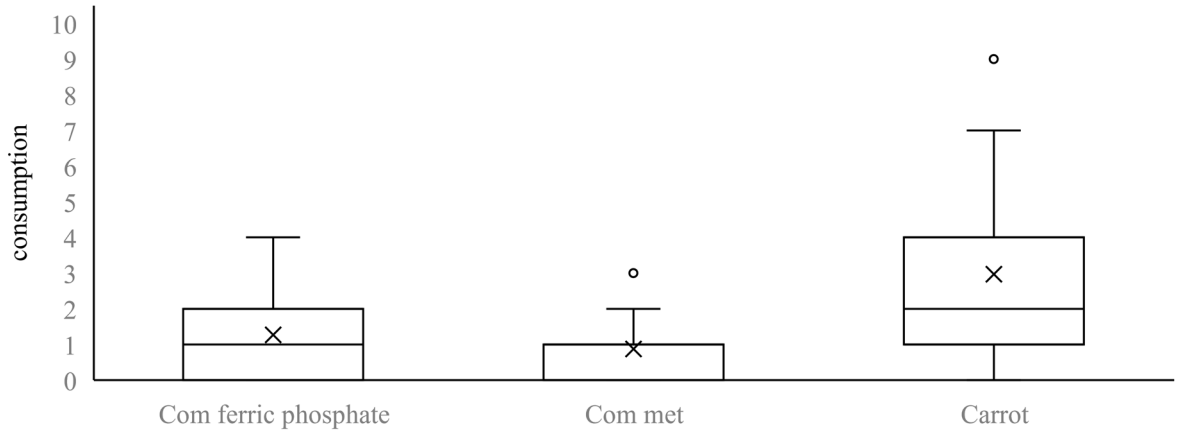

Figure 2.28 Consumption on scale of 0 – 10 (0 = none consumed, 10 = all consumed) of commercial ferric phosphate (n=30), commercial metaldehyde (n=30) and carrot (n=30) after 24 hours. The box indicates the upper and lower quartiles, the whiskers indicate the range of data outside the upper and lower quartiles. The black cross within the box indicates the mean. Circles and crosses outside of the box show outlying values.

Differences in consumption (24 – 96 hours)

Distribution of consumption levels were not similar for all groups, as assessed by visual inspection of a boxplot. Consumption was significantly different between the different pellet groups, $\chi^2(2) = 41.6, p < 0.001$ (Figure 2.29). Differences were found between commercial ferric phosphate and carrot as well as between commercial metaldehyde and carrot.

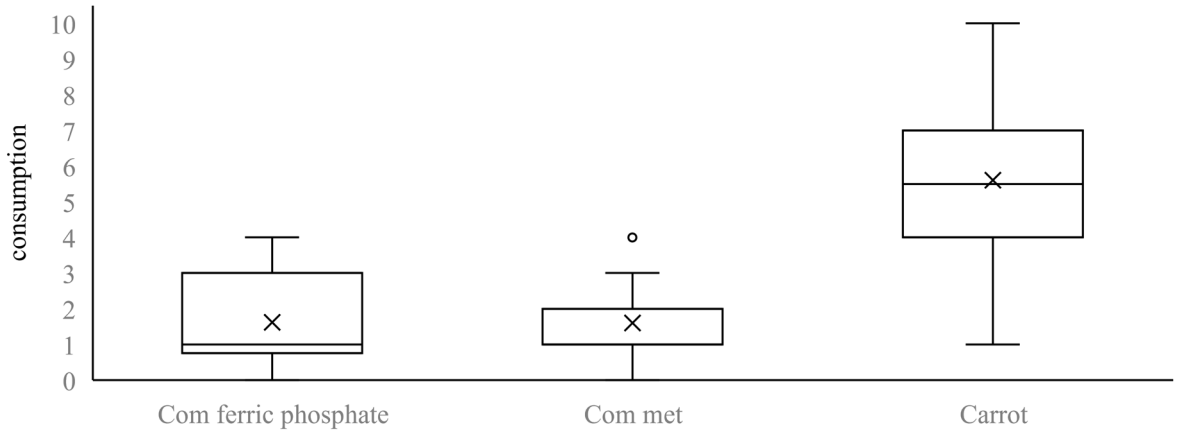

Figure 2.29 Consumption on scale of 0 – 10 (0 = none consumed, 10 = all consumed) of commercial ferric phosphate (n=29), commercial metaldehyde (n=27) and carrot (n=30) after 96 hours. The box indicates the upper and lower quartiles, the whiskers indicate the range of data outside the upper and lower quartiles. The black cross within the box indicates the mean. Circles and crosses outside of the box show outlying values.

Differences in consumption (96 – 144 hours)

Consumption was significantly different between the different pellet groups, $\chi^2(2) = 47.4$, $p < 0.001$ (Figure 2.30). Differences were found between commercial ferric phosphate and carrot as well as between commercial metaldehyde and carrot.

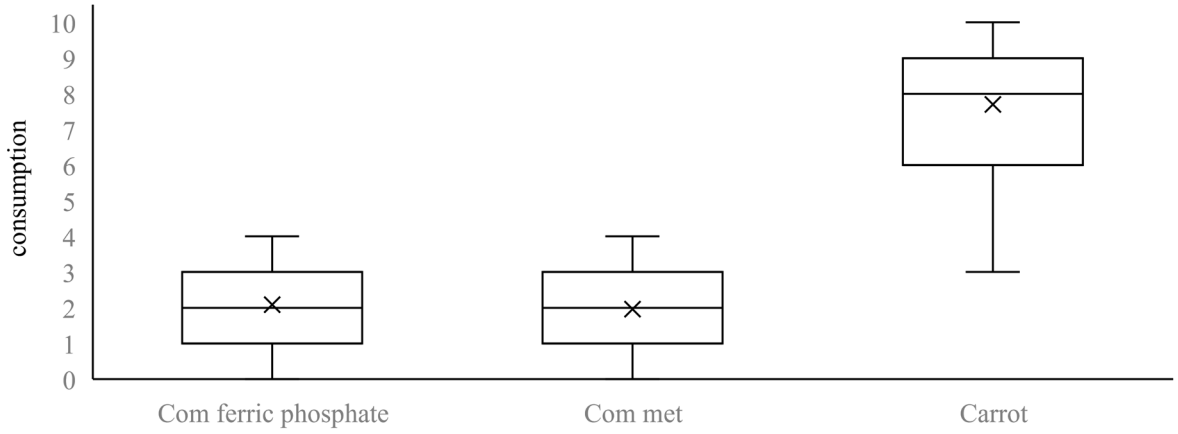

Figure 2.30 Consumption on scale of 0 – 10 (0 = none consumed, 10 = all consumed) of commercial ferric phosphate (n=26), commercial metaldehyde (n=24) and carrot (n=28) after 144 hours. The box indicates the upper and lower quartiles, the whiskers indicate the range of data outside the upper and lower quartiles. The black cross within the box indicates the mean. Circles and crosses outside of the box show outlying values.

Differences in survival (0 – 24 hours)

There was no difference in the survival percentage of slugs between formulation types at 0 – 24 hours, as assessed by Fisher's exact test, $p = 0.318$ (Figure 2.31).

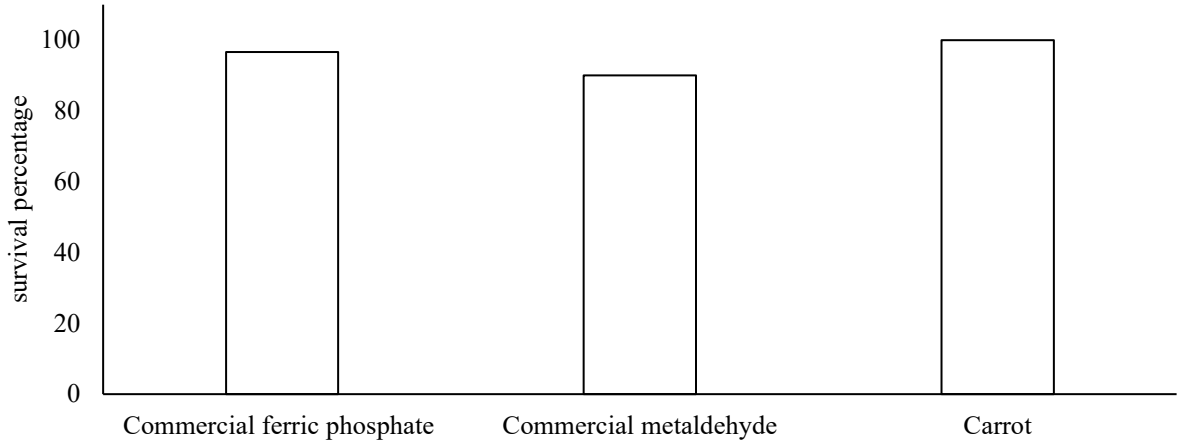

Figure 2.31 Survival percentage in pellet formulations, commercial ferric phosphate (n=30), commercial metaldehyde (n=30) and carrot (n=30) after 24 hours

Differences in survival (24 – 96 hours)

There was no difference in the survival percentage of slugs between pellet types at 24h – 96h, as assessed by Fisher's exact test, $p = 0.811$ (Figure 2.32).

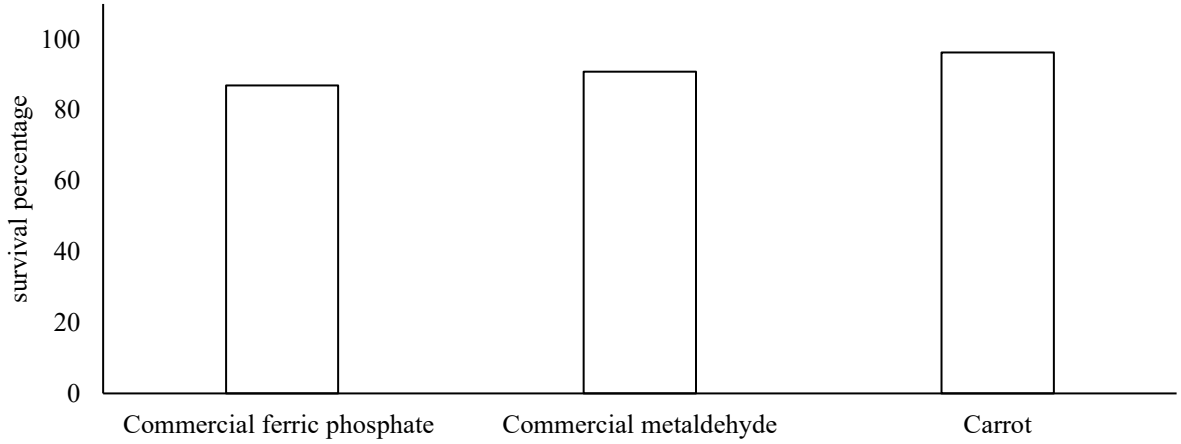

Figure 2.32 Survival percentage in pellet formulations, commercial ferric phosphate (n=29), commercial metaldehyde (n=27) and carrot (n=30) after 96 hours

Differences in survival (96 – 144 hours)

There was no difference in the survival percentage of slugs between formulation types at 96h – 144h, as assessed by Fisher's exact test, $p = 0.585$ (Figure 2.31).

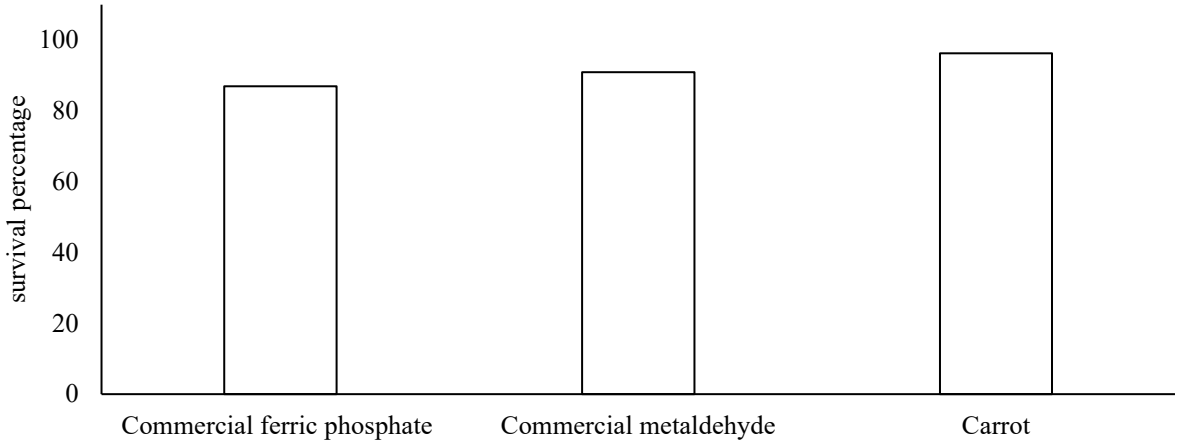

Figure 2.33 Survival percentage in pellet formulations, commercial ferric phosphate (n=26), commercial metaldehyde (n=24) and carrot (n=28) after 144 hours

2.9.3 Summary

Consumption

There were differences between toxic pellet types and the non-toxic control (carrot) after 24 hours. There was no difference between toxic pellet types.

Survival

There was no difference in the survival of slugs in any treatment group

2.9.4 Conclusion

No difference in efficiency were observed between toxic pellet types.

2.9 General Discussion

The overall aim of this chapter was to provide data and feedback regarding consumption and associated survival in *D. reticulatum* on several novel formulations compared to commercially available molluscicides using a standardised bioassay methodology. Feedback received after each experiment was used to evolve product testing specifics in real time, taking a stepwise approach to inform product selection and methodologies for subsequent experiments undertaken in this chapter. A summary of the results obtained is provided in Table 2.3.

Table 2.3 Results of bioassay experiments A – G, including formulations used and significant results for weight, consumption, and survival. The different letters within the same experiment denotes significant difference between formulation types.

Experiment	Formulation	Weight	Consumption			Survival		
			24	96	144	24	96	144
A	Novel control powder 1	a		a			ac	
	Novel metaldehyde powder 1	a		b			a	
	Laboratory metaldehyde 1%	ab		b			b	
	Laboratory control	b		a			c	
B	Novel metaldehyde powder 2 5%	a	a	ab		a	a	
	Novel metaldehyde powder 2 20%	a	a	a		a	a	
	Laboratory metaldehyde 0.28%	a	a	ab		a	a	
	Novel metaldehyde powder 1 20%	a	a	b		a	a	
C	Commercial metaldehyde pellet	a	a	a		a	a	
	Novel metaldehyde pellet	a	a	a		a	a	
	Non-toxic control	a	b	b		a	a	
D	Novel control pellet	a	a	a		a	a	
	Commercial control pellet	b	b	b		a	a	
E	Commercial ferric phosphate pellet	a				a	a	a
	Chicken feed	a				a	a	b
F	Commercial ferric phosphate pellet	a	a	a	a	a	a	a
	Carrot	a	a	b	b	a	a	a
G	Commercial ferric phosphate pellet	a	a	a	a	a	a	a
	Commercial metaldehyde pellet	a	a	a	a	a	a	a
	Carrot	a	b	b	b	a	a	a

A summary of the main conclusions would include:

1. Slug weight was not associated with consumption or survival for any pellet type
2. Novel metaldehyde powder 1 did not differ from control formulations
3. Novel metaldehyde powder 2 did not differ from control formulations regarding survival
4. Novel metaldehyde pellets did not differ from a commercial metaldehyde pellets
5. Novel control pellets were not consumed at all
6. Commercial ferric phosphate caused more slug mortality than non-toxic controls
7. Commercial ferric phosphate did not differ from commercial metaldehyde pellets

Novel formulations

Overall, none of the novel formulations showed potential as commercially viable molluscicides. Apart from novel control powder 1, consumption was generally low and none of the novel formulations produced a lower slug survival rate than commercial pellets. As discussed below, slug behaviour and feeding preference may vary between laboratory and field conditions. However, for the novel formulations described in this chapter, it is not anticipated that consumption and associated survival would vary greatly under field conditions. Poor consumption was observed during arena trials both here, as described in Chapter 3, and in other work (Campbell, 2020).

Metaldehyde and ferric phosphate

In work elsewhere, metaldehyde was found to cause a faster decline in slug health (measured through immobility and excessive mucus production) than ferric phosphate; however, the two treatments were not different from each other after ten days. The inclusion of both metaldehyde and ferric phosphate was found to reduce consumption when added to non-toxic food at equal speeds (Iglesias & Speiser, 2001). However, the concentrations of metaldehyde (5%) and iron phosphate (1%) used in experiments by Iglesias & Speiser (2001) were different to the those used in this thesis. Campbell et al (2021) found that survival did not differ between 5%, 3% and 1% metaldehyde pellets, so if only survival was considered, a 5% pellet may be comparable to those used in this thesis.

Deroceras reticulatum is well documented as generalist feeder, although mostly feeding on plant material, that can show preference or aversion to foods (Dmitrieva, 1969; Cook et al., 2000; Peters et al., 2000). There has been much research attempting to discover more on feeding preferences in pest slugs to increase molluscicide pellet consumption or deter slugs from seed coatings (Whelan, 1982; Bailey & Wedgwood, 1991; Cook et al., 2000; Langan et al., 2014). Feeding behaviour could be affected by prior learning, a physiological response to the current nutritional needs of the animal, inherited preferences, or a combination of factors. Neophilia, or an interest in novel foods, has been reported in slugs even when the item is later deemed less palatable (Whelan, 1982; Bailey & Wedgwood, 1991). In preliminary experiments under daytime laboratory conditions, slugs were alternately offered two novel foods (toxic pellets and non-toxic pellets). As the toxic pellets were produced specifically for this thesis and the non-toxic pellets are not available in the UK, it is not possible that any slug tested may have fed on either pellet prior to testing. Only the non-toxic pellets were accepted, with no attempt to feed on novel toxic pellets observed, even in slugs that were eager to feed. Slugs fed on non-toxic pellet before the toxic pellet was offered and fed on non-toxic pellets after toxic pellet was refused. These observations agree with the findings of (Cook et al., 2000) who suggested that a perceived preference for novel food items is a response to counteract an imbalanced diet. However, it is generally agreed that neither novelty, nor the addition of attractants or incitants, is consistently able overcome an aversion to foods, as well documented through slugs' distaste for most molluscicides.

In another example of neophilia, slugs were found to have consumed more 'unacceptable' plants during their first exposure to them as food, but showed reduced feeding upon a second exposure, suggesting that they may display some aspect of avoidance learning (Whelan, 1982). However, reduced feeding (including on normally highly palatable food material) after feeding on toxic material has been well documented in slugs (Wedgwood & Bailey, 1988; Mills et al., 1990; Bailey & Wedgwood, 1991). It could be argued that the higher consumption of unacceptable plant species resulted in less 'fit or healthy' slugs that could not feed as much, rather than an aspect of learning. Further support for a single exposure causing aversion is documented in reduced preference for a previously attractive odour after it was paired with an unattractive one (Sahley et al., 1981).

Cordoba et al (2018) also suggested that former feeding may influence future feeding preference in *D. reticulatum*. Slugs fed on cucumber for at least two weeks continued to show a preference for it, while the same preference was not observed for those fed on carrots. Further examples of conditioning include preferential feeding on cereals to other foods in snails fed on cereals for 2 to 6 weeks (Daguzan et al., 1985). Further field experiments or laboratory experiments that include a more varied diet would provide further insight. Also similar observations have been noted in other in other mollusc species (R. P. Croll & Chase, 1977; Desbuquois & Daguzan, 1995; Teyke, 1995).

Survival

Seemingly unexplained mortality was observed in some non-toxic treatments (2C 24-96, 2E 24-96) but none, or very little observed in others. As slugs were collected from the field, the age of slugs used in experiments was unknown. Weight can indicate age to some extent but is also dependent on a variety of other factors and cannot be relied upon as a true indicator (Schley & Bees, 2003; Kim et al., 2009b; Shirely et al, 2020). Slugs may have been approaching their natural end of life at the time of collection; handling stress or changes in environment could have caused or expedited death. Unexplained slug mortality was also observed prior to experiments (resulting in different numbers of available slugs for each experiment) and during preliminary experiments. Similar high control mortality rates have been reported between the months of November and April, in Frick, Switzerland, where the average temperature is comparable to Newcastle upon Tyne, United Kingdom (Iglesias & Speiser, 2001). Experiments 2A through to 2G are presented chronologically, and 2C and 2E were carried out within a few weeks of each other. However, this does not explain the 100% survival observed in experiment 2D. *D. reticulatum* has a life cycle of about one year, with egg production, in the UK, suggested to be peak during late spring and early autumn (the months of April – September) (South, 1982, 1989). Resultantly, many adult animals would approach the natural end of life towards the months of September – April. Additionally, during the months of November - February, collection of specimens from the field was more difficult. Smaller slugs that may have been excluded or overlooked in May – August due to an abundance of larger individuals, may have been more apparent in November – April and more likely to be accepted against a backdrop of general scarcity in numbers. Slugs are reported to decrease in size and weight in the post reproductive stages at the end of their life cycle, so smaller slugs may have been juveniles, or approaching end of life (South, 1989). Consistent with the literature, when comparing the

average weight of the slugs in each experiment, slugs were larger in experiments in May – August (summer) than those found in winter months. Research specific to the Northumberland region (from where the slugs were collected in this thesis), although dated, was consistent with the life cycle explanation above (Hunter, 1966). For a more complete discussion of the lifecycle of *D. reticulatum* refer to South (1982 and 1989), Schley & Bees (2003), Choi et al (2004) and Clemente et al (2008).

General methods

The feeding behaviour of slugs under laboratory conditions may differ greatly to conditions in the field. Standardisation of the methodology between studies could be easily achieved, although accounting for variation associated with slug origin, age, size and season is more difficult. The digestive system of slugs starved for 48 hours was found to be completely empty (Dobson & Bailey, 1982). Most bioassay, olfactory and feeding experiments do starve slugs, with some variation in the length of starvation ranging from 48 hours (2 days) to 6 days (Glen et al., 2000; Marigómez et al., 1986). In these experiments slugs were not starved for 48 hours (see section 2.2.1 and 2.2.2 for details of collection and storage), for experiments to fall within facility access hours. Preliminary experiments[10] found no difference in consumption of non-toxic foods between slugs starved for 24 hours and slugs starved for 48 hours. Similar bioassay style experiments also starved slugs for 24 hours (Desbuquois & Daguzan, 1995; Clark et al., 1997; Birkett et al., 2004; Baker et al., 2012).

[10] used methodology as described in 2.2.1 with the variation of time length of starvation (n=5). These did not find a difference in consumption or mortality. Pellets used were commercial metaldehyde pellet and non-toxic control pellet (see table 2.2 for pellet composition).

2.10 Conclusion

The methodology described above was an established, easily repeatable, and inexpensive way to compare novel and existing molluscicide formulations. The results described in this chapter were consistent with those of preliminary experiments, results described in Chapter 3, and currently unpublished arena trials (Campbell, A. 2020). Poor consumption of the novel formulation types suggests that the components of the formulation deter feeding, which may explain the high survival rates observed with novel metaldehyde formulations.

Chapter 3. Bioacoustics

Part of this chapter has been published as de Silva, S.M., Chesmore, D., Smith, J. & Port, G. (2021) Listening to Slugs: Acceptability and Consumption of Molluscicide Pellets by the Grey Field Slug, Deroceras reticulatum. *Insects*. 12 (6), 548.

3.1 Introduction

3.1.1 Invertebrate bioacoustics

The term bioacoustics refers to topics that cover the production, effect, and detection of sound by living organisms. A vast amount of literature is available on topics such as communication and learning in animals, but this introduction will focus on a much smaller subsection. Abundant literature on bioacoustics is available on marine invertebrates, including marine molluscs, but due to the differences in environment, it will not be discussed in this thesis. Further information on general bioacoustics can be accessed in the Bioacoustics journal, published by Taylor and Francis in association with The British Library Sound Archive ('Bioacoustics', n.d.).

When considering terrestrial invertebrates, as many are considered pests, there has been much interest in using sound to identify and monitor pest species, alongside damage caused, automatically (Cardim Ferreira Lima et al., 2020). Acoustic and optical (also referred as machine vision systems) methods are the most common techniques (Liu et al., 2017; Azfar et al., 2018). Modern equipment used to detect sound or ultrasound related to insects includes "accelerometers, piezoelectric sensors, microphones and ultrasonic transducers", where Liu et al (2017) provide a good account of technical equipment details. There has been much interest in using sound to identify and monitor pest species automatically, due to the push for streamlined integrated pest management (Cardim Ferreira Lima et al., 2020). This has been particularly successful with borer insects - the automatic detection of the red palm weevil (*Rhynchophorus ferrugineus*) has been commercialised in several countries (Hetzroni et al., 2016).

There is also interest in the impact of anthropogenic related sounds on invertebrates (Hugel, 2012; Buxton et al., 2018; Raboin & Elias, 2019). Considerably less literature is available on the impacts of human disturbance than is available for pest control.

3.1.2 Sounds in slugs

It is difficult to determine exactly how much food a slug has consumed, although difficulties in measuring food intake is not exclusive to slugs. Estimating grazing in roaming livestock is essential to improving forage grazing systems for animal husbandry. Previous techniques to estimate this were labour intensive and not cost effective, although reliable automated methods have since been suggested (Clapham et al., 2011; Navon et al., 2013; Tani et al., 2013; Andriamandroso et al., 2016). For slug pellet consumption, water evaporation and mucus contamination means that there is no reliable way to measure the weight of the pellet in an open system where the slug is free roaming. It is also not feasible to weigh the slug due to the effect on slug weight of evaporation (Prior et al., 1983; Hommay et al., 1998; Triebskorn & Ebert, 1989; El-Danasoury et al., 2016). When a slug feeds, food fragments are broken off by the buccal mass, the organ responsible for coordinated feeding and mastication (Mackenstedt & Märkel, 2001). Sounds of the food fragments being broken off can be recorded and quantified. Due to the difficulties in measuring slug/pellet weight, acoustic information produced during consumption can be used as a proxy for consumption, which allows comparison of different pellet types. The amount of substance a slug has consumed is important information for pellet developers and manufacturers, particularly to establish whether feeding occurs at all.

Acoustic techniques to quantify bite data were first used by Wedgwood and Bailey (1986). Pellets were glued to a piezo-electric gramophone pick-up, the disturbances were viewed on an oscilloscope and the recording played back on an audiocassette. A very similar method for recording the audio was used for later experiments, but instead of an oscilloscope and audiocassette, a BBC microcomputer was used (Wedgwood & Bailey, 1988; Mills et al., 1990; Bailey & Wedgwood, 1991), Table 3.1. Audio and video technology have improved considerably since the publication of this initial bioacoustics work. The methodology developed in this paper produces more detailed data on slug consumption, advancing the pioneering methods described above

Table 3.1 Published papers with acoustic methodology to detect bites in molluscs

	Wedgwood & Bailey (1986)	Wedgwood & Bailey (1988)	Mills et al (1990)	Bailey & Wedgwood (1991)	de Silva et al (2021)
Slug species		*A. hortensis, D. caruanae, D. reticulatum*	*Lymnaea stagnalis*	*Arion distinctus* and *D. reticulatum*	*D. reticulatum*
Slug collection	May – June	Un-baited tile traps between October and January			Hand collection and chicken feed baited traps, from a variety of field sites around Newcastle upon Tyne (UK)
Slug storage	plastic boxes lined with damp tissue paper cycle	plastic boxes 13 x 7 x 6 cm lined with damp paper towelling	5-gallon tanks of aerated tap water		plastic containers, lined with damp tissue paper
Storage conditions	12: 12 day: night cycle at 16:12 °C temperature cycle	12: 12 day: night cycle at 16:13 °C temperature cycle	17-21°C	reversed 12 : 12 night: day cycle at 13 - 16 °C in a	12:12 day: night cycle at 15 ° C for 24h only
Pre-conditioning	ad lib cabbage or carrot, starved overnight	Ad lib sliced carrot	Lettuce ad lib, starved 48 hours prior	Starved for 48 hours	Starved from the time of collection (36 – 24h)
Size	Slugs of similar size, smaller slugs discarded	250 - 450 mg	similar shell size of approximately 2-5 cm length were used	*D. reticulatum* 254 and 752 mg, *A. distinctus* weighing between 1I5 and 414 mg	Weight range available for reference, smaller slugs discarded when possible
Pellet composition	soft wheat flour, 0.1% dehydroacetic acid fungicide and 5% gelatine dissolved in warm water	maize flour (Cheshire Foods) 5% gelatine and warm water	maize flour was mixed with 50 g kg- ' gelatine and warm water	maize, wheat, pea or potato flours and 5% gelatine (some had 2%)	pre-made by industrial partners
Pellet formation	extruded through a spaghetti maker, dried and broken into 1 cm pellets	extruded through a spaghetti maker, dried and broken into 1 cm pellets (3mm diameter)	a spaghetti mixer and the 2-mm diameter strands allowed to dry in air. The strands were then broken into 2-cm length pellets and stored in tightly sealed jars.		commercial pre-made pellets
Active ingredient	metaldehyde (0, 2, 4, 6, 8%) or methiocarb (4%)	0, 0.5, 1, 2,4, 6, or 8% metaldehyde	0, 5, 10, 20, 40, 60, 80 or 120g metaldehyde	4% metaldehyde or methiocarb	metaldehyde (3%) and ferric phosphate 2.97

Experimental conditions	daytime at room temperatures	daylight at temperatures between 17 and 21 °C	diurnal phase at room temperature (17- 21 °C)	daytime in a darkened cabinet *D. reticulatum* 13.5 and 18 °C, *A. distinctus* weighing between 12.7 and 23.8 °C	daytime at room temperature (15–21 °C, 17–22 °C)
Experimental audio equipment	piezo-electric gramophone set up, the disturbances were viewed on an oscilloscope and the recording played back on an audiocassette	piezo-electric gramophone set up which amplified the sounds and fed into an audio cassette recorder and an oscilloscope - output was fed into a signal conditioner and BBC microcomputer	piezo-electric transducer and the amplified signal of a slug biting was recorded on a tape recorder and displayed on an oscilloscope		audio-sensitive plate was connected to an independent built-in power supply (PP3 9V battery) and a recording device (Tascam DR-05, TEAC Corporation, Guildford, UK)

3.1.3 Aims and objectives

The amount of substance a slug has consumed is important information for pellet developers and manufacturers. In Chapter 2, it was observed that slugs died where little or no food seemed to be consumed, and it was not possible to provide conclusive feedback if this was due to passing an active ingredient consumption threshold, physical contact with the pellet or natural causes with no relation to the active ingredient. This chapter was developed due to a desire to provide more accurate data on the amount of pellet consumed by slugs. Novel metaldehyde formulations use a silica matrix to coat pellets and therefore reduce the release of metaldehyde into water courses during rain events. The aim was to control the release of metaldehyde and to prevent slugs detecting the metaldehyde initially, and hence succumb to premature paralysis. In theory, this would allow more slugs to consume a lethal amount, resulting in a higher death rate and consequently less damage to crops. The overall objective of this paper was to assess the acceptability and consumption of experimental and commercial pellets, as a precursor for the successful commercial development of the novel products. Additionally, the methodology described in this chapter may be used to provide further insight to any novel slug or snail pellet produced. The objectives of the work reported in this chapter were:

1. Develop a standardised laboratory-based bioacoustics methodology to test the consumption of any pellet or formulation by *D. reticulatum* or any other slug species

2. Provide feedback on pellet acceptability and consumption in relation to the length of bites, number of bites, and general patterns of slug feeding

3. Compare novel pellet formulations to current commercially available products

4. Investigate whether the environment in laboratory trials had an impact on the results, as well as explore if it would be possible to use this methodology under field conditions

Multiple small-scale experiments to improve recording quality were carried out to achieve objective 1, resulting in the methodology below. [11]

[11] A professional recording room or studio would produce the highest quality recording. In lieu of these facilities a small quiet room was used. Future experiments should compare various locations and settings on the recording device to achieve a high recording quality.

3.2 Materials and methods

Two types of experiment were done. Laboratory trials involved recording the feeding of individual slugs to assess their feeding on all the formulations shown Table 3.2. Novel formulations were developed by Lucideon. Arena trials involved groups of slugs feeding on commercial formulations in a situation more similar to that where slugs encounter a pellet in nature.

3.2.1 Slug collection and maintenance

Slugs were collected, using hand collection and chicken feed baited traps, from a variety of field sites around Newcastle upon Tyne (United Kingdom), approximately 36 hours prior to use in trials. Prior to use, slugs were stored in plastic containers, lined with damp tissue paper to maintain humidity level, in a refrigerator (3 – 5 °C). The time spent in storage varied from 0 - 48 hours, within and between experiments. Twenty-four hours before the experiment, slugs were transferred to a controlled temperature room at 15 °C with a day:night cycle of 12:12. Pellets that were to be used in feeding trials were allowed to take up moisture from a damp filter paper under the same conditions for 24 hours (Table 3.2). Slugs were randomly assigned to a formulation treatment for both laboratory and arena experiments. Slugs were removed from the controlled temperature room approximately 30 minutes before entering the trial. No slugs in the results described were preconditioned (i.e. no dummy pellets were used).

Table 3.2. Text reference, formulation name and AI percentage (w/w) for pellets used in laboratory and area trials

Text reference	Formulation	AI percentage (w/w)
Non-toxic control	Axcela ® cereal	
Commercial metaldehyde	Axcela ® metaldehyde	3
Novel non-toxic control	Silica coated Axcela cereal	
Novel metaldehyde	Silica coated Axcela metaldehyde	3
Commercial ferric phosphate	Sluxx HP ®	2.97

3.3.2 Laboratory trials

A plastic box (57 x 39 x 28 cm) was used to house the recording equipment (Figure 3.1). Acoustic foam (10 cm acoustic foam panel - Advanced Acoustics AS4) lined the interior of the plastic housing and accompanying lid forming a sound-proof chamber. An audio sensitive plate, hereafter referred to as the sensor, was used to record sounds produced. The sensor had an independent built-in power supply (PP3 9V battery) and was connected to a recording device (Tascam DR-05- TEAC Corporation). The sensor, power supply unit and Tascam DR-05 were placed on the bottom surface of the acoustic foam chamber. The apparatus was housed in an isolated room and no other work was conducted in the laboratory during the recordings. Simultaneous video recordings were taken using cameras (Samsung video camera, SCB_2001), an Inspire four-channel digital security recorder (INS-DVR04V2-250) and an infrared light.

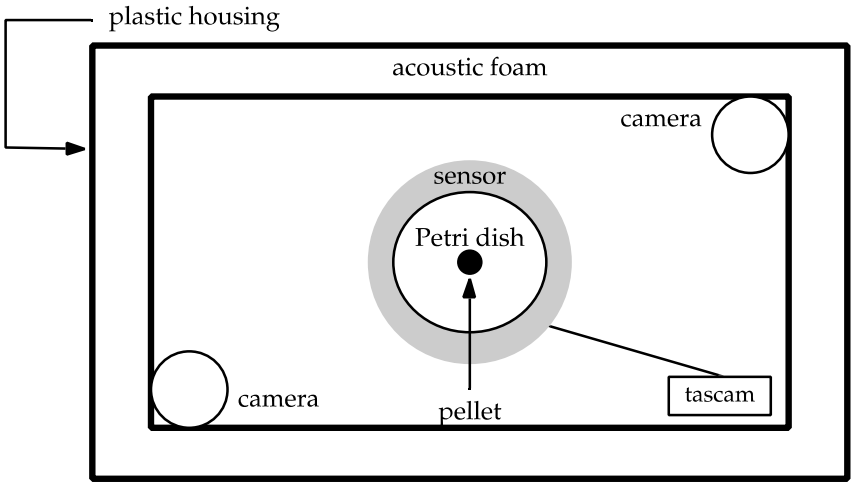

Figure 3.1 Audio and video equipment used to record bioacoustics laboratory trial experiments, not to scale

After the recording equipment was started, the Petri dish (9cm) containing the pellet, glued to the base, was placed into the centre of the sensor and, if necessary, the cameras were refocused on the pellet. Pellets were glued to ensure good contact with the base of the Petri dish and minimize noise. A single slug was placed in the Petri dish, where the locations of placement were constant throughout all trials. The lid of the Petri dish was then placed, upside down, on the base, to prevent slug escape without hindering visibility. Each trial was allowed to run for 30 minutes regardless of pellet discovery or consumption. Laboratory trial recordings were analysed for ten minutes, beginning immediately after the first bite. Recordings where no bites were taken were not analysed any further. No slug was fed on more than one pellet type and a new pellet and petri dish were used in each trial. The identifying slug number, pellet type and experiment number were recorded before the start of each trial. After every third trial a five-

minute recording of the background noise level was taken, without a slug present (control recording). To maintain a stable temperature (17°C – 22°C) within the setup the lid of the box was left open for at least 30 mins before the next trial. Thirty replicates were run for each formulation (Table 1) with treatment order dictated by a randomised block design. If any disturbance was noted, the trial ended, and a replacement trial run afterwards. There were no observed slug mortalities during experimentation.

3.3.3 Arena trial

A plastic box (50 x 77 x 19 cm) was filled with soil (to a depth of 13 cm), that was collected from Cockle Park Farm, Morpeth (NE61 3DZ). The soil was a sandy loam texture from the Rivington series. This is typical of many agricultural soils and supports a range of arable crops. A well was formed in the middle of the box and a cylindrical plastic container that matched the diameter of the sensor was used to support it at the level of the soil (Figure 2). This was done to prevent the moist soil from damaging the plate. The wired power supply and recording device were elevated on a shelf above the arena. The soil was kept visibly damp, but not wet, using distilled water. The arena was housed in an isolated laboratory at room temperature (15°C – 21°C). No other work was conducted in the laboratory during the recordings. Simultaneous video recordings were made of slugs under study using cameras (Brinno – TLC 200 Pro) and an infrared light; the camera was fixed to allow the entire arena to be seen in the viewfinder. The dampened pellets were glued to a microscope cover slip placed onto the centre of the sensor immediately prior to the slugs entering the trial.

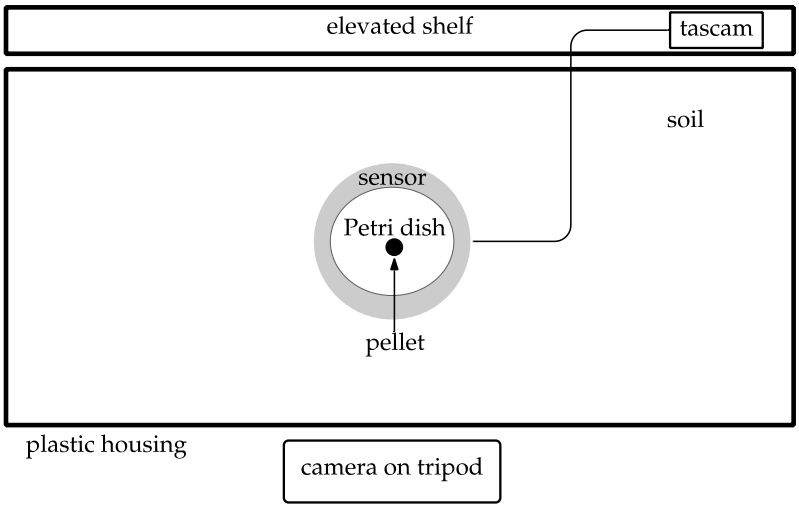

Figure 3.2 Audio and video equipment setup used to record bioacoustics in arena experiments, not to scale

Slugs were removed from the control temperature room approximately 30 minutes before the recording started. Twenty slugs were placed on the soil; the location of slug placement was constant throughout all trials. Trials were set up around 16:00 – 17:00 and allowed to run into the next morning. Each trial was allowed to run regardless of pellet discovery or consumption. Immediately before or after each trial a five-minute control recording was taken. Five trials were run for each of three formulations: non-toxic control, commercial metaldehyde, and commercial ferric phosphate, in a randomised block design.

3.3.4 Audio analysis

Waveform Audio (WAV) files for each session were imported into Audacity 2.3.0 - Audacity®. The control recording was used to create a noise profile for every session which allowed for background noise reduction. Individual trial WAV files containing only the recording for one experiment were created. The beginning of the data collection was defined as commencing after the acoustic box was closed, perceivable from both the audio and video records. Laboratory trial recordings were trimmed into ten-minute sections that began immediately after the first bite. Recordings where no bites were taken were not analysed any further. In arena trials, the audio recording analysed was from 18:00 to 06:00 the next day. If feeding began before the defined beginning point, these bites were disregarded. Individual trial recordings were imported into a graphical.mlapp application for analysing slug bites which recorded the length of each bite.

3.3.5 Statistical methods

All statistical analysis was conducted using IBM SPSS Statistics 26.0.

Laboratory trials

To determine if there was a difference between the mean length of bite between treatment groups, and between individual slugs within treatment groups, a nested ANOVA was used. Outliers were measured by the inspection of a boxplot, where if there were any present, they

are described. Groups were checked for normality and homogeneity of variances using the Shapiro-Wilk ($p > 0.05$) and Levene's Test ($p > 0.05$). Neither are reported unless the assumption is violated. Data is presented as mean ± standard deviation. For analyses that showed statistical significance a Tukey HSD Test was used to identify significant differences between pairs of means. A Kruskal-Wallis test was conducted to determine if there were differences in the number of bites between treatments groups. Distributions of bites was not similar as assessed by visual inspection of a boxplot. Pairwise comparisons were performed using Dunn's (1964) procedure with a Bonferroni correction for multiple comparisons. Adjusted p-values are presented. A Fisher's exact test (2 x c) procedure with Monte Carlo simulations was performed to examine the relationship between the number of bites taken during 60 second intervals between pellet formulations. Multiple Fisher's exact tests (2 x 2) were run to determine which pellet groups significantly differed.

Arena trials

A Welch ANOVA was conducted to determine if there were differences in the number of bites between treatments groups. Distributions of bites were not similar as assessed by visual inspection of a boxplot. A Games-Howell post hoc analysis was used to determine pairwise comparisons. A Chi-square test of homogeneity was performed to examine the relationship between the number of bites taken during 60-minute intervals between pellet formulations. A z-test of two proportions with a Bonferroni correction for multiple comparisons was performed to produce pairwise comparisons.

3.4 Results

3.4.1 Laboratory trials

An equal number of trials for each formulation was run, but the number of recordings where slugs fed (useable recordings) varied (Table 3.3). Recordings where no bites were recorded were not suitable for analysis.

Table 3.3. Number of useable and total bioacoustics recordings in laboratory trials

Formulation	Useable recordings	Total recordings
Non-toxic control	24	30
Commercial metaldehyde	11	30
Novel non-toxic control	7	30
Novel metaldehyde	9	30
Commercial ferric phosphate	10	30

Length of bite

The mean lengths of slug bites between different formulations are shown in Figure 3.3. There were no significant differences in the length of slug bites between different formulations ($F_{4, 268} = 1.012$, $p = 0.402$) but there was a difference between individual slugs within formulations ($F_{56, 731} = 1.496$, $p < 0.05$).

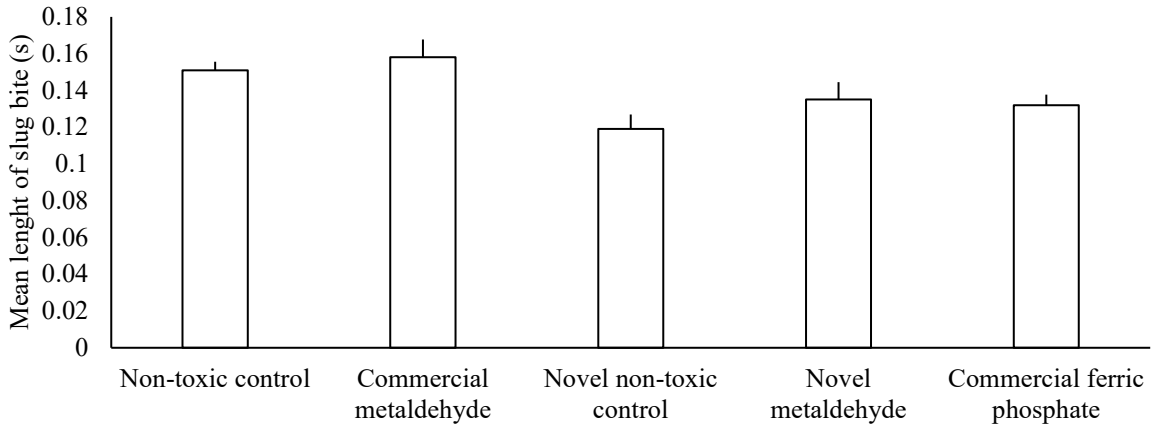

Figure 3.3 Mean lengths (+ SD) of slug bites between groups non-toxic control (n=24), commercial metaldehyde (n=11), novel non-toxic control (n =7), novel metaldehyde (n=9) and commercial ferric phosphate (n=10)

Number of bites

The number of bites differed between formulations, as assessed by visual inspection of a boxplot (Figure 3.4) with this difference being found to be statistically significantly, $\chi 2$ (4) = 24.219, $p < 0.001$.

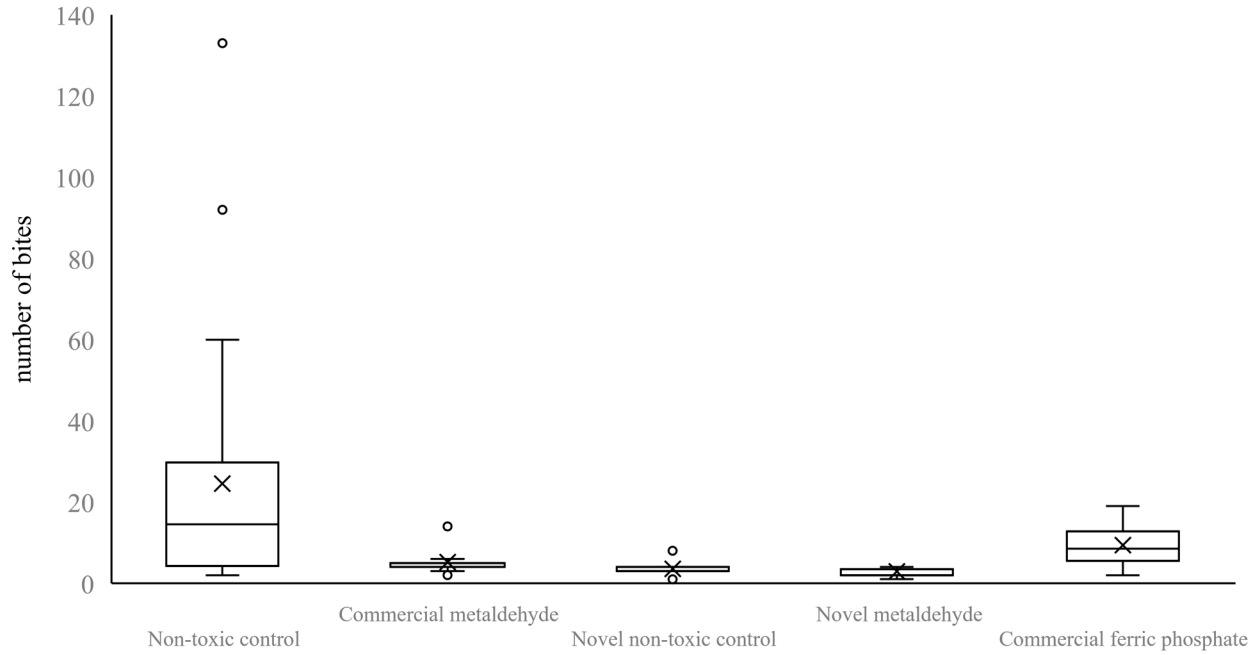

Figure 3.4. Mean number of slug bites on non-toxic control (n=24), commercial metaldehyde (n=11), novel non-toxic control (n =7), novel metaldehyde (n=9) and commercial ferric phosphate (n=10) in laboratory trials. The box indicates the upper and lower quartiles, the whiskers indicate the range of data outside the upper and lower quartiles. The black cross within the box indicates the mean.

The number of bites at each 60 second time interval between pellet groups was statistically significantly different, $p < 0.01$ (Figure 3.5). There were significant differences between all groups ($p < 0.05$) except for commercial metaldehyde and commercial ferric phosphate, as well as novel non-toxic control and novel metaldehyde.

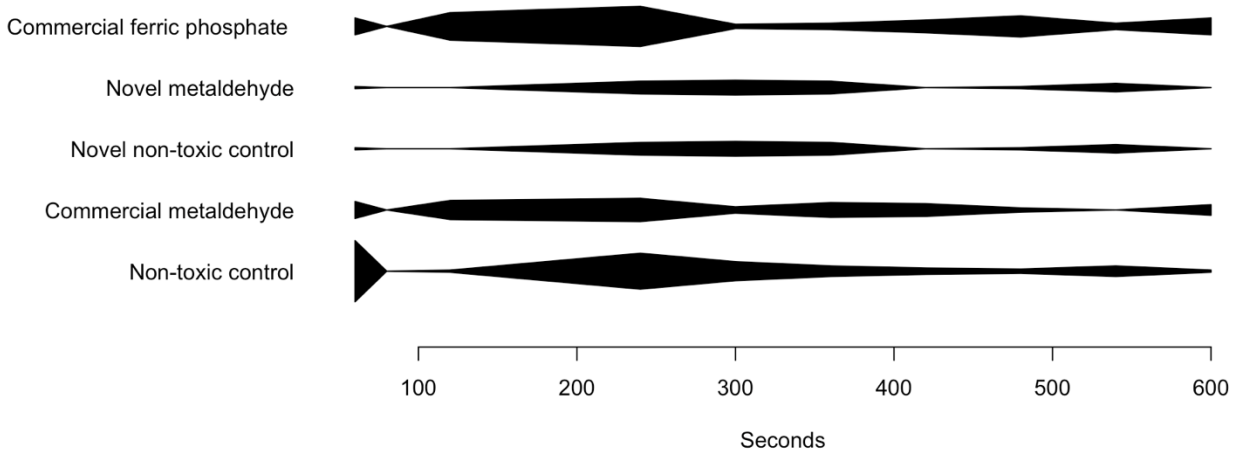

Figure 3.5 Total number of slug bites on non-toxic control (n=5), commercial metaldehyde (n=5) and commercial ferric phosphate (n=4) in laboratory trials. Thickness along the x-axis indicates relative number of bites at time point along y-axis (seconds).

3.4.2 Arena trials

Due to constraints on equipment access it was not possible to run an equal number of recordings across the arena trials, where for the non-toxic control n = 5, the commercial metaldehyde treatment n = 5 and for the commercial ferric phosphate n =4. However, all recordings were usable ensuring adequate levels of replication for analysis.

Length of bite

The arena trial was not conducted in a sound proofed environment which resulted in a lower quality of recording. Increased background noise or external sounds combining with the recording of the bites led to the recording of exceptionally high values (> 0.4 seconds). This may also be due to merging of several bites in quick succession or noise combining with the recording of the bite. The frequency of these outlying values was far greater in the arena trials than in the laboratory trials. Due to this, the length of bites for arena trials has not been analysed. It is not anticipated that there would be a significant difference in the length of slug bites between pellet types in the arena trials, where when disregarding all bites over > 0.4 seconds, the average length of bites for the laboratory and arena trials was similar.

Number of bites

The number of bites between pellet groups was statistically significantly different (Welch's $F_{(2, 5.118)} = 5.790$, $p = 0.049$). Statistically significant differences were observed between the non-toxic control and commercial metaldehyde, as well as between the non-toxic control and commercial ferric phosphate (Figure 3.6).

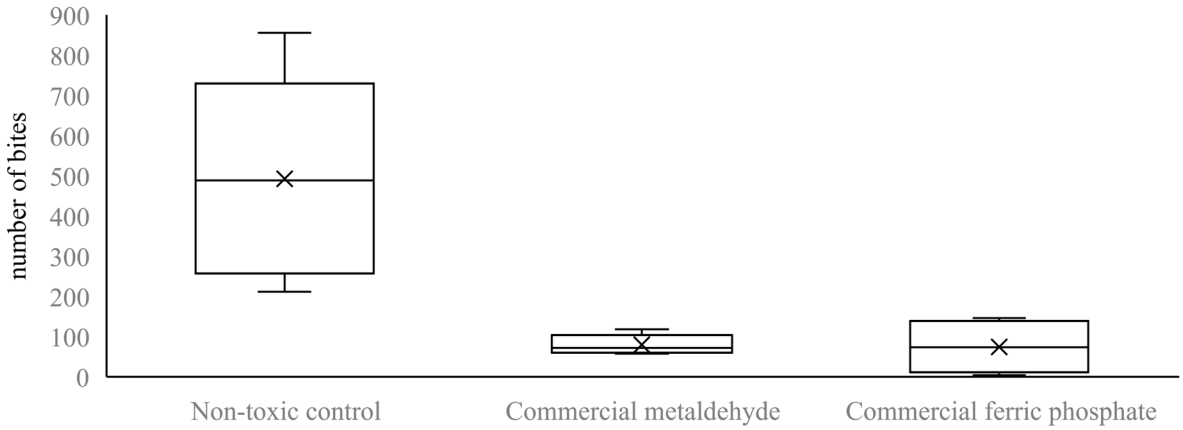

Figure 3.6 Mean number of slug bites on non-toxic control (n=5), commercial metaldehyde (n=5) and commercial ferric phosphate (n=4) in arena trials. The box indicates the upper and lower quartiles, the whiskers indicate the range of data outside the upper and lower quartiles. The black cross within the box indicates the mean. Circles and crosses outside of the box show outlying values.

The number of bites between pellet groups was statistically significantly different, $p < 0.01$ (Figure 3.7).

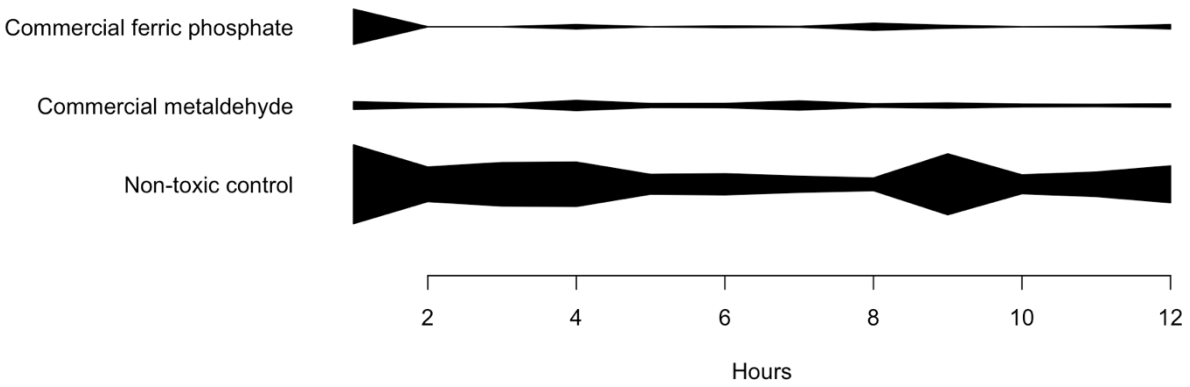

Figure 3.7 Total number of slug bites on non-toxic control (n=5), commercial metaldehyde (n=5) and commercial ferric phosphate (n=4) in arena trials per hour. Thickness along the x-axis indicates relative number of bites at time point along y-axis (seconds).

There were statistically significant differences between the numbers of bites taken within each hour (Table 3.4)

Table 3.4 Different letters denoting groups (time in hours 1-12) that differ significantly between pellet categories (non-toxic control, commercial metaldehyde, commercial ferric phosphate) in arena trials

Time (hours)	Non-toxic control	Commercial metaldehyde	Commercial ferric phosphate
1	a	b	c
2	a	a	b
3	a	b	c
4	a	b	a
5	a	a	b
6	a,b	b	a
7	a	b	c
8	a	b	c
9	a	b	b
10	a	a	b
11	a	a,b	b
12	a	a	a

3.5. Discussion

In the experiments described, we found that *D. reticulatum*, when feeding on a range of pellets, did not show any change in the length of the bites taken between formulations, but did make fewer bites on pellets containing toxins or coated with a silica matrix. Slugs found non-toxic control pellets the most acceptable, resulting in significantly more usable recordings for this formulation. The lower acceptance of toxic pellets observed in laboratory trials is finding that differs from the literature, where slugs were reported to nearly always accept pellets, regardless of molluscicide presence (Wedgwood & Bailey, 1988). This could be due to different molluscicide formulations being used between studies, general variation between the slug populations used, or variability in experimental conditions. Additionally, previous experiments screened slugs to select those more likely to feed through the use of a dummy pellet (Wedgwood & Bailey, 1986, 1988; Bailey & Wedgwood, 1991; Mills et al., 1990). A dummy pellet (any non-toxic pellet) is one that would be offered to a slug before entering the trial to test a willingness to feed, with only slugs willing to feed on the dummy pellet entering the trial. The use of a dummy pellet could mask the true results of acceptability between pellet types, by selecting slugs more likely to feed, rather than representing a varied feeding spectrum, as would be found in field conditions. These previous studies do not disclose how many slugs were excluded after refusing the dummy pellet to obtain the results, so we are unable to compare our results on acceptance with other work (Wedgwood & Bailey, 1986, 1988; Mills et al., 1990; Bailey & Wedgwood, 1991). Our unpublished preliminary work found that the use of a dummy pellet prior to a trial was not useful for increasing the number of usable recordings for toxic or novel pellet types. It was observed that slugs that would accept a dummy pellet (non-toxic control) would reject a novel pellet or toxic pellet offered immediately after.

The least accepted pellet[12] was the novel non-toxic pellet, suggesting that slugs are deterred from feeding by the novel components in the formulation, or from a by-product formed during the coating process. The main materials used to produce the novel coating were silica-based products (S. Newman, personal communication). Increased silica or silicon content has been observed to cause general health problems in animals, including molluscs (Mayland & Shewmaker, 2001; Massey & Hartley, 2009; Selvi et al., 2015; Hartley & DeGabriel, 2016; Jeer et al., 2018). In slugs, increased silicon content in food was suggested to cause reduced consumption (Wadham & Parry, 1981; Griffin et al., 2015). This may be due to decreased leaf digestibility or wear on the feeding apparatus, with similar effects observed in other invertebrates (Jeer et al., 2018; Wadham & Parry, 1981; Griffin et al., 2015). However, the Golden Apple Snail, *Pomacea canaliculata*, showed no feeding aversion to plants with higher silicon content, indicating that an increased silicon content may not affect feeding in all mollusc species, and that any reduced feeding may be due to other factors (Horgan et al., 2017). Due to the nature of renewable teeth in molluscs, it could be argued that wear on the radula would only be a minor inhibition to feeding (Horgan et al., 2017; Krings et al., 2019). It is possible that the particle size of the silica in the novel products may be so fine that the product is not degraded further by the radula and that other components of the novel pellet may be the cause of aversion (S. Newman, personal communication). Further work comparing formulations with silica of different particle sizes could provide a better explanation of the results.

In previous work on slugs, bite size has been estimated from the body weight of the slug; slugs were allowed to take a set number of bites, from a known quantity of food, before the meal was interrupted and the food reweighed (Wedgwood & Bailey, 1988; Bailey & Wedgwood, 1991). When feeding on a non-toxic pellet, it was found that the bite size was not directly proportional to slug weight, and an allometric relationship was observed in *Deroceras* species (Wedgwood & Bailey, 1988; Bailey & Wedgwood, 1991). Bite size as also been shown to vary with the concentration of metaldehyde in a pellet, and between slug species (Bailey & Wedgwood, 1991). Due to the difficulties in accurately measuring pellet and slug weight, we suggest that the size of the bite may be better reflected in the bite length (time where sound is produced during each bite). Longer lasting bites may reflect a higher food volume intake per bite, while shorter bites reflect a lower volume. While this has not been proposed before, there is merit in comparing the same characteristic (bite length) between formulations. One explanation for previously observed variation in bite size is a difference in consistency between pellets. Pellets

[12] Least accepted pellet refers to the pellet the least number of slugs accepted or fed on after initial contact

of a softer consistency allow for greater volume in each individual bite, resulting in a greater total volume consumed in a fewer number of bites (Bailey & Wedgwood, 1991). As all pellets in this experiment were more or less of the same consistency (subject to minor manufacturing variations), this may explain why no difference was observed between the length of bites between formulations. There was, however, a difference between the length of bites between individual slugs feeding upon the same formulation. This could be related to the size of the slug, which varied within formulations.

As the lengths of bites between pellet types were similar, it can be assumed that variation in the amount consumed comes from the number of bites taken. A greater number of bites (assuming each at a similar volume) would suggest a larger (and usually longer) meal with more of the pellet consumed (Bailey & Wedgwood, 1991). However, meal length cannot necessarily be considered a reliable indicator of consumption of pellets of different hardness, as softer pellets would be more readily consumed than harder pellets (Wedgwood & Bailey, 1988; Bailey & Wedgwood, 1991). In our experiments, meal length was not considered as slugs were only observed in the laboratory trial for a set period. Further arena trials, with individual slugs feeding freely, may present a greater understanding of meal length. Similarly, video recordings have been suggested to overestimate the length of the meal if the time in contact with the pellet was considered as the feeding time (Bailey & Wedgwood, 1991). The consumption of pellets containing metaldehyde causes slug paralysis (Bourne et al., 1990; Mills et al., 1990; Bailey & Wedgwood, 1991; Triebskorn & Florschütz, 1993; Campbell et al., 2021) and this could easily be misinterpreted by video methods as feeding if the slug were to be paralysed in contact with the pellet. All slugs in the novel non-toxic control in laboratory trials were observed to have made physical contact with the pellet, however, only 23% were recorded taking bites from the pellet. Ferric phosphate was not observed to cause immediate paralysis due to a different mode of action, which may result in more reliable video analysis.

In the current study, the presence of a toxin (or novel component) decreased the palatability (number of bites taken) of a food substance, similar to the results found by other authors (Wedgwood & Bailey, 1986, 1988; Bailey & Wedgwood, 1991). The paralysis of the feeding apparatus immediately after metaldehyde consumption may be the cause of low bite numbers in commercial and novel metaldehyde pellets (Wedgwood & Bailey, 1988). No difference was observed in the number of bites taken between commercial metaldehyde, novel metaldehyde, or the novel non-toxic control, suggesting that the novel pellets used in this study are not better at improving the palatability of pellets. No difference in the number of bites was observed

between commercial metaldehyde and commercial ferric phosphate. However, this does not suggest that the products are equal when considering their usefulness as molluscicides, due to different modes of action. Metaldehyde and ferric phosphate pellets are suggested to require distinct levels of consumption to be effective as molluscicides (Speiser & Kistler, 2002). Due to different modes of action, after a threshold amount is consumed, slugs were observed to take longer to die when consuming ferric phosphate when compared to metaldehyde. The observation of slugs that fed on any of the toxic pellets two to three days after the laboratory trials suggested that they did not consume enough pellet to cause mortality during the trials (Campbell, 2020). Obtaining a greater number of recordings of toxic pellet types would improve the interpretation of these results. Further work could include longer feeding sessions to understand the relationship between the number of bites of a toxic pellet and paralysis, as well as mortality.

The main purpose of arena trials was to determine whether the artificial conditions in laboratory experiments had an impact on consumption. We were not able to collect any useable recordings with novel pellet types. Preliminary work suggested that there may be difficulty collecting useful results due to lower chances of a slug encountering a single pellet while in the arena. This could be remedied with the use of multiple sensors with multiple food sources, increasing the likelihood of a slug encountering a pellet. Due to constraints on equipment and time, multiple sensors could not be investigated, but multiple slugs were used in each arena trial. Slugs took significantly more bites from non-toxic control pellets than from commercial pellets containing active ingredients, similar to results observed in the laboratory trials. While it is not anticipated that the results would differ greatly, further work with individual slugs in an arena trial would allow the laboratory and arena experiments to be directly comparable. A further improvement of the arena trial methodology would be to conduct feeding experiments in a specialised recording room to improve sound quality and compare the results with the method presented in this paper, or those taken in the field. It may also be possible that the feeding behaviour of slugs may have been altered by the temperature and humidity conditions within the laboratory (Dainton, 1954; Wareing & Bailey, 1985; Grimm & Schaumberger, 2002). Due to noise from air-handling units, it was not possible to conduct experiments in a controlled temperature room; the methodology could be improved with the use of a noise-reduced controlled temperature room.

3.6 Conclusion

Within the limits of this study, no commercially useful differences between toxic pellet types were observed. Poor acceptance and the consumption of the novel non-toxic control suggests that the components of the formulation deter feeding. Based on the results above, the reformulation of the novel pellet would be suggested and further work into the individual components of the novel formulation may be useful to the manufacturers. An ideal toxic pellet would show consumption similar to the non-toxic control. The methodology described has been useful in comparing pellet formulations and may be of use in the development of future pellet formulations.

Chapter 4: Leaching

4.1 Introduction

4.4.1 General pesticide use

The use of any pesticide in the UK must be authorised by the Chemicals Regulation Division following a range of pesticide regulations. These are in place, amongst other reasons, to protect human health and the environment. There are often different regulations governing usage depending on if pesticides are used in a home garden or in a professional capacity, such as in agriculture and horticulture ('Chemicals Regulation Division', n.d.).

All pesticides are subject to a range of tests, including those that cover, but are not limited to, toxicity, degradation rates and leaching ('Pesticides: The basics', n.d.). However, many pesticides are produced in liquid form, with spray application being the most common application method and the testing protocols reflect this. Protocols that are relevant to liquid pesticides may not yield accurate or useful information for those manufactured and applied in alternate forms, such as pellets and seed coatings (Keighley et al., 2021).

4.1.2 Pesticides and water

Pesticides from professional use can be detected in bodies of water, such as rivers, tributaries and groundwaters. Over time, as concern for the effect of pesticides on health and the environment has grown, increasingly stringent regulation governing usage has been implemented. A set standard of $0.1\mu g/l^{-1}$ per individual pesticide and $0.5\mu g/l^{-1}$ total pesticides (considering all the substances detected in a sample) was set as the limit by the European Drinking Water Directive ('Drinking water legislation - European Commission', n.d.). Despite this, agriculture and associated practices are the principal cause of water quality failure in the UK and EU member states (Jönsson et al., 2014). Entry of diffuse pollutants, including metaldehyde, to water bodies is difficult to regulate due to difficulties identifying a single source of contaminants and the consequent shared management-responsibility between agricultural landowners and water companies (Ibrahim et al., 2017; Asfaw et al., 2018). Metaldehyde presents a major problem to the water industry. In 2009, peak metaldehyde concentrations up to $0.1\mu g/l^{-1}$ in raw waters resulted in 73 water treatment works failing the drinking water pesticide standard ('Emerging Pesticides; What Next?', n.d.).

4.1.3 Metaldehyde and water

Preventative measures to limit metaldehyde concentration in potable water include reducing the amount of metaldehyde applied to fields and managing the amount of metaldehyde in water taken in for treatment from the source (catchment management); rather than collecting all standards of water and relying on water treatment after collection (Ibrahim et al., 2017, 2019; Le Moigne & Short, 2019; Cooke et al., 2020; Whelan et al., 2020). Product substitution, where the substitution was taken up by farmers, was shown to be effective at limiting the amount of metaldehyde applied to land (Lu et al., 2017; Ibrahim et al., 2019). Notably, investment in specialist advisors, consideration of commercial interests, transparency of risks and active communication were key to improving involvement in successful product substitution trials (Ibrahim et al., 2019; Hobbs., 2021). For metaldehyde mitigation case studies conducted by water providers in the UK see Ibrahim et al (2017) and Ibrahim et al (2019) for Anglian water, Le Moigne & Short (2019) and Lu et al (2017) for Thames water, and Cooke et al (2020) for Severn Trent water. Product substitution is discussed further in Chapter 5.

Detecting metaldehyde, at source within a timeframe to manage water entering the drinking water system, is difficult and cannot be completely relied upon (Castle et al., 2018). A quick turnaround time is essential for catchment management, however manually collecting a sample and subsequent laboratory analysis generally does not allow for timely management (Rabiet et al., 2010; Gong et al., 2018). The opportunity to manage water with a high metaldehyde content would have passed in the time the result from routine sampling is available. Routine sampling is relatively low cost, but to detect metaldehyde 'surges' associated with rainfall events, increasing the number of samples taken is necessary, which come with associated costs. Increased routine sampling generally provides a clear retrospective view of metaldehyde levels but does little for future management. Automatic samplers and sensor-based sample methods are available but are not typically used in the field due to maintenance and access difficulties. For a review on catchment management strategies see Davey et al (2014). More recently developed passive samplers tend to be favoured over spot sampling due to being low cost and easy to deploy in field, due to not requiring accompanying specialist equipment or energy (Sánchez-Bayo et al., 2013; Townsend et al., 2018). In 2018, a passive sampler (trade name - Chemcatcher®) was developed to detect metaldehyde in surface waters. For a comprehensive account of developing a passive metaldehyde sampler, see Castle (2018). For a more complete

review of passive sampling technology for a broad range of pesticides see Vrana et al (2005), Namieśnik et al (2005) and Valenzuela et al (2020). For a more complete review of monitoring metaldehyde see Kay & Grayson (2013), and for a comparison of metaldehyde monitoring methods see Castle et al (2019).

Removal of metaldehyde from water

Once present in water, metaldehyde is generally difficult and expensive to remove. Preventing metaldehyde from entering the system is therefore commercially preferable to removal, although removal *per se* is possible. The absorption of metaldehyde by activated carbon beads has been shown to reduce the metaldehyde concentration in water (Tao & Fletcher, 2013). However, the level of metaldehyde absorption (71 mg g^{-1}) may be too low, with too high a cost for commercial interest. Variations of this method show stronger absorption, but again may be at too costly to be commercially viable (Busquets et al., 2014; Salvestrini et al., 2017; Rolph et al., 2018). However, these methods may be beneficial if considering the benefit of removing other general pollutants as well as metaldehyde (McKay et al., 1985; Baudu et al., 1991; Salvestrini et al., 2017; Jeirani et al., 2017).

Degradation of metaldehyde by advanced oxidation processes is possible and includes use of ultraviolet (UV) irradiation to produce unstable radicals from titanium dioxide and hydrogen peroxide. For further information on the chemical process see Autin et al (2012). In waters that include organic matter, UV and hydrogen peroxide performed better than UV and titanium dioxide (Autin et al., 2012, 2013). Further work on the effective degradation of metaldehyde has been presented, but this method has not been pursued on a large scale due to costs associated with removal, and issues surrounding secure by product disposal (Jefferson et al., 2016; Semitsoglou-Tsiapou et al., 2016; Tang et al., 2016; Nguyen et al., 2017).

Two microbes that degrade metaldehyde have been identified from the genera *Acinetobacter* and *Variovorax* (Thomas et al., 2013; Thomas, 2016). Since this time, other strains have been isolated, and factors that impact their microbial capacity to degrade metaldehyde have been identified (Balashova et al., 2020; Castro-Gutiérrez et al., 2020). Nevertheless, it is thought that it is unlikely that the capacity of soil microbes will be sufficient, or could be depended upon, to maintain metaldehyde at acceptably low levels in agricultural or horticultural situations. Further unpublished work on biological metaldehyde degradation includes Fuller (2021, personal communication).

4.1.4 Aims

The amount of metaldehyde leaching from a pellet is of great interest to pellet developers and water industry stakeholders. Little information on leaching of pellets is provided by manufactures and current environmental fate data may not accurately reflect leaching of pellets in the field.

This work reported in this chapter was done to provide data on whether the novel formulations used in this project released metaldehyde more slowly than currently commercially available pellets. Novel metaldehyde formulations use a silica matrix to incorporate metaldehyde, or to coat pellets, with the aim of slowing the release of metaldehyde into water courses during rain events. The objective of the work reported in this chapter was to:

1. Compare leaching of novel pellet formulations to current commercially available products

Due to limited access to facilities during the COVID-19 pandemic, it was not possible to complete the analysis of some experiments (4C).

4.2 Materials and methods

4.2.1 Soil column preparation

The soil was collected from Cockle Park Farm, Morpeth (NE613DZ). The soil was a sandy loam texture, and the series was Rivington. The soil was air-dried, and the finer particles were separated using a 1/16-inch sieve. Larger clumps and particles were discarded. Soil columns were created using a 2.2cm x 22cm capacity glass syringe barrel. Each column contained 35g of sieved soil. Burrette clamps were used to hold the barrel in place, with the end of the tip inserted around 0.5 cm into a conical flask.

To prevent an excess of dry soil in the leachate, the tip was temporarily sealed using tape. Soil columns were rehydrated with 100ml of distilled water, added incrementally. The tape was removed once the soil was completely saturated, and the columns were allowed to drain for 3 hours under laboratory conditions. The pellet or power was placed on the surface and 15ml of distilled water was then poured over and the leachate collected in a clean conical flask. Table 4.1 sets out the details of the different experiments.

The experiments did not take place simultaneously as each consecutive experiment was devised based on the results of the previous.

Table 4.1 Experiments 4A – C, including text reference, metaldehyde concentration, formulation name and number of replicates used

Experiment	Text reference	Metaldehyde concentration % w/w	Formulation name	Replicates
4A	Commercial metaldehyde pellet	3	Axcela metaldehyde	5
4A	Commercial control pellet		Axcela cereal	5
4A	Novel control pellet		iCRT3 Axcela cereal	5
4A	Novel metaldehyde pellet	3	iCRT3 Axcela metaldehyde	5
4A	Novel metaldehyde powder 1	3	iCRT1 Axcela metaldehyde	5

4B	Distilled water			1[6]
4B	Soil column water			1[6]
4B	Novel metaldehyde pellet		Axcela metaldehyde	1[13]
4C	Commercial metaldehyde pellet	3	Axcela metaldehyde	3
4C	Commercial control pellet		Axcela cereal	3
4C	Novel control pellet		iCRT3 Axcela cereal	3
4C	Novel metaldehyde pellet	3	iCRT3 Axcela metaldehyde	3
4C	Distilled water			3

4.2.2 Leachate analysis

Metaldehyde content was determined Nathan Keighley (Fera Science) using LC-MS analysis methodology. The methodology used can be referred to in Keighley et al, (2021).

4.2.3 Statistical analysis

A Kruskal-Wallis H test was performed. Pairwise comparisons were performed using Dunn's (1964) procedure with a Bonferroni correction for multiple comparisons. Adjusted p-values are presented.

[13] Two of three replicates not suitable for analysis

4.3 Results

4.3.1 Experiment 4A

Novel metaldehyde powder 1 was included in Figure 4.1 but was not included in analysis (see section 4.4).

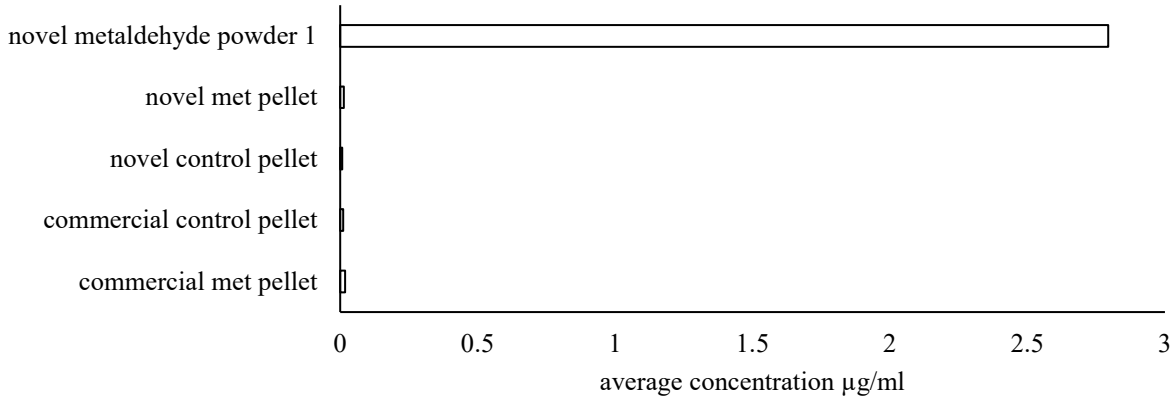

Figure 4.1 The average concentration of metaldehyde detected in leachate samples from novel metaldehyde powder 1 (n=5), novel metaldehyde pellet (n=5), novel control pellet (n=5), commercial metaldehyde pellet (n=5), commercial control pellet (n=5)

Metaldehyde concentration was statistically significantly different between novel control pellet and commercial metaldehyde, $\chi^2(3) = 12.148$, $p < 0.05$, Figure 4.2

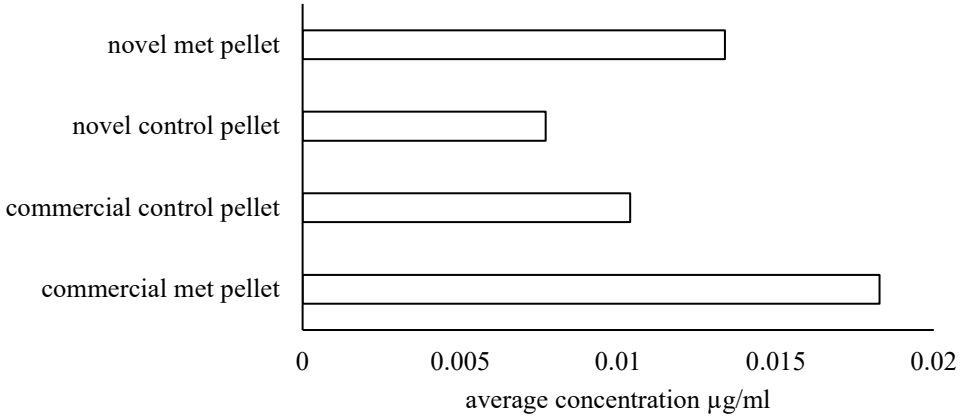

Figure 4.2 The average concentration of metaldehyde detected in leachate samples from novel metaldehyde pellet (n=5), novel control pellet (n=5), commercial metaldehyde pellet (n=5), commercial control pellet (n=5)

4.3.2 Experiment 4B

Metaldehyde was only detected in commercial metaldehyde pellet samples, Figure 4.3.

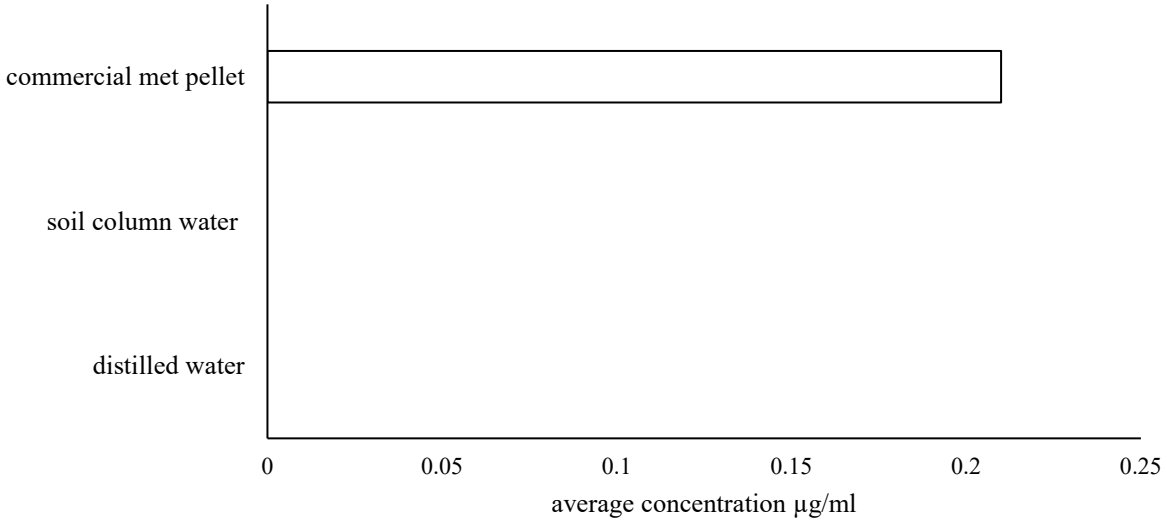

Figure 4.3 Average concentration of metaldehyde detected in leachate samples from distilled water (n=1), soil column water (n=1), commercial metaldehyde pellet (n=1)

4.3.3 Comparison of 4A and 4B

More metaldehyde was detected in the 4B commercial metaldehyde pellet sample than the 4A commercial metaldehyde pellet, Figure 4. 4.

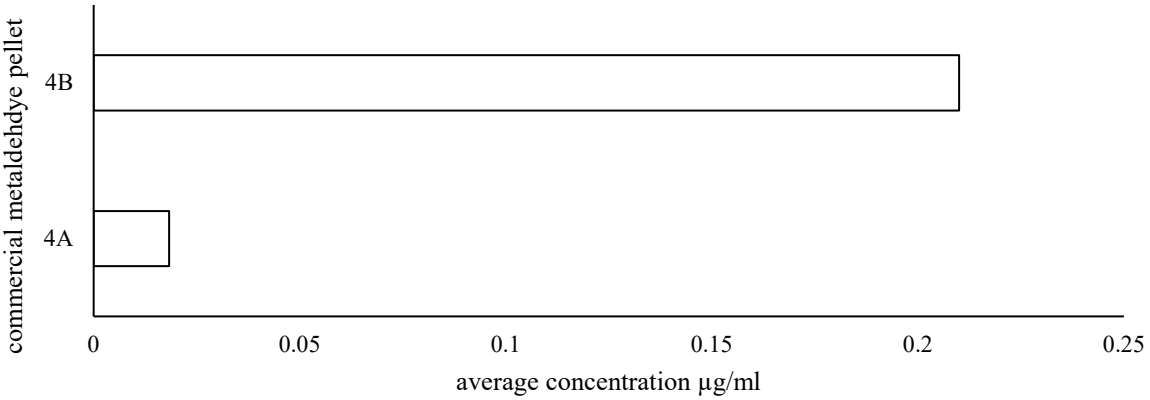

Figure 4.4 The concentration of metaldehyde detected in leachate samples from commercial metaldehyde pellets in 4A and 4B

4.4 Discussion

Novel metaldehyde pellets did not leach less than commercial metaldehyde pellets. Commercial metaldehyde pellets leached significantly more than novel non-toxic control pellets. No other differences in metaldehyde leachate were observed in Experiment 4A. Novel control powder 1 was included in Figure 4.1 but was not included in analysis because there is a substantial difference between the concentration of metaldehyde detected in the novel metaldehyde powder 1 and the other formulations. This may be due to powder formulation, which may have moved directly into the leachate. As a result, the value for novel metaldehyde powder 1 may not be comparable with the pellet formulations and was excluded from further analysis.

Unexplained metaldehyde was detected in the leachate for non-toxic pellet types in experiment 4A. As the soil for the soil column was collected from grounds where metaldehyde may have been applied in close proximity, or may have had historical metaldehyde application, is was initially suspected that there was metaldehyde contamination of the soil. Nevertheless, in Experiment 4B no metaldehyde was detected in water that had passed through the same soil, using the same procedure as that used in 4A. No metaldehyde was detected in distilled water in Experiment 4B either. Although only one replicate of each treatment was possible in Experiment 4B, this suggests that neither the soil, nor the distilled water, were contaminated with metaldehyde. Consequently, it may be more likely that metaldehyde was contaminated into the leachate in Experiment 4A through experimental error. It may be possible that metaldehyde entered the soil after it was collected and before use in the soil column. It could be possible, for example, that due to disturbances, novel metaldehyde powder 1 was accidently transferred into the soil or directly into the leachate of novel control pellet and the commercial control pellet. However, this is considered unlikely as the soil columns were performed consecutively and with different equipment. It may also be possible that there may have been contamination during LC-MS analysis, although this is also considered unlikely due to stringent protocols and blind analysis of samples in a randomised order.

Another explanation for detecting metaldehyde in non-metaldehyde containing treatments in Experiment 4A could be that there was metaldehyde present in the soil when collected for Experiment 4A, but that it had dissipated from the soil by the time samples were collected from the same sites for Experiment 4B. The period between collecting the soil for Experiments 4A and 4B was over 50 days, so it may be possible that if the soil had been contaminated for the

4A but any metaldehyde present could have degraded in storage over the period between 4A and 4B (Bieri, 2003; Whelan et al., 2020; Keighley et al., 2021). Metaldehyde was detected in water from the commercial metaldehyde treatment in 4B at over 10X the concentration of that detected in 4A. As no metaldehyde was found in the soil column leachate, nor the distilled water, it is unlikely that additional metaldehyde contaminated the leachate sample through these means.

4.5 Conclusion

The results of experiment 4C are not available[14]. Experiment 4C was conducted to provide clarity on the potential of a metaldehyde contamination and as the experiment could not be completed it is not possible to draw firm conclusions on the results of experiments 4A and 4B. If metaldehyde was detected using the pour over method in commercial control pellets and novel control pellets, this would suggest that the pellets may have been contaminated during production, storage or handling. The level of metaldehyde in the commercial metaldehyde pellet in experiment 4C would not be directly comparable to those of 4A and 4B due to the different. However, it would have been useful to compare the level of metaldehyde between the commercial metaldehyde and novel metaldehyde pellets.

[14] Analysis equipment was not available due to COVID-19 pandemic

Chapter 5: Is a Novel Metaldehyde Pellet Commercially Viable in the United Kingdom?

5.1 Introduction

The world population is expected to increase by at least 2.3 billion people by 2050, and although a slower growth rate is expected for the future than has been seen in the last few decades, this population increase is placing enormous pressure on food production (UN, 2022; FAO, 2009). Resultantly there is growing pressure on pesticide development to aid food production - as pesticides contribute significantly to maintaining the level of food produced. It is estimated that 35% of crops are lost to pest damage before harvesting, excluding loss to prevalent post-harvest pests that cause further losses most important grain crops (wheat, rice and maize) (Oerke, 2006). On top of this, rising global temperatures could decrease the growing season for crops, and also increase the amount of crops lost to pest damage further. In the most important grain crops, losses may increase from current levels by 10 - 25% per degree Celsius of temperature increase (Deutsch et al., 2018).

For control of slugs in the UK it has been estimated that without control measures, the loss and damage to crops would cost the UK up to £100 million each year, with up to £43.5 million for losses in oilseed rape and wheat alone (Nicholls, 2014). Without the use of molluscicides, growers will bear the financial brunt of these losses, with those growing the most prevalent crops[15] facing estimated losses of 1.1 - 2.4% of the total crop value.

5.1.1 Metaldehyde in the UK

This section will briefly outline the history of metaldehyde in the UK. For further information, comprehensive reviews of the subject include the chapter "Chemical Control of Terrestrial Gastropods" in Barker (2002) and Castle et al (2017).

Metaldehyde was first described in 1835 by Justus von Liebig (Castle et al., 2017). Its molluscicidal properties were discovered in the early 1930's and it was the most popular gastropod bait by the 1940's (Barker, 2002). Metaldehyde was initially produced on an

[15] Oilseed rape and wheat, in the UK

industrial scale in 1920, not as a molluscicide, but as a solid fuel, with production for this purpose continuing until 1983 (by Lonza). Lonza are still the main manufacturers of metaldehyde, under the trade name Meta® Metaldehyde. The use of metaldehyde in the UK rose from 1990 onwards, where it is generally applied from September until December due to increased mollusc activity in wet weather. Peak metaldehyde levels in potable water were detected in 2008, prompting establishment of "The Metaldehyde Stewardship Group (MSG)" in 2008 to encourage best practice usage of metaldehyde pellets in the UK as part of an IPM approach ('Get Pelletwise!', 2021).

On 19 December 2018 the ban on metaldehyde for outdoor use was announced by the Department for Environment, Food & Rural Affairs, Health and Safety Executive, and The Rt Hon Michael Gove MP (Department for Environment & Affairs, 2018). This was reversed on 30 July 2019, following a legal challenge from Chiltern Farm Chemicals which led the High Court to declare that the ban issued on 19 December 2018 was unlawful. Sale and use of metaldehyde then continued, although it was speculated that a further ban was to follow, or that authorisation for metaldehyde products would not be renewed. A further announcement was made on 18 September 2020, confirming a renewed ban on the outdoor use of metaldehyde in Great Britain by the Department for Environment, Food & Rural Affairs, Health and Safety Executive, and Victoria Prentis MP (Department for Environment & Affairs, 2020). From 31 March 2021 metaldehyde cannot be sold by manufacturers, but those in possession of metaldehyde pellets can continue to use up stock until 31 March 2022 (including the sale of current stocks by distributors). From 1 April 2022 metaldehyde products cannot be sold or used outdoors in the UK, where instead alternative methods of slug management are encouraged.

5.1.2 Pesticide development

The main benefits of pesticide usage are related to improved direct returns on crop yield, while problems associated with using pesticides include the potential hazards to human health and the environmental, plus economic costs associated with purchasing and applying products. Strict legislation and policies concerning the use of pesticides are in place to alleviate the health/environment risks of usage. Within the UK, risk to human health from pesticide poisoning has greatly lessened since the mid 20th century (Casey & Vale, 1994). The herbicide Paraquat was responsible for 56% (570 of 1012) of pesticide related deaths in the UK from

1945 and 1989 but was withdrawn from use in 2007. Within this time, 73% of deaths were due to intentional consumption of the pesticide (Casey & Vale, 1994). Pesticides have also played a role in poisoning of domestic animals, though most cases can be attributed to poor storage, rather than exposure after application. Metaldehyde related deaths in humans and animals are discussed in detail in Chapter 1.

5.1.3 Screening

There has been a steep decline in the discovery of new pesticide active ingredients - not to be confused with 'new pesticides', which may refer to a variation in formulation of an existing active ingredient. Out of a multitude of compounds tested, very few display the features required in a pesticide - this being referred to as the screening or success rate. The search for new pesticides is now often an active one, contrary to accidental discovery of past products. In the mid 20th century, around 1/1,800 compounds displayed pesticidal qualities, but screening success in 2012 was estimated at 1/140,000 (CropLife International, n.d.; Sparks, 2013). Although, this change may reflect development of improved screening techniques that could allow compounds to be tested more efficiently or cheaply (Devlin, 1997; Cordoba et al., 2018). This decreased screening success has a knock-on effect on the time taken to discovery, with this being 3.7 to 4.2 years on average for the discovery of new insecticides (Sparks, 2013).

Due to increased legislative and screening costs, the cost of developing an active ingredient into a marketable product can be as much as $250 million dollars (£188 million). The cost of research and development in leading agrochemical companies has a compound annual growth rate of 8.2%, resulting in a combined expenditure of $6,728 million in 2012 (CropLife International, n.d.).

5.1.4 Chapter motivation

The sponsors of this PhD, UKWIR, were interested in the economic aspects of a novel iCRT based slug pellet. As the project progressed, the results of Chapters 2, 3 and 4 indicated that the formulations tested within this thesis may not be commercially viable. Additionally, the ban on outdoor use of metaldehyde meant that there would be little value in further developing a metaldehyde pesticide for the UK market. However, as the sponsors were still interested in the development costs of an iCRT pellet, this information is included alongside a more general discussion that may be of use to those interested in pellet development. Metaldehyde is still in use in many other countries worldwide, including most EU nations. As metaldehyde was the dominant chemical molluscicide during the period over which this PhD was written, this chapter still focuses on metaldehyde. Ferric phosphate is expected to become the dominant molluscicide in the UK, with similar usage trends to metaldehyde once the metaldehyde ban commences. Consequently, this is also commented on in this chapter.

The aims of this of this chapter were to:

1. Investigate trends in the use of metaldehyde on arable crops in the UK from 2015-2016
2. Discuss developmental costs of a new pellet, focusing on an iCRT pellet

Chapter 5 has been dived into 5A and 5B. Chapter 5A covers the use of metaldehyde on arable crops in the UK from 2015-2016 including the relationship between the percentage strength of metaldehyde applied and crop type, differences in the type of application between crop types, factors that can be used to predict the weight of active substance and product applied. Chapter 5B covers the production and cost of a novel metaldehyde pellet type, as well as the costs and use of alternative molluscicides types (ferric phosphate and biological control).

5A: Metaldehyde applications on arable crops (2015 – 2016)

5.2 Method

The data analysed in section 5A was kindly provided by colleagues at FERA. It was collected by the Pesticide Usage Survey (PUS) Teams at Fera Science Ltd, the Scottish Agricultural Science Agency and the Agri-Food and Biosciences Institute of Northern Ireland. The programme IBM SPSS Statistics 26.0 was used for statistical analysis.

5.2.1 Data overview

Crops that are more frequent in number within the dataset (and overall may be treated more with molluscicide) will be more important when considering subsidisation and the impact of metaldehyde on crops. In the data set discussed in this chapter, the number of records available per crop type was unbalanced (Figure 5.1).

An overview of all crops will be discussed initially, but the main analysis will focus on the primary crops grown in the UK (wheat and winter oilseed rape), with some discussion of secondary crops (potatoes ware and winter barley) as these crops will have the biggest impact. The impact of other (tertiary) crops on overall metaldehyde application in the UK is considered minimal. It should be noted that this report only concerns arable crops to which metaldehyde was applied in 2015-2016. It may not reflect the actual number of arable crops grown in the UK in 2015 – 2016 as it does not consider crops that were treated with a different molluscicide, or not treated at all. Further information on the growth of arable crops in the UK can be referenced at FERA PUS with specific reports on the years 2018, 2016, 2014 and 2012 available (survey report numbers 284, 271, 263, 250).

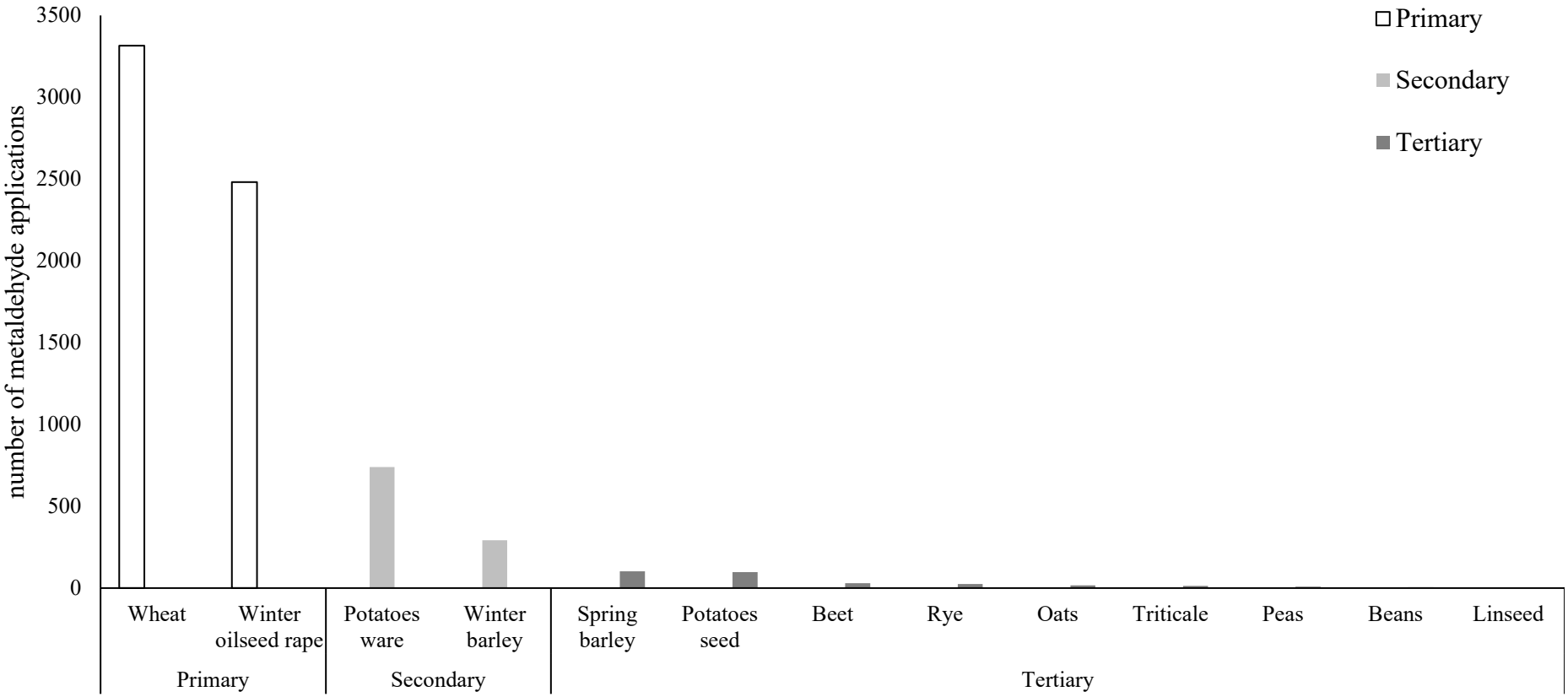

Figure 5.1 Segregation of crops into classes (primary, secondary, and tertiary) based on number of metaldehyde applications available for arable crops treated with metaldehyde in the UK (2015-2016)

5.2.2 Statistical analysis

A chi-square test for association was conducted to determine if there was a relationship between the percentage strength of metaldehyde applied between crop types, and if there was a relationship between the type of application between crops and between the rate of application between crops. All expected cell frequencies were greater than five.

A multiple regression was conducted to determine what factors could be used to predict the weight of active substance and the weight of product applied. Linearity was assessed by regression and studentized residuals against the predicted values plots.

A Durbin-Watson test indicated independence of residuals. Homoscedasticity was assessed by plot of studentized residuals versus unstandardized predicted values. There was no evidence of multicollinearity and normality indicated by a Q-Q plot.

5.3 Results

5.3.1 Is there a relationship between percentage strength of metaldehyde applied and crop type?

There was a statistically significant association between crop type and the percentage strength of metaldehyde applied $\chi^2(9) = 382.974$, $p < 0.001$. There was also a weak association between crop type and the percentage of metaldehyde applied, $\varphi = 0.137$, $p < 0.01$.

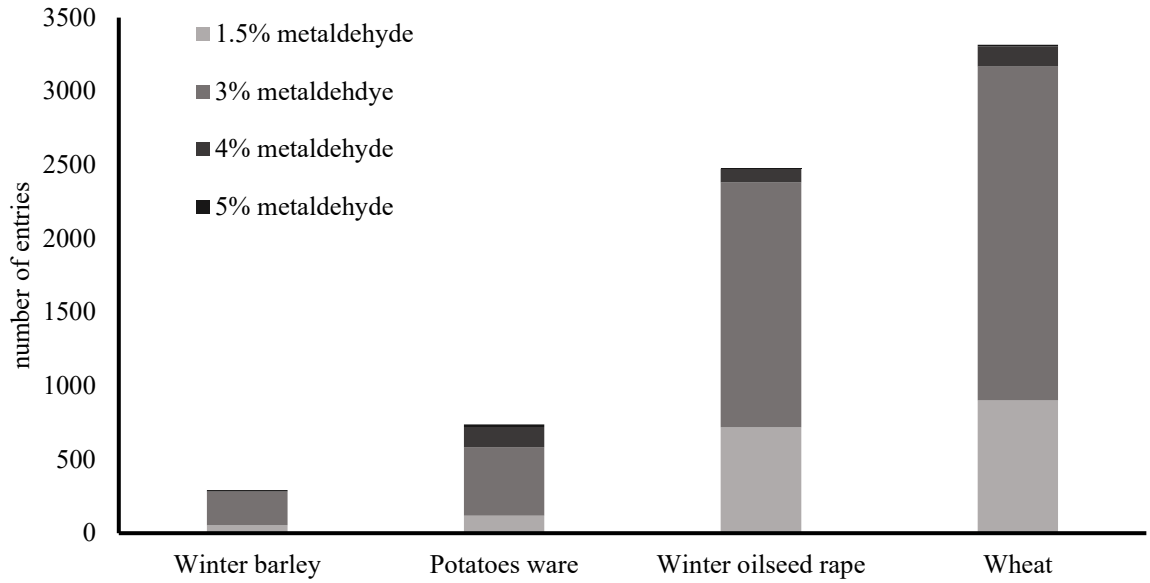

Figure 5.2 Number of entries and percentage of metaldehyde applied to crop types in 2015- 2016

Application of higher (4% and 5%) percentage strength metaldehyde pellets was considerably less than lower percentage strength (1.5% and 3%). While a weak association between crop type and percentage of metaldehyde was observed, as higher strength pellets were applied in smaller quantities the resultant impact on overall metaldehyde applied to the field is thought to be low. Little benefit to overall reduction of metaldehyde content would be observed in further limiting the application higher percentage strength pellets.

5.3.2 Is there a difference in the type of application between crop types?

There was a statistically significant association between crop type and the method of molluscicide application. $\chi^2(3) = 17.664$, $p < 0.001$. There was a weak association between type of application between crop types, $\varphi = 0.051$, $p < 0.01$.

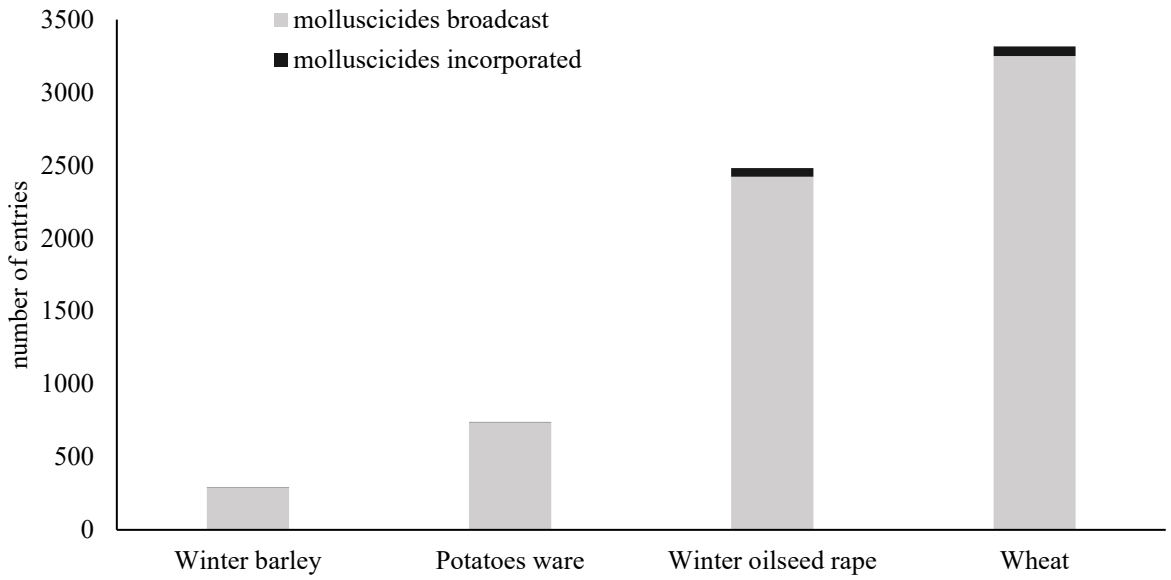

Figure 5.3 Number of entries and type of metaldehyde application to crop types in 2015- 2016

While a weak association between type of application and crop types was observed, there were comparatively less applications of metaldehyde incorporated than metaldehyde broadcast.

5.3.3 What factors can be used to predict the weight of active substance applied?

Crop type, region, crop stage (at time of application) and the rate of product application did not significantly contribute to the prediction of the model. Once these were removed, a multiple regression was run to predict weight of active substance applied from percentage of active ingredient applied, area treated, and weight of product applied. The multiple regression model significantly predicted the weight of active substance, $F_{(3, 6823)} = 9.746e+04$, $p < 0.001$, adj. $R^2 = 0.97$. All three variables added statistically significantly to the prediction, $p < 0.001$, Table 5.1.

Table 5.1 Multiple regression results for weight of active substance applied, SE = standard errors; CI = confidence interval; LL = lower limit; UL = upper limit.

Weight of active substance	Estimate	SE	95% CI	
			LL	UL
Model				
Intercept	-1.45	0.018	-1.48	-1.41
Percentage of active ingredient	0.05	0.01	0.49	0.51
Area treated	0.00	0.01	0.00	0.00

Weight of product	0.03	0.00	0.03	0.03

Crop type, region, crop stage (at time of application) and the rate of product application were not useful indicators to predict weight of active substance applied. Weight of active substance applied incorporates pellets of all percentage strengths and the overall weight of product. There may be a relationship between percentage strength and overall weight of product, but this relationship may vary between manufacturers. The results indicate that factors such as crop type and region, which may remain consistent between years, cannot be used to predict the weight of active substance applied.

5.3.4 What factors can be used to predict the weight of product applied?

Percentage of AI and region did not significantly contribute to the prediction of the model. Once these were removed, a multiple regression was run to predict weight of product applied from crop type, crop stage (at time of application), area treated, rate of product applied, and weight of active ingredient applied. The multiple regression model significantly predicted the weight of active substance, $F_{(5, 6821)} = 4.113e+04$, $p < 0.001$, adj. $R^2 = 0.97$. All variables added statistically significantly to the prediction, $p < 0.001$, Table 5.2

Table 5.2 Multiple regression results for weight of product applied, SE = standard errors; CI = confidence interval; LL = lower limit; UL = upper limit.

Weight of product	Estimate	SE	95% CI	
			LL	UL
Model				
Intercept	-15.15	0.86	-16.86	-13.47
Crop type	0.97	0.18	0.61	1.33
Crop stage	0.73	0.14	0.45	1.01
Area treated	0.94	0.02	0.90	0.98
Rate of product applied	3.86	0.16	3.55	4.16
Weight of active ingredient applied	25.16	0.18	24.78	25.51

Percentage of AI and region were not useful indicators to predict the weight of product applied. As discussed above, weight of product applied may not accurately reflect the weight of active ingredient applied. Metaldehyde stewardship guidelines are provided on weight of active substance due to variation in the weight of product applied. Crop type, crop stage alone and

area treated alone, could not contribute to a useful model, but improved the model above which included rate of product applied and weight of active ingredient applied.

5B: Development of a Novel Molluscicide

5.4 Introduction

All pesticides undergo an approval process before they are authorised and registered for use in the UK. This is to demonstrate that the pesticide does not pose a risk to human health and the environment, including wildlife and domestic animals. Strict safety standards are enforced by the Health and Safety executive covering labelling, protective clothing during handling, facilities, and training for professional users. Separate legislation exists for pesticide products developed for amateur use (home growers). Pesticides in the UK are reviewed regularly; renewal and registration of a potential slug pellet (as it is with other pesticides) can be referred to at https://www.hse.gov.uk/pesticides/ (Health and Safety Executive, n.d.). Ensuring that a new pesticide will not have any detrimental effects on the environment, human and animal health is costly and time consuming. More thorough testing is required for a newly discovered active ingredient.

The media reports that growers are unhappy with the ban on metaldehyde, mostly due to the product cost and incurred associated storage and usage costs if ferric phosphate is less efficient than metaldehyde in preventing slug damage (Case, 2018; National Farmers Union, 2018, 2020). Growers may share similar concerns when testing a new slug pellet product. Research has suggested that product loyalty among growers in the USA is high for both capital inputs and consumables, which would include pesticides and molluscicides (Harbor et al., 2006, 2008). Grower buying behaviour may differ between countries due to difference in legislation and product availability. Buying behaviour of growers in the UK are more likely to compare to those of growers in the EU (Burgert, 2011). When considering UK growers purchasing farm equipment, it was found that the brand accounted for 38.95% of the decision, this being more important than price which was the deciding factor in purchases for only 25.98% of growers. This may differ when considering molluscicides, an expendable cost, so further research would be needed before generalisations can be made (Walley et al., 2007).

5.4.1 Production of a novel pellet type

The following section assumes that a new metaldehyde iCRT, or any other novel metaldehyde product, works as anticipated, where it prevents leaching and is consumed moderately by slugs.

It is based on personal communication with industry experts. The transcripts of these interviews are available upon request. It also contains public sector information published by the Health and Safety Executive and licensed under the Open Government Licence.

To incorporate metaldehyde into an iCRT product, Lonza would supply Meta® Metaldehyde to Lucideon. Lucideon would be able to incorporate Meta® Metaldehyde into an iCRT product within days (at the current facility) if all raw materials were available. If new equipment were required at a different location, it would take several months to start production and significant capital investment, as well as technical expertise. It is not possible to determine an exact cost to construct new facilities as the details of the raw materials and methods used to produce the iCRT are commercially sensitive.

If the main component of the iCRT product were to be fusion glass, it would be quenched, producing a frit type material loaded with Meta® Metaldehyde and milled to the required particle size. It would be important to determine if particle size has an impact on palatability and leaching properties. Leaching and consumption experiments, similar to those described in this thesis, in collaboration with Lonza, would allow product refinement. Lonza does not anticipate that particle size would impact physical creation of the pellet and that any solid active ingredient could be incorporated. Once Meta® Metaldehyde is incorporated into an iCRT product (metaldehyde-iCRT), it would be transported to Lonza, or any other pellet manufacturer, for incorporation into a pellet. In the UK, the manufacturers of slug pellets are Adama Agricultural Solutions UK; Certis Europe; Chiltern Farm Chemicals; De Sangosse; Lonza and Sharda Cropchem. Alternatively, if a new factory were to be used to then time and costs for transportation would need to be allocated.

The general stages of pellet manufacture can be simplified into mixing and pelletizing. As pellet manufacturers in the UK are not legally required to disclose inert ingredients on labelling, only general ingredients are known. These are discussed in further detail in Section 1.4.2 and some examples of laboratory made pellet formulation are available in Table 3.1. It is anticipated that there will be no problems adding an iCRT powder to the mixture of dry ingredients prior to pelletizing. There are two main processes for pelletizing: dry pressing and wet extrusion. Wet extruded pellets have been suggested to have a higher longevity in field condition and reduced leaching (Fisher, 2006; Mohammad Bari, 2006). For wet extrusion processing, all the ingredients are mixed and kneaded into a dough before pressing. Cylindrical pellets are cut to shape and dried until hard. Pellets take up moisture when in field conditions, allowing them to

become palatable to molluscs. While dry pressed pellets are cheaper to manufacture, the superior quality of wet pressed pellets means they are the prevalent type used in professional agriculture with a growing home growers' market. Not considering the cost of manufacturing an iCRT, the cereal carrier material is the highest cost, as high-quality wheat flour is generally used (Durum wheat by Lonza). The time it takes to produce a wet extruded pellet is around five hours, due mostly to drying time.

If a metaldehyde iCRT pellet were to be produced in a single complete factory the anticipated time for a batch production could vary from 2-3 days. However, until sales of product can be relied on, it would make most sense for the iCRT to be produced by Lucideon, as the equipment, technical knowledge and raw materials are already being used for other industrial purposes.

5.4.2 Cost of novel metaldehyde pellets

One of the main concerns for pellet developers is the cost of the product for the end user. It is anticipated that, at least initially, the cost of producing iCRT pellets would be higher than the cost of conventional pellets. This would be mostly due to the costs of research and development. As the equipment required to produce the iCRT is already available at Lucideon, additional equipment costs would be minimal. From a grower's perspective, to make a profit, costs must be kept as low as possible while maintaining standards. Slug pellets, purchased by growers, are expendable goods that (in areas with slug pressure) are required to maintain standards; once purchased and used, they cannot be reused, nor provide future value. However, without them (in areas of slug pressure) the loss of crops through damage is far greater than the cost of the currently available commercial slug pellets (Nicholls, 2014). Growers are willing to purchase and use molluscicides if necessary, but will not see long term benefits from their use as there is no evidence that slug pressure declines over time with repeated use of molluscicide in a field setting (Hata et al., 1997; Gavin et al., 2009).

The current cost of metaldehyde, ferric phosphate and other molluscicidal products marketed to home growers is freely accessible and is not discussed further in this thesis. The costs of professional products are not accessible in the public domain, and the same product may vary in cost between distributors. Registered pesticides of Great Britain and Northern Ireland can be searched for by active substance and by use at https://secure.pesticides.gov.uk/pestreg/. At least

one attempt was made to contact all marketing companies listed under the search features "professional", "metaldehyde" and "ferric phosphate" at the time of the search[16]. There were few responses and additional information was sought from wholesale distributors (Table 5.3).

Table 5.3 Cost in GBP per kg of metaldehyde and ferric phosphate molluscicides of different AI w/w. Roman numerals denote different anonymised sources correct in 2020. Product IDs with the same letter denote the same Marketing Company as detailed on the pesticide register and different numbers denote different formulations.

Metaldehyde					Ferric phosphate			
Product ID	AI w/w	cost ex vat (£ /kg)			Product	AI w/w	cost ex vat (£ /kg)	
		I	II	III			I	II
A1	1.5	1.4	-	1.5	E1	2.97	2.7	-
A2	1.5	2.8	-	3.3	E2	2.97	2.7	3
A3	1.5	2.8	-	3.3	F1	3.7	2.1	-
A4	1.5	-	3.1	3.3	G1	2.42	3.7	-
A5	1.5	-	3.1	3.3	G2	2.42	2.8	-
A6	1.5	2.8	-	3.3	G3	2.42	3.2	3.48
A7	1.5	1.4	-	1.5	G4	2.42	3.1	-
A8	1.5	1.4	-	1.5	-	-	-	-
B1	3	2.5	-	-	-	-	-	-
B2	3	-	2.6	-	-	-	-	-
B3	3	-	1.5	-	-	-	-	-
C1	3	-	1.8	-	-	-	-	-
C2	4	-	3.6	-	-	-	-	-
D3	3	-	1.3	-	-	-	-	-

It may be useful to analyse the price changes in molluscicides products over time. It would be particularly useful to analyse changes in ferric phosphate (as the last major addition to the molluscicide market in the UK) from time of registration, to understand changes in cost over time. It would also be useful to consider the impact of ferric phosphate as an alternative molluscicide (sometimes subsidised) on the cost of metaldehyde. However, it was not possible to include this information as the data is not publicly available, and all companies contacted either did not respond, or declined to respond.

[16] May – July 2020

5.4.3 Historical use of ferric phosphate

A new metaldehyde product, as described above, could be encouraged in areas of high metaldehyde leaching, as ferric phosphate is at present. Ferric phosphate has been actively encouraged in areas of high metaldehyde leaching, through subsidies. These have generally been successful (Ibrahim et al., 2017; Le Moigne & Short, 2019; Ibrahim et al., 2019). Ferric phosphate was first registered in the UK in 2004. The first recorded use of ferric phosphate under the pesticide usage survey was only 1kg of product being applied, when compared to 417 kg of product applied in 2009 (Fera Science Ltd. (Fera), n.d.).

The total weight applied (kg) and total area treated (ha) with ferric phosphate increased rapidly from 2010 onwards ('Pesticides Register of Authorised Plant Protection Products', n.d.). Similar increasing trends have been observed for other crop groups. A large increase in both the areas treated with ferric phosphate and total applied weight of product were observed between the years 2013 and 2015 (Figure 5.4). There was a 216.7% percentage increase total area treated in the same crop groups between the previous year of measurement and 2013. As the crops surveyed in 2016 are unknown, the percentage change from the previous year of measurement cannot be calculated, but there was a 102.407% increase from the second highest measurement.

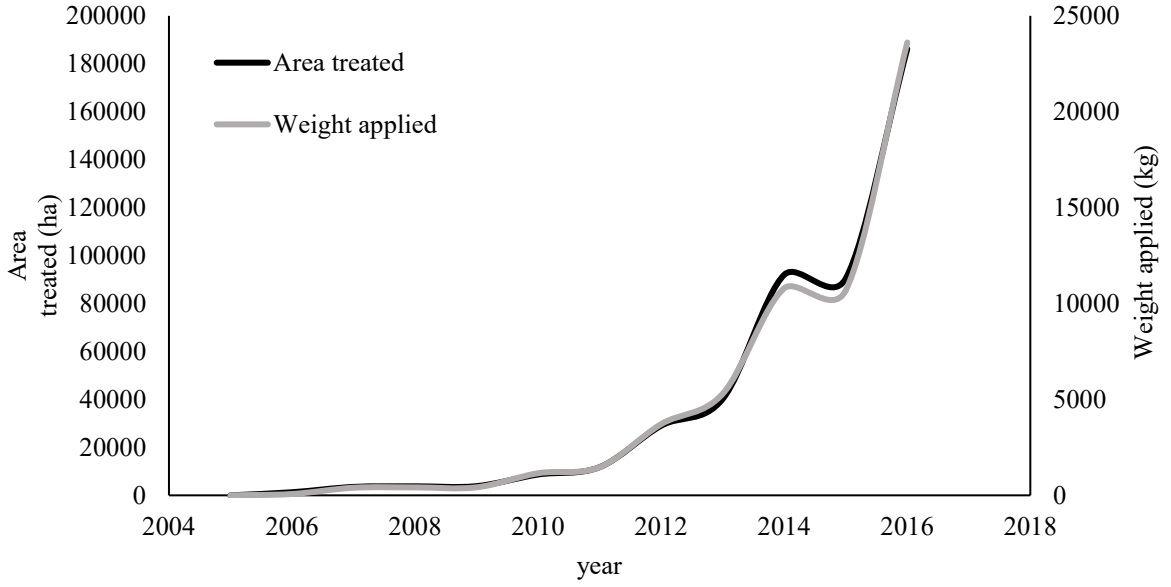

Figure 5.4 Area treated (ha) and weight applied (kg) of ferric phosphate (calculated using percentage of each active substance within a formulated product) in all crops in Great Britain from 2004 until 2016. Data sourced from the Pesticide Usage Survey.

5.4.4 Costs of biological slug control

High-cost slug protection is generally used by growers of high value or organic produce. If an iCRT pellet were to cost more than conventional metaldehyde and ferric phosphate pellets, this may result in a more niche product, restricting the customer base to growers who produce high value or organic crops.

Biological control of slugs using nematodes (Nemaslug®) costs significantly more than pellet molluscicides. The cost of treating a single hectare once with Nemaslug®[17] at the recommended rate would cost £2899, whereas the average cost of treating the same field with ferric phosphate would be £20.6, and £18.7 with metaldehyde[18]. Crops that have been treated with Nemaslug®, include soft fruit, brassicas, lettuce, peas and beans (Garthwaite et al., 2009; Mace et al., 2017). As these are higher value crops, growers may prefer to use a more effective product despite the increased cost. In more recent Pesticide Usage Survey reports (2018 onwards), all biopesticides are grouped as either insecticides or fungicides, so it is not possible to comment specifically on the use of *P. hermaphrodita* in more recent years (Ridley et al., 2020). However, older reports that comment specifically on *P. hermaphrodita* use on edible protected crops, suggested the nematode accounted for only <1% of area treated, within those areas treated with biological control agents *per se*. The use of *P. hermaphrodita* increased by 476% from the previous year of survey (2013), which may have been due to exceptionally high slug pressure in years 2014 and 2015 (Garthwaite et al., 2016).

[17] Nemaslug® value calculated from Nemaslug - 100 sq.m (£28.99) correct at the time of publication. It is not known if growers can purchase Nemaslug® at a cheaper rate in commercial quantities.
[18] Values compared are maximum individual doses per hectare. Ferric phosphate and metaldehyde values calculated from figures in table 5.3 considering concentrations of 3% for metaldehyde.

Chapter 6: Overall Summary and Conclusions

The overall aim of this thesis was to investigate slug response to and commercial potential of novel metaldehyde formulations. Due to the results of experimental work and changes in legislation, the project developed to incorporate further work on an alternative slug pellet, containing ferric phosphate. Novel metaldehyde formulations considered were based on inorganic Controlled Release Technology (iCRT) formulations that aimed to release metaldehyde more slowly than commercially available formulations. The benefits of this would be that the quantity of metaldehyde entering the water system at any given point would be reduced, allowing water companies to maintain set/legal standards more easily, and that more slugs would consume a lethal amount of metaldehyde, resulting in a higher death rate and consequently less damage to crops. Currently available metaldehyde formulations leach metaldehyde under certain conditions, which may result in non-compliance with standards and may not be effective at controlling mollusc pests, enabling further damage to crops and consequential financial loss in agricultural and horticultural industries.

Initial experimental work aimed to provide data and feedback on the consumption of, and associated survival of slugs after consuming, novel metaldehyde formulations, comparing results to current commercially available products. The formulations tested were generally unpalatable to slugs in bioassay experiments, consistent with preliminary experiments and described results. Poor consumption of the novel formulation types suggests that the components of the formulation deter feeding, either via the individual components themselves, or the creation of products resulting from the interaction between the powder and metaldehyde, or that slugs may not be able to perceive the pellet as a food substance. Low consumption may explain the high survival rates observed with novel metaldehyde formulations. Nevertheless, this work succeeded in establishing the methodology described in Chapter 2, which proved to be an easily repeatable and inexpensive way to compare the acceptability, palatability, and associated slug survival after feeding on, novel and existing molluscicide formulations in *D. reticulatum*. It could be expected that this methodology could also be applied to any other slug species.

Bioassay experiments were not able provide conclusive feedback as to whether previously observed slug death associated with low (observable) palatability was due to AI ingestion passing an active ingredient consumption threshold, physical contact with the pellet, or natural

causes with no relation to the AI. A standardised laboratory-based bioacoustics methodology, to test the consumption of any pellet or formulation to *D. reticulatum* or any other slug species, was developed to provide data and feedback on the acceptability and consumption of pellets in relation to the length of bites, number of bites, and general patterns of feeding, comparing novel formulations to current commercially available products. Consistent with the results of the bioassay experimental work of Chapter 2, slugs fed less on novel formulations or pellets containing toxins (metaldehyde or ferric phosphate). There was no change in the length of the bites between formulations, but slugs did make fewer bites were made on toxic pellets. Similarly, there was measured lower acceptance of toxic or novel pellets in experimental work described in Chapter 3, as was hypothesised in Chapter 2. Further work investigated whether the environment in laboratory trials had an impact on the results, as well as exploring if it would be possible to use this methodology under field conditions. The methodology described was useful in comparing pellet formulations and may be of use in the development of future pellet formulations.

Deroceras reticulatum was observed to actively avoid consuming foods containing iCRT, metaldehyde and ferric phosphate when compared to foods not containing these components. The iCRT was not able to mask metaldehyde and acted as a deterrent even when used in isolation. Based on these results, the reformulation of the novel pellet would be suggested and further work into the individual components of the novel formulation may be useful to the manufacturers. An ideal toxic pellet would show consumption levels akin to those achieved with the non-toxic control. The methodologies described in Chapters 2 and 3 can be used to compare consumption between novel pellet types and commercial formulations.

The amount of metaldehyde leaching from a pellet is of great interest to pellet developers and water industry stakeholders, as metaldehyde has repeatedly been the main cause for failure to achieve drinking water standards. Relatively little information on leaching from pellets is provided by manufactures and current environmental fate may not accurately reflect leaching of pellets in the field. Even with the poor consumption observed in other experimental work, it was of interest to compare leaching of novel formulations to current commercially available products.

Novel formulations did not seem to prevent metaldehyde leaching into the soil any more than currently available formulations in laboratory trials. Due to the overall conclusions of Chapters

2, 3 and 4, it can be concluded that the novel formulations containing inorganic Controlled Release Technology were not able to improve on currently available pellet formulations. Therefore, the novel formulations described in this thesis are unlikely to be of interest to manufacturers, this being further exacerbated due to their increased cost of production, as well as their minimal improvement on efficiency.

The ban on outdoor use of metaldehyde suggests that there will be little use in further developing a metaldehyde pesticide for the UK market at the current time. Ferric phosphate is expected to become the dominant chemical molluscicide with similar usage trends to metaldehyde being realised once the metaldehyde ban commences. However, improvements in screening and pesticide development may allow for the discovery of novel active ingredients against molluscs and improvements in currently available formulations. Furthermore, metaldehyde is still in use in many other countries worldwide, including most EU nations, and information on the costs of developing a novel formulation (like the iCRT formulations) may thus still be of interest to manufacturers. If a novel formulation pellet were to cost more than conventional pellets, this may result in restricted sales to more niche marketplace – e.g., to growers who produce high value or organic crops. Future work for those interested in developing a novel molluscicide could benefit from analysing changes in the cost of ferric phosphate (as the last major addition to the molluscicide market in the UK) from time of registration, to better understand changes in cost over time. For future work on iCRT, reformulation of the novel pellet would be suggested, as would further experimental work isolating the individual components of the novel formulation, which may provide useful information to manufacturers.

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