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Geomalacus maculosus:

An assessment of trapping methods, forestry management impacts, and feeding preferences

A thesis submitted to the National University of Ireland, Galway for the degree of Doctor of Philosophy

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Table of Contents

Declaration

I, Erin Johnston, hereby verify that this thesis is all my own work and that I have not obtained a degree in this university or elsewhere on the basis of this work.

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Abstract

The EU protected Lusitanian slug species *Geomalacus maculosus* Allman (Gastropoda: Arionidae) is found only in northern Iberia and the west of Ireland. Once thought to inhabit undisturbed habitats such as blanket bogs and forests, in 2010 was found to be breeding in commercial forestry, a cause for concern in relation to the adequate conservation of the species. Chapter 1 provides an overview of the current knowledge of the species' distribution, ecology and associated legislation and identifies areas in need of further research.

Chapter 2: Given that automated behavioural analysis has never been undertaken for *G. maculosus*, a procedure to allow for Ethovision XT10 behavioural software use with *G. maculosus* was established. Observational trials indicated that when *G. maculosus* was in contact with food, it was actively feeding for 95% (median) of the time. Ethovision XT10 software was used to establish contact time with a range of lichens, mosses and liverworts, and analyse feeding behaviour for the first time under darkened conditions. While preferences for some bryophytes and lichens were observed, overall results indicate that *G. maculosus* is a generalist lichen and bryophyte herbivore with no differences in feeding preferences shown by the two colour morphs, associated with forested and open habitats respectively. Maximum mean distance moved in the laboratory over two hours by *G. maculosus* was 6.7m ($\pm$ 2.9 SE) with mean meander by colour morphs of open habitats being significantly greater ($P = 0.008$) than that of colour morphs of forested habitats. The results of this study are discussed in the context of maximising the food resource of *G. maculosus* during the forest cycle.

Chapter 3 examines the efficacy of De Sangosse refuge traps across three habitats frequently found associated with commercial forest plantations in Ireland, comparing them with hand searching, a commonly used method for slug monitoring. Catch data during different seasons and under different weather conditions indicate that autumn is the optimal time for sampling *G. maculosus*. Refuge traps placed at 1.5m on trees in mature conifer plantations and directly on exposed rock in blanket peatlands result in significantly greater

catches, however hand searching is the most successful approach for clear-fell areas. Hand searches in clear-fell preceded by rain are likely to result in greater numbers caught.

Chapter 4: The discovery of EU protected *G. maculosus* in commercial plantations requires knowledge regarding implications of forestry practices on the species in the context of sustainable management in Ireland. The aims of the study were to compare *G. maculosus* captures across mature planted, existing clear-felled and unplanted habitats, assess models for estimating *G. maculosus* population sizes, and determine impacts of clear-felling on *G. maculosus*. Mean catches of *G. maculosus* were greatest in mature forest compartments. The Schnabel model for estimating population size was most suited for mature forest stands but could not be utilised for other habitats. BACIP analysis showed 95% reductions in *G. maculosus* mean catches post-felling where no individuals marked prior to felling were recaptured compared to 21% recapture rates at the control site. Greater tree circumference correlated with greater catches.

Chapter 5 summarizes and discusses the main outcomes of the three preceding chapters through the establishment of key points, examines potential mitigation measures that could be established for *G. maculosus* and highlights areas in need of further research.

Chapter 1: General Introduction

General Introduction

1.1. Scope and objectives

"The most unique feature of Earth is the existence of life, and the most extraordinary feature of life is its diversity" (Cardinale *et al.* 2012). However, levels of this diversity are in decline (Jones-Walters 2008) and the preservation of biodiversity presents a huge challenge. The conservation of flora and fauna relies largely on sets of legal instruments, which are in force at a number of levels ranging from international to local. Bouchet *et al.* (1999) reported that invertebrates are generally poorly represented within these pieces of legislation, due to a variety of factors including insufficient knowledge, existing knowledge being poorly represented in the documents, and unsuccessful lobbying from invertebrate conservationists at the appropriate levels of decision-making. As a group, molluscs are facing what has been described as being an unprecedented survival crisis (Bouchet *et al.* 1999), making up 42% of all animal extinctions since the 1500s (Lydeard *et al.* 2004). In Ireland, 150 native non-marine molluscs have been evaluated for conservation status and *Geomalacus maculosus* Allman, 1843 is one of six of these species that has been afforded protection under EU legislation (Byrne *et al*. 2009). Despite the designation of this species as protected, relatively little is known about *G. maculosus*, and in recent years the species has been found in habitats associated with commercial forestry. The legal conservation obligations set forth by the current legislation for *G. maculosus* mean that the presence of the species in commercial forestry is a cause for concern. Current guidelines for the species in forestry plantations have yet to be updated following the discovery of the species in conifer plantations (Forest Service 2009). In addition, finding practical measures to protect the species in commercial forestry is difficult as so little is known about the species in these habitats.

The aim of this thesis is to expand our knowledge of *G. maculosus* to inform management guidelines for commercial forestry with a view to improving the conservation of the species in Ireland. This study examined three different aspects

1. Due to the lack of information on the feeding preference and behaviour in *G. maculosus* this study established an automated experimental procedure using Ethovision XT10 behavioural software. This allowed for the assessment of food preference in the species, based on which food preferences were examined and ranked.

2. Given the paucity of data which compare different trapping methods for *G. maculosus*, the efficacy of De Sangosse refuge traps and hand searching across three habitats frequently associated with commercial forestry in Ireland was examined. The optimal time of year for surveying, along with optimal temperature and weather conditions were determined for future monitoring programmes.

3. Finally, differences in captures rates of *G. maculosus* across three habitat types associated with commercial forestry in Ireland were assessed, together with models for estimating population size. The impacts of clear-felling on the species was also determined.

1.2. Literature Review

1.2.1. Characteristics and life history of *G. maculosus*

Geomalacus maculosus is a member of the family Arionidae. The genus *Geomalacus*, of which *G. maculosus* is the type species, is relatively small, consisting of just four species. The three other species found within the genus are *G. anguiformis*, *G. malagensis*, and *G. oliveirae*, all of which are endemic to Iberia (Castillejo *et al.* 1994; Reich *et al.* 2015) with *G. maculosus* the only species found in Ireland (Platts and Speight 1988).

As with all the members of the genus *Geomalacus*, *G. maculosus* has a long dorso-laterally compressed body (Platts and Speight 1988). Castillejo e*t al.* (1994) reported that adult specimens measure up to 70mm with a mantle length of 30mm, while juveniles measure 30mm with a mantle length of 10mm. These measurements, however, may not be accurate as individuals have an ability to elongate and flatten to fit into narrow crevices, meaning animals measured at rest at 40 or 50mm may be as much as 120mm when fully extended (Platts and Speight 1988). *Geomalacus maculosus* adults have a distinctive spotted colouration with two colour morphs (Fig. 1). These are described by Platts and Speight (1988) as either brown with yellow spots, or a grey/black with white spots. The difference in this colouration is thought to be related to the habitats they inhabit: those coloured brown with yellow spots generally found in closed habitats such as woodlands, while the grey/black specimens are found in habitats that are more open, i.e. bog and heath, although some crossover may occur. In juveniles the colour difference is less pronounced and two dark lateral stripes are present, which become less obvious and fade with age (Mc Donnell and Gormally 2011). A unique trait of *G. maculosus* is its ability to roll into a defensive ball when threatened (Fig. 2), a feature which distinguishes the slug from other members of the Arionidae (Platts and Speight 1988).

Figure 1 Two colour morphs of *G. maculosus* found in forests (L) and bogs or clear-fell (R) (A. O'Hanlon)

Figure 2 *G. maculosus* curled into characteristic defensive ball (G. Kindermann)

A hermaphroditic and self-fertilising species (Mc Donnell and Gormally. 2011), *G. maculosus* deposits its eggs in batches of up to 30 eggs between February and October (Wisniewski 2000) (Fig. 3). These eggs are at first translucent in colour, turning darker over time until hatching occurs six to eight weeks later (Fig. 4) (Wisniewski 2000). Maturity is reached after two years, and specimens have been found to live for up to six years (Rogers 1900; Wisniewski 2000).

Figure 3 Cluster of *G. maculosus* eggs newly laid on damp filter paper

Figure 4 Cluster of four week old *G. maculosus* eggs and freshly hatched juvenile (G. Kindermann)

Geomalacus maculosus, or the Kerry Slug, was first discovered on the shores of Lake Caragh in County Kerry in 1842, and described as a new species the following year (Allman, 1843; Allman, 1844; Allman, 1846; Platts and Speight 1988). The species was found to be present in northern Spain in 1868, and was subsequently discovered in northern Portugal in 1873 (Platts and Speight 1988). The single report of the species being present in Brittany, France (Mabille, 1867), has been dismissed as erroneous (Platts and Speight 1988; Falkner *et al.* 2002) and the same is the case for a record of the species from the Netherlands (Bos, 1914). Due to this disjunct and limited distribution with no intermediate populations, *G. maculosus* is considered a Lusitanian species (Castillejo *et al*. 1994). Within its Iberian range the species is found from the North-Western coast to the Serra da Estrela in Portugal and as far east as Pamplona in Spain (Fig . 5a) (Castillejo *et al.*, 1994; Reich *et al*. 2015).

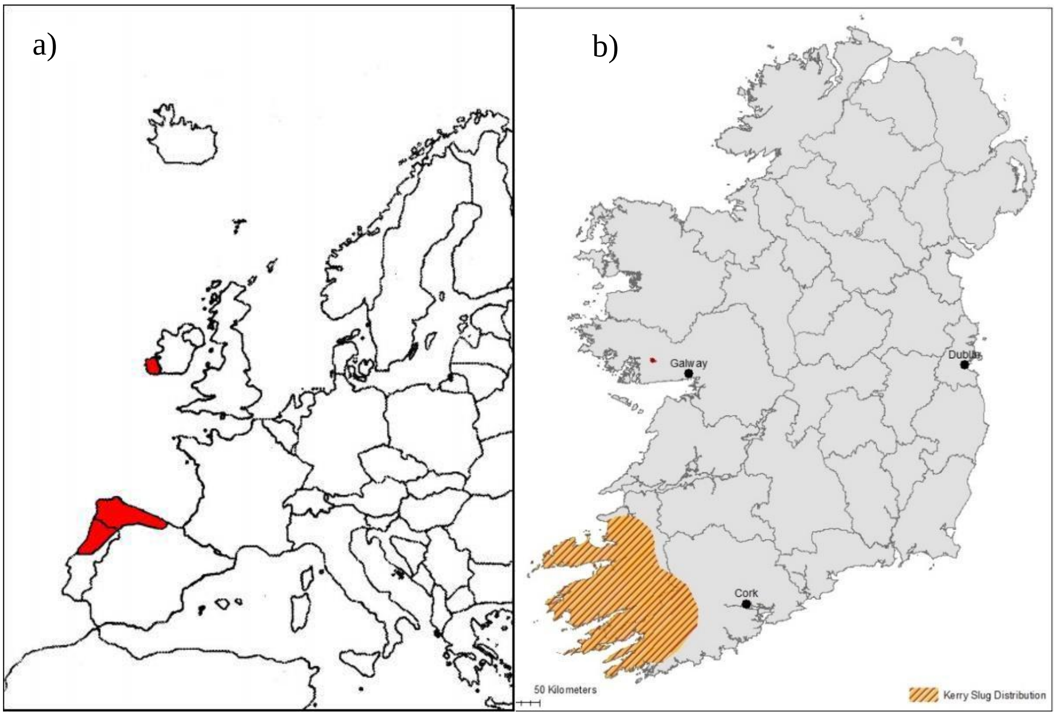

Figure 5 a) Global distribution of *G. maculosus* (NPWS 2010) and in Ireland (b) (Gesche Kindermann)

Reich *et al*. (2015) found that the Irish populations of *G. maculosus* are genetically closely related to those found in Spain and that there is very low genetic variability within the Irish populations. This suggests that the populations in Ireland are descended from a few founding individuals introduced from Spain (Reich *et al*. 2015). In Ireland, the species is found throughout the southwest of Ireland, predominantly in counties Kerry and Cork (Fig. 5b). Within this range, it was long thought that *G. maculosus* was restricted to a very narrow range of habitats including native deciduous woodland, blanket bog, and oligotrophic open moor and lake shores (Platts and Speight 1988; NPWS 2010; McDonnell and Gormally 2011). Within these habitats, *G. maculosus* was previously considered to be restricted to areas with outcrops of Devonian Old Red Sandstone with suitably humid conditions (NPWS 2010). Recent studies by McDonnell *et al.* (2011) however have shown that, despite reports that populations of *G. maculosus* disappear in areas of limestone and reappear in areas of sandstone (Platts and Speight 1988), the slug appears to have no preference for sandstone, and is also found on granite. Most recently, *G. maculosus* was found to be breeding in a conifer plantation in Galway, some 200km north of what was considered its distributional range in Ireland (Fig. 5b) (Kearney 2010). As no intermediate populations were found and the population was localised to one particular plantation (Reich *et al* 2012), the presence of the slug is deemed to be due to introduction via anthropogenic sources, likely associated with the ongoing forestry operations (Reich *et al* 2012). Following this discovery, McDonnell and Gormally (2011) found further populations in coniferous plantations in both counties Cork and Kerry as well as in forestry clearfell sites, thus indicating that the species may have a wider habitat distribution than previously thought.

In Iberia, *G. maculosus* inhabits montane forests and groves of chestnut, oak and pine trees, but is also found on rock outcrops and walls near houses, gardens and agricultural fields (Rodriguez *et al*. 1993; Castillejo *et al.* 1994; Patrão *et al*. 2015). Optimal temperatures for *G. maculosus* are thought to be between 8 and 12°C (Rodriguez *et al.* 1993). When temperatures or moisture levels are not suitable, *G. maculosus* is thought to take refuge in a number of places, which vary depending on the habitat in which it is found. When found in unimproved open moor or blanket bog in Ireland, *G. maculosus* often seeks shelter within deep moss and in rock crevices, while in woodland environments they take refuge beneath bark and moss on tree trunks (Platts and Speight 1988; McDonnell and Gormally 2011; Reich *et al*. 2012). The species is considered to be crepuscular and in Iberia *G. maculosus* is primarily only active after dark. Given the high atmospheric humidity in the west of Ireland, *G. maculosus* is regularly found to be active during the day, often after rain (Platts and Speight 1988). While this information on activity in *G. maculosus* is often cited, very little is known about the optimal weather conditions for *G. maculosus* in Ireland, or indeed what exactly drives their distribution within habitats.

1.2.2. Feeding

To date, feeding preferences of *G. maculosus* have not yet been fully determined. Platts and Speight (1988) stated that the species has no specific food plant, listing lichens, fungi and algae as general food sources for the slug. When held in captivity, the species has been found to consume a variety of vegetable matter and may be sustained on a diet containing porridge oats, mushroom, celery, lettuce, potato, beetroot and carrot (Platts and Speight 1988). In Spain *G. maculosus* was listed by Castillejo (1996) as being a species known to damage cultivated vegetables, however there has been little further evidence to support this. In Ireland a report was made of *G. maculosus* damaging beet seedlings, but the report has since been dismissed (Godan 1983; NPWS 2010). Incidences of *G. maculosus* being carnivorous have also been reported, with *G. maculosus* being observed to attack and eat the snail *Vitrina pellucida* and other, similar snail species (Taylor 1906). Reich *et al.* (2012) noted assumed incidences of cannibalism between test individuals. This was observed through the numbers of captive-held individuals decreasing within a holding container, with no ways for individuals to escape and no corpses found. Further, there was no indication as to whether these missing individuals were consumed while alive or already dead.

Feeding trials were carried out by Reich *et al.* (2012) to determine the feeding preferences of *G. maculosus* in Ireland. A total of 28 different species made up of lichens, liverworts, mosses, a heather and a fern were collected from forests and granite outcrops within the known habitats of the species and were presented to *G. maculosus*. These trials found that lichens and liverworts were the most important food sources for *G. maculosus.* The slug consumed all forest lichen species presented, including both fructose and foliose growth forms, Out of the crustose species found on granite most of the lichen species were consumed except for *Porpidia flavocruenta*, and *Verucaria* sp. Reich *et al.* (2012) suggested this could be due to the growth form of the lichen, or due to secondary compounds accumulating in the lichen. They noted while just two out of ten species of moss were consumed, *Campylopus introflexus* and *Pleurobium schreberi*, moss still appears to be of importance for the slug with the authors finding correlations between *G. maculosus* abundance and bryophyte/lichen cover on trees, as well as observations of comparably more individuals being found on trees with higher bryophyte cover.

Although Reich (2012) found that lichen is the preferred food type of *G. maculosus*, with more species of lichen consumed than moss, there have been, to date, no investigations regarding how this choice is made. Lawrey (1983) investigated how slugs which are lichen herbivores discriminate between species of lichen and presented two hypotheses. The first hypothesis is that of avoidance which suggests that herbivores sample lichens almost randomly, avoiding those species which produce defence compounds that reduce palatability. The second hypothesis is the preference hypothesis. Under this hypothesis herbivores feed most frequently on available lichen species which have the highest quality resources. Lawrey's findings, however, suggest that lichen herbivores tend to "avoid" lichens due to unpalatable defence compounds rather than "choose" lichen species on the basis of their nutritional content. Overall, feeding preferences in *G. maculosus* are not yet fully understood and the feeding plasticity in the species is yet to be examined, including an investigation of what defence compounds they avoid, if indeed they do avoid certain species

1.2.3. Legislation associated with *G. maculosus*

Geomalacus maculosus is listed as vulnerable in Spain (Verdú and Galante 2006), and reports show that populations are declining within the overall Iberian range (Platts and Speight 1988; Byrne *et al.* 2009) as habitats for the species are suffering from severe fragmentation and deterioration (Patrao *et al.* 2015). In addition, a reduction in precipitation levels and increase in temperature is predicted for the Mediterranean basin (Kelemen *et al.* 2009) indicate that suitable habitat for *G. maculosus* is likely to further decrease in future. The susceptibility of the Iberian population to the loss of habitat highlights the importance of the Irish populations for the ongoing conservation of the species.

Due to its limited distribution and global rarity, *G. maculosus* is listed in Appendix II of the Bern Convention, as well as Annex II and Annex IV of the European Union Habitat Directive (92/43/EC). The primary objective of the Habitats Directive is to promote the preservation of biodiversity in the European Union and requires Member States to adopt suitable measures to sustain or restore natural habitats and wild species that are listed on the Annexes of the directive to Favourable Conservation Status (EC 2007). Member States are required to establish Special Areas of Conservation (SACs) for species listed in Annex II of the Habitats Directive, and ensure the protection of the species listed in Annex IV wherever they occur; this includes populations within and outside of SACs. *Geomalacus maculosus* is protected in 64 NATURA 2000 sites across the three countries in which it is found (EUNIS 2016). Three of these sites are located in Portugal, two in the Norte and one in the Centro administration zones, 54 in Spain and in Ireland there are seven protected areas (Table 1). While the designated sites represent a sizeable amount of the known range of *G. maculosus* (NPWS 2010), a large part of the known distribution of *G. maculosus* falls outside of this area.

Table 1: List of *Geomalacus maculosus* protected sites in Ireland (NPWS 2015)

Site Number	Site Name	Location
IE0000090	Glengarriff harbour and woodland	Co. Cork
IE0000093	Caha mountains	Cos. Cork and Kerry
IE0000102	Sheep's head	Cos. Cork
IE0000365	Killarney National Park, Macgillycuddy's Reeks, and Caragh river catchment	Cos. Cork and Kerry
IE0000370	Lough Yganavan and lough Nambrackdarrig	Co. Kerry
IE0001342	Clonee and Inchiquin loughs, Uragh wood	Co. Kerry
IE0002173	Blackwater River	Co. Kerry

1.2.4. Trapping

Prior to 2011, the recommended method for surveying for the species in Ireland was through hand searching (National Roads Authority (NRA) 2009). As with the forestry guidance documents, the guidelines put forward by the National Roads Authority (now called Transport Infrastructure Ireland) do not reflect the current situation that *G. maculosus* is present within commercial, conifer plantations and is also found in County Galway. The guidelines state that "fixed route transects at 20m intervals throughout oak woodland or bog habitat at night using torchlight" be carried out as surveys. While this may be an effective method for confirming presence of *G. maculosus* in these habitats, an effective trapping method has since been determined by Mc Donnell and Gormally (2011). Trials involving refuge traps made of wood, Styrofoam, and DeSangosse refuge traps were undertaken by Mc Donnell and Gormally (2011) who found that DeSangosse traps were the most effective in trapping *G. maculosus* in both bog and forested habitats as the traps could be placed on both rocks and tree trunks. Following this study Reich *et al*. (2012) successfully utilised DeSangosse traps placed in woodland and clear-fell sites to capture *G. maculosus* and undertake a capture-mark-recapture assessment.

While these studies established that DeSangosse refuge traps were effective in the capture of *G. maculosus*, none of them compared refuge traps with hand searching or compared the effectiveness of placing traps at different heights on tree trunks. In addition, although *G. maculosus* has been successfully captured using DeSangosse refuge traps in both clear-fell (Reich *et al.* 2015) and peatland habitats (Mc Donnell *et al.* 2011) neither of these studies has examined their effectiveness across different habitats. The establishment of the optimal trapping method for the species, particularly in habitats associated with commercial conifer plantations is imperative, not only to enable effective monitoring of the species, but to establish potential impacts on the species. Further, establishing the optimal season, weather, and temperature conditions for trapping in Ireland is necessary to ensure the greatest chance of catch success. The current threat response plan for *G. maculosus* (NPWS 2010), lists the main threats to *G. maculosus* as "forestry management (including afforestation), invasion of woodland habitat by *Rhododendron ponticum*, agricultural improvement (reclamation), and fragmentation of habitat and isolation of populations by major infrastructure such as roads". Further information on trapping efficacy in different habitats utilised by *G. maculosus* would allow for more specific guidelines on trapping the species to ensure that populations of *G. maculosus* are not overlooked through sampling inefficiencies and would also allow for better monitoring of populations in areas where threats occur.

1.2.5. Impacts of forestry

Ireland is currently one of the least forested countries in Europe (Bullock and Hawe 2013) with only 10% of the country covered by forestry (Forest Service 2007). The Forest Service aims to increase forest cover in Ireland to 17% by 2030, primarily through conifer plantations (Forest Service 2008). These coniferous plantations are typically made up of single species woodland, usually containing *Picea stichensis* (Sitka spruce), and *Pinus contorta* (Lodgepole pine), and are generally of low biodiversity value. In the NPWS species action plan for *G. maculosus* (2010), commercial forestry is listed among the key pressures threatening the species. The plan put forward that planted habitats would become increasingly unsuitable for the slug as conifer trees grow; the closing canopy would cause a reduction in light levels required for the growth of bryophytes and lichens that the slug needs for food and shelter (NPWS 2010). This was supported by the view that *G. maculosus* did not inhabit conifer plantations, which was only recently contradicted (Mc Donnell *et al.* 2011; Reich *et al.* 2012). As such, the level to which commercial forestry will actually impact *G. maculosus* remains uncertain. While to date no information is available on population numbers in areas with very young trees, populations seem to thrive in mature plantation and persist even in clear-felled areas albeit in lower numbers than the mature tree sections (Reich *et al.* 2012).

Under current commercial forestry practices in Ireland there are a number of points in the forestry cycle (Fig. 6), which may cause damage to habitats both directly and indirectly. This environmental damage has the potential to lead to the deterioration of *G. maculosus* habitats, which is in breach of the terms of the Habitats Directive and the conservation requirements for the species. The major steps which may cause environmental change within the site include the thinning, felling, and subsequent extraction of the timber from the sites (Forestry Service 2000).

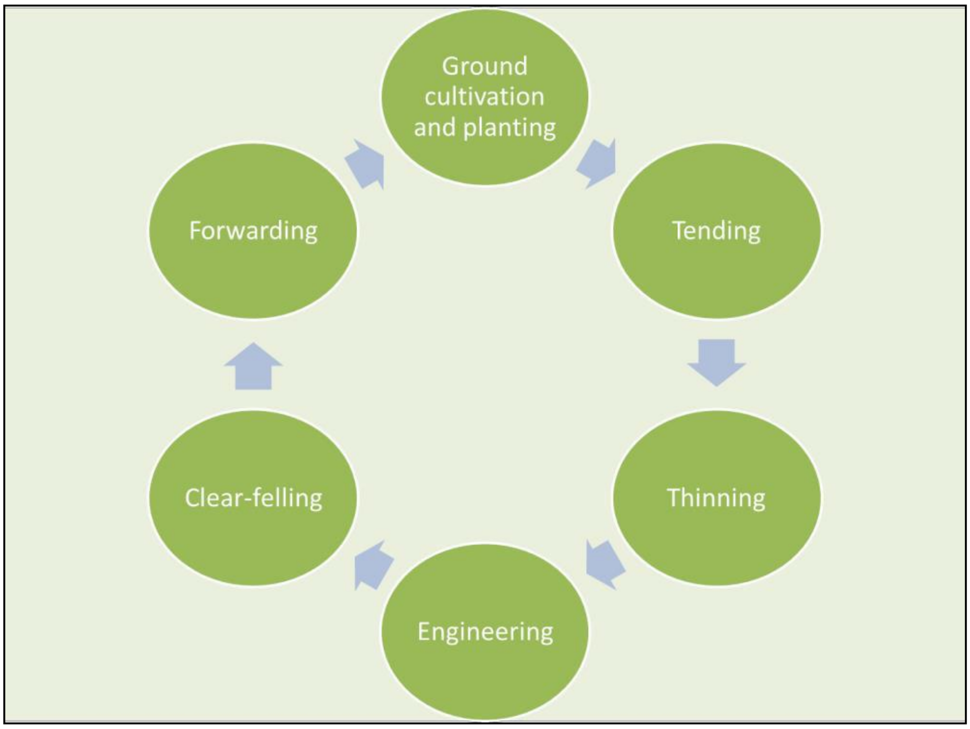

Figure 6 Forestry cycle in Ireland (Amended from Coillte 2016)

Thinning can take place at several points throughout the lifetime of the plantation, involving the removal of suppressed, diseased and damaged trees, the timing of which can determine the ultimate quality of the harvest (Forest Service 2000). Aside from the physical removal of trees, the Forest Service (2000) noted that additional environmental impacts may be caused where best practice is not observed and the risk of wind damage causing trees to be up rooted or broken (windthrow) is increased. Following thinning, harvesting takes place at maturity (30 – 50 years) of the plantation trees (Horgan *et al*. 2003; Teagasc 2016). The most frequently used method of harvesting in Ireland is that of machine clear-felling where entire stands are felled (Forest Service 2000). The environmental impacts of clear-felling can vary depending on factors including structure and composition of the forested stand itself, combined with the topography, geology and the climate of the individual sites (Keenan and Kimmings 1993). Clear-felling can cause a considerable build-up of debris and extensive soil disturbance where the removal of the tree canopy causes an increase in run off leading to a risk of sediment, debris and harmful chemicals entering nearby aquatic zones (Forest Service 2000). The most commonly used method of extracting felled timber in Ireland is through forwarding using a machine designed specifically for timber extraction (forwader) (Teagasc 2016). With good operation in an appropriate site, the forwarder can aid in the reduction of site damage although if used improperly or in an unsuitable site, forwarding can cause further soil compaction and rutting due to continuous use of tracks (Sutherland 2003). A good harvesting operation will leave low stumps between 5 and 10cm in height, and remove the full trunk of the tree often only leaving branches as brashing behind (Forest Service 2000). Following the harvesting operation an excavator with a grab bucket arranges the leftover brashing into long narrow rows in a process called windrowing (Clark *et al*. 2015). During this process, additional excavation may also be required to recreate drains and provide mounds for planting (Forest Service 2000). This along with the disturbance caused by lifting brash and exposing and scraping soil below (Clark *et al*. 2015) can have additional negative impacts on nearby aquatic systems as well as the site itself (Forest Service 2000). Following clear-felling, the stand is then often replanted beginning the forestry cycle anew.

Given the restricted movement of *G. maculosus*, the removal of trees, and therefore the physical habitat of the species, at any point in the forestry cycle, has the potential to negatively impact the species. Although the NPWS (2013) states that *G. maculosus* is “resilient” to clear-felling, no before-after-impact-assessment has been carried out to date to investigate any changes in population as a result of the process. Given that clear-felling causes a rapid transformation of a forested landscape into an open one, considerable disturbance of the physical environment can occur (Larsen 1995). In addition, the low mobility of slugs (Strayer *et al.* 1986) and their susceptibility to dehydration (Prior 1985) mean that changes may impact adversely on populations in disturbed areas. Given the lack of knowledge of the impacts on *G. maculosus*, and that Reich *et al.* (2012) found lower numbers in previously clear-felled compartments, further research is needed to fully establish the extent, if any, of the impact on populations in commercial forestry, particularly in the south-west of Ireland. Addressing this important gap in the knowledge will inform forestry managers through guidelines on how to properly meet the obligations set under the previously mentioned legislation.

1.2.6. Forestry Guidelines

The Forestry and Kerry Slug Guidelines (Forestry Service 2009) have been developed to provide information on management procedures in forested areas where *G. maculosus* has been found. The guidelines are designed to enable forestry workers to use a flow chart (Fig. 7), along with background information on the species and its ecology, to determine which sites may contain, or are likely to contain *G. maculosus*. The guidelines then recommend a number of steps to protect the species. Where possible the area that contains *G. maculosus* habitat or potentially suitable habitat should be avoided. The Forest Service will not issue approval or license for forest management measures to take place in areas where *G. maculosus* was found. Where populations are confirmed but forestry operations must still proceed, a derogation license must be sought from the Minister of the Environment, Heritage and Local Government under Regulation 25 of the European Communities (Birds and Natural Habitats) Regulations 2011. This derogation can be issued where a satisfactory alternative cannot be provided and where management activity is not detrimental to the maintenance of populations. Where *G. maculosus* is discovered during ongoing forestry work, this work needs to be halted, the Forest Service notified, and correct procedure applied.

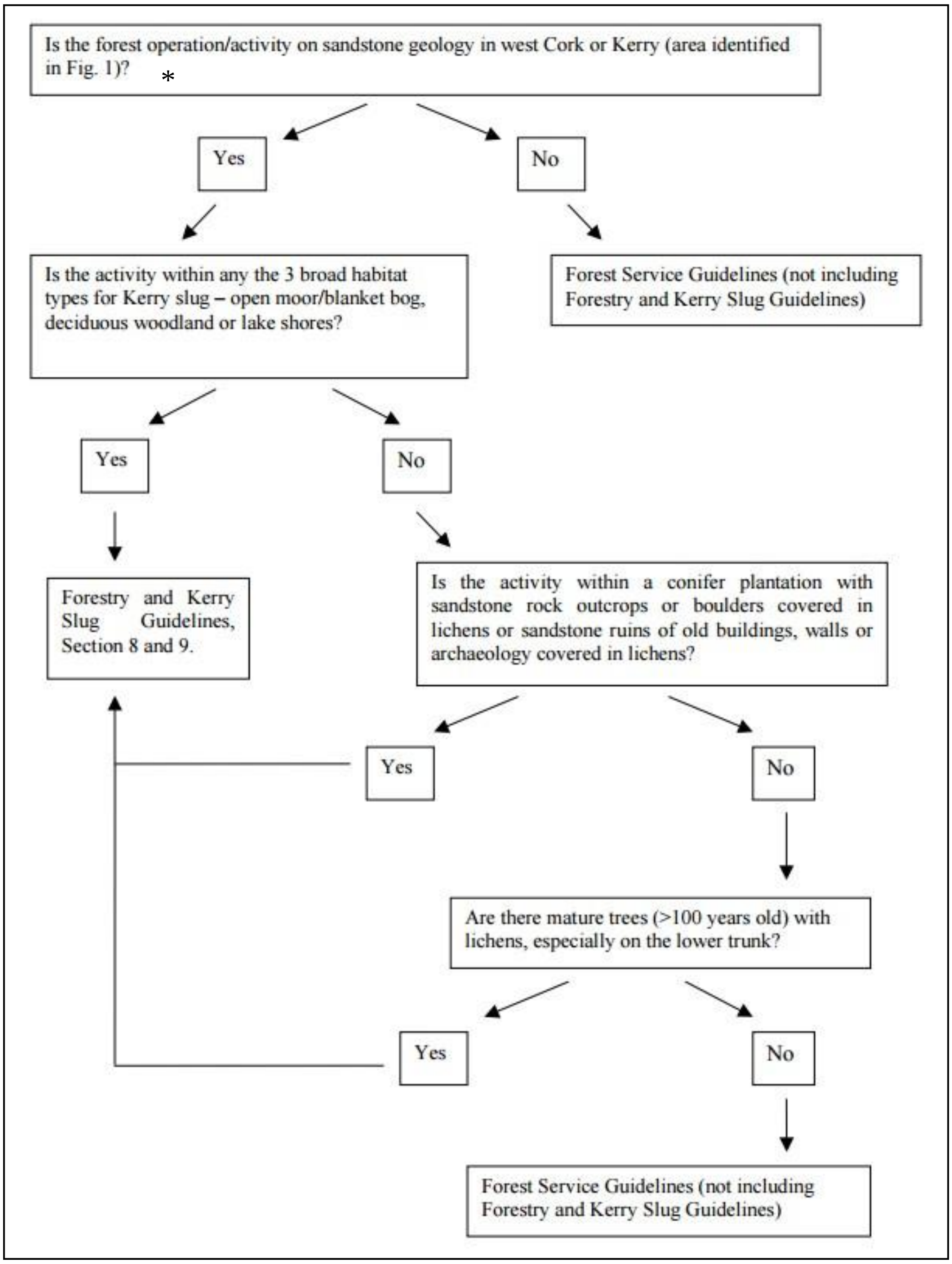

Figure 7 Screening chart used in The Forestry and Kerry Slug Guidelines (Forest Service 2009) to aid foresters in determining whether a forest activity has the potential to impact on a *G. maculosus* habitat (in the absence of a confirmed record/sighting). *Fig. 1 refers to Irish distribution without the County Galway population included.

The presence of *G. maculosus* in commercial conifer plantations in Ireland means that forestry managers are legally obliged to protect the species while undertaking day-to-day forestry practices (e.g. clear-felling). Up to date guidance regarding the species and forestry is required. Current guidelines have not been updated during the past seven years and information such as the inclusion of conifer plantations as a potential habitat for *G. maculosus* and up to date information on the distribution of the species, in particular the inclusion of the population found in County Galway, are essential. These gaps in the guidelines may result in populations of *G. maculosus* in conifer plantations being overlooked by forestry managers, which may be detrimental to these populations. Of additional concern is that out of 45 management plans compiled by Coillte (2016) for commercial forestry sites within the distribution area of *G. maculosus*, only one plan, for Killarney Forest KY13, made specific reference to *G. maculosus*. This is despite many of the plans referring specifically to other protected species including bats and the pearl mussel (*Margaratifera margaratifera*). These plans, along with current guidance documents, are in need of revision, which should be based on solid scientific data informed by relevant research findings.

Planned increases in forestry in Ireland will likely cause an increase in interactions between forestry and *G. maculosus*. Considering the lack of up-to-date guidelines and the lack of knowledge regarding the impacts of forestry practices on the species, research investigating the impacts of commercial forestry management on the species is imperative to ensure that Ireland continues to meet its obligations in protecting *G. maculosus*. In addition, the establishment of effective mitigation measures must be explored to effectively protect the species before negative impacts can occur. The NRA (2009) notes that translocation and habitat enhancement schemes should not be utilised due to the vulnerability of *G. maculosus* to changes in its habitat, due to how much the species relies on lichens as a food source. However, the suitability of these mitigation measures have to date not been explored fully and further information regarding the ecology of *G. maculosus* is required.

1.3. Structure of thesis

This thesis addresses the need for more information on *G. maculosus*, and is presented in the style of a paper-based thesis. This will consists of three separate papers, each following the format guidelines for the target journal to which they have been submitted, preceded by a general introduction (Chapter 1) and followed by a general discussion (Chapter 5).

Chapter 2: Establishes a procedure to analyse *G. maculosus* behaviour under dark conditions using Ethovision XT10 software, and examines the feeding preferences and mobility in *G. maculosus.*

Chapter 3: Investigates the efficacy of trapping methods for *G. maculosus* in three habitat types associated with commercial conifer plantations, and looks at factors that influence trapping success including temperature and rainfall.

Chapter 4: Looks at the impacts of commercial forestry management on populations of *G. maculosus,* investigates factors driving the distribution of populations within conifer plantations, and examines population estimates of the species.

The sampling sites and the same methods of data collection were used in papers 3 and 4. Therefore there is, of necessity, overlap and some repetitive sections of the individual papers.

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Chapter 2: Feeding preferences and mobility studies of the EU protected Kerry Slug, *Geomalacus maculosus*: automated behavioural analysis and barrier assessment for laboratory trials

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2. Feeding preferences and mobility studies of the EU protected Kerry Slug, *Geomalacus maculosus*: automated behavioural analysis and barrier assessment for laboratory trials

2.1. Abstract

Geomalacus maculosus is an EU protected species with a disjunct Lusitanean distribution, restricted to Northern Iberia and the west of Ireland. In recent years the species was discovered in commercial conifer plantations in Ireland. The legal obligations to protect the species within its natural range mean that an understanding of the food preferences of *G. maculosus* is required to ensure the protection of the species in commercial forestry. Given that automated behavioural analysis has never been undertaken for *G. maculosus*, a number of barriers for laboratory trials were tested resulting in salt impregnated paper being selected as the most appropriate. Observational trials indicated that when *G. maculosus* was in contact with food, it was actively feeding for 95% (median) of the time. Using Ethovision XT10 software to record contact time with a range of lichens, mosses and liverworts, *G. maculosus* behaviour was recorded and analysed for the first time under darkened conditions. While preferences for some bryophytes and lichens were observed, overall results indicate that *G. maculosus* is a generalist lichen and bryophyte herbivore with no differences in feeding preferences shown by the two colour morphs, associated with forested and open habitats respectively. Maximum mean distance moved in the laboratory over two hours by *G. maculosus* was 6.7m ($\pm$ 2.9 SE) with mean meander by colour morphs of open habitats being significantly greater ($P = 0.008$) than that of colour morphs of forested habitats. The results of this study are discussed in the context of maximising the food resource of *G. maculosus* during the forest cycle.

2.2. Introduction

Slugs are frequently referred to as generalist feeders (Boycott 1934; Chatfield 1976; Albrectsen 2004), but a degree of specialisation in food choice by slugs has been noted by Jennings & Barkham (1975); Dirzo (1980); and Fritz *et al.*

(2001). Feeding rate and selection can also show a great deal of disparity at the individual level (Hanley 1995; Hanley *et al.* 2003). In addition to differences found in feeding specificity, experimental set-ups to examine feeding behaviours in terrestrial gastropods vary greatly. In particular, numbers of test subjects cited in the literature range from 12 (Cook *et al.* 1997) to more than 600 individuals (Barlow *et al.* 2013) with Speiser (2001) stating that the optimal number of test subjects in molluscan feeding trials has yet to be fully established. In addition, treatment of molluscs prior to feeding experiments, while important (Bailey 1989; Mølgaard 1986), show little consistency. In some instances molluscs are subjected to extensive starvation regimes e.g. 9 days (Croll & Chase 1977) prior to experiments while in other studies (Briner & Frank 1998), molluscs are not starved. Different containment methods for mollusc feeding trials have also been employed ranging from sealed containers (e.g. Briner & Frank 1998) to salt impregnated barriers (Bailey, 1989) to barriers composed of polytetrafluoroethylene (Fluon) (Schüder *et al.*, 2004; Ribadula Nogueira, 2011), the latter two methods being used to facilitate the use of the automated behavioural analysis software Ethovision. Ethovision is an "integrated system for automatic recording of activity, movement and interactions of animals" (Noldus *et al.* 2001). The use of automated observation systems, like Ethovision, allow for a more systematic and accurate method of analysing behaviour, particularly over long periods of time, as algorithms in the software do not suffer from observer fatigue or drifts (Noldus *et al.* 2001). The software allows for extraction of quantitative measurements of behaviour (Spink 2002) while vastly reducing labour intensity (Spruijt & Gispen 1983). Ethovision software has been utilised successfully to study behaviour in a range of different animals (Noldus *et al.* 2001) including, in recent years, slugs and snails (Schüder *et al.* 2004, Ribadulla Nogueira 2011). The increasing use of software like Ethovision has improved our knowledge of behaviour in slow moving animals such as terrestrial gastropods through the provision of unbiased, less invasive and less labour intensive methods of assessment. Creating a better understanding of the feeding and foraging behaviour in a species can provide valuable information, particularly in the context of conserving protected species (Caro 2007). Since the 1500s, 42% of all extinctions have been molluscan species (Lydeard *et al.* 2004) with the number of molluscan species extinctions greater than birds, mammals, reptiles and amphibians combined (Bouchet *et al.* 1999). It is, therefore, critical that our knowledge of molluscan species at risk be improved to ensure the effectiveness of future conservations efforts. Only six species of non-marine molluscs, of which the Kerry Slug (*Geomalacus maculosus* Allman) is one, are currently protected in Ireland under European legislation (Byrne *et al.* 2009).

Geomalacus maculosus was first discovered in Co. Kerry in the south-west of Ireland by Allman in 1842. The species was subsequently found in 1886 in Spain and in 1873 in Portugal (Platts & Speight 1988). This restricted distribution, with no intermediate populations (Reich *et al.* 2015), has resulted in the protection of the species under Appendix II of the Berne Convention, and Annexes II and IV of the European Union Habitats Directive (92/43/EC). *Geomalacus maculosus* is listed as vulnerable in Spain (Verdú & Galante 2006) and populations are reported as severely threatened and declining within its Iberian range (Platts & Speight 1988; Byrne *et al.* 2009). The habitat for the species in this region has become increasingly fragmented and has deteriorated in recent years (Patrão *et al.*, 2015) with the result that populations in Ireland are now of international importance. Within Ireland, *G. maculosus* was once considered restricted to the southwest of the country, where it was thought to be limited to areas of native deciduous woodland, blanket bog, unimproved oligotrophic open moor and lake shores edges (Anon 2010). In 2010, however, the species was found, approximately 200km north of its assumed distribution in Co. Galway (Kearney 2010) and *G. maculosus* has since been discovered in numerous conifer plantations throughout the south-west of Ireland (Mc Donnell *et al*. 2011; Reich *et al*. 2012).

A large and distinctive slug, *G. maculosus* has a spotted colouration with two colour morphs: brown and grey/black (Platts & Speight 1988). Although some crossover has been noted (Platts & Speight 1988), the brown specimens are commonly found in closed woodland habitats, while the grey/black specimens are found in more open habitats such as blanket bog and heath (Rowson *et al.* 2014). Little is known about the origin of these colour morphs and while there is evidence that colouration in some species of slugs can be influenced by diet

(Jordaens 2000), this has not yet been determined in *G. maculosus.* The species has also been found to survive on a variety of food types in captivity, including mushrooms, oats, lettuce, and carrots (Rogers 1900; Wisniewski 2000) and will consume most vegetable matter offered (Platts & Speight 1988). In the wild, specimens have been observed to consume algae, bryophytes, lichen and fungi (Platts & Speight 1988) and preliminary feeding trials by Reich *et al*. (2012), found that the species feeds predominantly on lichens. In the latter study, slugs fed on 18 lichen species which were presented to them with the exception of the crustose lichens *Porpidia flavocruenta* and *Verucaria* sp. which grow on granite rocks. A range of ten moss, three liverwort, two heather and one fern species were also presented to *G. maculosus*. The slugs were observed to feed on two of the mosses, *Campylopus introflexus* and *Peurozium schreberi* and on all three of the liverworts (*Frullania dilatata*, *Metzgeria furcata*, and *Saccogyna viticulosa*) while rejecting the remaining eight moss species and both heather and fern species presented.

Ranking preferences for a range of food species requires comparing them all in a sequence of choice tests to establish true rankings (Mølgaard 1986). The methods employed by Reich *et al.* (2012), while hugely valuable in their establishment of food species that *G. maculosus* consumes, only recorded feeding behaviour as point observations with multiple test subjects and food choices within the same arena under daylight conditions. As *G. maculosus* is regarded as a crepuscular species (Platts & Speights 1988), the analysis of feeding behaviour under dark conditions may provide less stressful and more realistic conditions for the species. In addition, the trials by Reich *et al.* (2012) did not distinguish between the preferences of the two colour morphs of *G. maculosus.* Understanding differences between the colour morphs could be useful with a view to informing possible mitigation measures such as translocation to protect the species. While the previous studies are valuable in contributing to our understanding of the diet of *G. maculosus*, a more systematic pairwise comparison of food choices is required to clarify fully food preferences (if any) of the slug, thereby aiding future conservation efforts for the species. In addition to the scarcity of information on feeding preferences in the species, very little is known about movement and potential dispersal of *G.*

maculosus. Mc Donnell *et al.* (2011) provide the only movement estimate of the species, estimating the distance travelled by *G. maculosus* individuals in oak-birch woodland in southwest Ireland as 0.55m per day. While a low estimate is not unusual given the locomotive costs in terrestrial gastropods (Denny 1980) this study did not constantly monitor individuals over a set time-period, but instead employed a mark-recapture approach to assess distance travelled by individuals from an initial release point over a 64-day period. However, the advent of automated observational systems such as Ethovision means these can now be used to track (quantitatively) slug movements more accurately than by human observation alone (Spink *et al.* 2001).

Previous studies of terrestrial gastropods have made use of an acceptability or palatability index to rank feeding preferences. These indices were calculated using the area of a food item consumed divided by the area eaten of a reference item, with the reference item corresponding to a control in these studies (Dirzo 1980; Cates & Orians 1975). Given a suitable reference species has never been established for *G. maculosus*, and that this reference material may have an influence on the outcome of rankings (Richardson & Whittaker 1982), this approach unlikely to be suitable for *G. maculosus* until further research has taken place. Other studies have used food weight as a measure of the amount eaten (Richardson & Whittaker 1982; Wareing 1992; Clark *et al.* 1997). However, the small quantities of lichen and bryophytes eaten by *G. maculosus* combined with slug mucus deposition on the food items suggests that weight would not be a reliable method of determining food consumption as food items (including slug mucus) can sometimes weigh more following feeding by *G. maculosus* (I. Reich *pers. comm.*).

Given the legal obligations to protect the species within its natural range, including within commercial conifer plantations, an understanding of the feeding behaviour and food preferences of *G. maculosus* is necessary to further our understanding of the ecology of the species. Utilising behavioural analysis software like Ethovision XT10 will allow for a systematic, unbiased, pairwise analysis of feeding choice and movement parameters in *G. maculosus*. Given that this has never been undertaken for *G. maculosus*, a suitable experimental procedure is required. The lack of data regarding important aspects of *G. maculosus* feeding behaviour and the requirement for a suitable experimental procedure to undertake behavioural studies provided the incentive for this study, which aims to:

- Design, for the first time, an effective experimental procedure to analyse *G. maculosus* behaviour under dark conditions using Ethovision XT10 software
- Establish ranked feeding preferences for *G. maculosus* with a view to identifying habitats with optimal food sources to ensure continued protection of the species
- Quantify the mobility of *G. maculosus* in the laboratory with a view to establishing potential dispersal rates.

2.3. Materials and Methods

Adult *G. maculosus*, both forest and open habitat colour morphs (hereafter referred to as “forest” and “open” morphs), were collected during daytime, for safety reasons, from conifer plantations and open habitats (e.g. peatland and clear-felled areas) in counties Galway, Cork and Kerry, Ireland. *G. maculosus* roll into a defensive ball when disturbed, as was the case when removed from traps. Slugs greater than 1cm in diameter when rolled into a defensive ball were deemed to be adults for the purposes of this study (adapted from Reich *et al* (2012). Dissection to determine sexual maturity was not an option as this study required live subjects. Neither was weighing of individuals in the field as there were no stable surfaces on which to place scales and because humidity levels could affect the weight of slugs (A O Hanlon, pers. Comm). Individuals of the same colour morph were maintained in groups of eight in plastic containers lined with damp filter paper for a minimum of one week prior to the commencement of feeding trials. Test subjects were fed (*ad libitum*) a diet of oats and carrots (Platts & Speight 1988) to prevent any bias in subsequent feeding trials (Wareing 1993). The feeding trials tested slug preferences for a range of lichens, mosses and liverworts all of which are present in habitats where *G. maculosus* occurs.

2.3.1. Recording Set-up and Ethovision XT10

Eight experimental arenas (21cm x 21cm) were constructed following the design of Ribadulla Nogueira (2011) with two arenas placed in each of four fully enclosed wooden chambers (94cm x 66cm x 60cm) to prevent external light contamination. Experiments were undertaken in the dark since *G. maculosus* is known to be crepuscular (Platts & Speight 1988) and has been observed to spend more time feeding in the laboratory in the dark than under natural daylight conditions (Crowley *pers. comm.*). Each chamber was lit with two infra-red illuminators (Abus TV6700**)** and a single infra-red capable miniature CCD camera (Colour Sony SUPER HAD II CCD) was mounted above the two arenas. Arenas were separated by a sheet of Plexiglas to confine the individuals within the chamber and permit the use of a single camera (per chamber), thereby doubling the number of test subjects that could be recorded at any one time. A layer of damp cotton wool covered with damp filter paper was placed at the base of each arena to provide suitable humidity for slug activity (Dainton 1954). Experiments took place at room temperature.

The camera footage was examined using Ethovision XT10 software, which tracks animal movement, spatially and temporally, within each arena and has been successfully utilised to examine behaviour in slug species previously (e.g. Schüder *et al*. 2004; Ribadulla Nogueira 2011). Areas with the food options were marked digitally and since the position of the slug was detected using the centre point of the animal, the final digitised food zone included a 0.5 cm border to ensure that all instances of contact with food were noted. Individual slugs were then tracked and the amount of time spent in each zone quantified using Ethovision XT10. All recorded footage was reviewed to substantiate the tracking. Data were then analysed for: a) time taken to make contact with the food zones (latency to first); b) time spent in food zones (cumulative duration); c) number of times individuals entered a zone (frequency); d) total distance moved; and e) levels of sinuosity in the recorded tracks (meander) within the two hour trials.

2.3.2. Optimisation of experimental conditions

Containment

As slugs have the capacity to move upwards onto the underside of any lid and the cameras only provide a 2D view of the arenas, it was necessary to containindividuals at the bottom of the lidless arenas. Five barriers found to be effective for terrestrial gastropod containment in previous studies were assessed as barriers for *G. maculosus* within the experimental arenas. Barriers included Insect-A-Slip (a fluoropolymer resin (PTFE-30), also sold as Fluon®) (Symondson 1993; Ribadulla Nogueira 2011); salt impregnated blotting paper (Bailey 1989; Grimm & Schaumberger 2002), birch tar oil (Lindqvist 2010); copper tape (Schuder *et al.* 2003); and aluminium oxide sand paper (Bound 2004). Sixty *G. maculosus* were randomly selected and divided into groups of ten individuals. Each group was assigned to one of the barrier types, with one group acting as a control (no barrier). To encourage slug movement and consequently contact with the barrier, no food was provided and the base of the arena was saturated with water as observations by the authors indicate that *G. maculosus* tends to move away from overly wet substrates.

Salt paper barriers were created using filter paper (Whatman ®, 180-µm thickness) cut into 20 x 21cm strips. These strips were soaked in a 20% salt solution and air-dried over night. Aluminium oxide sandpaper was cut into 20 x 21cm strips prior to application. Insect-A-Slip, birch tar oil, and copper tape barriers were applied to 20 x 21cm strips of clear acetate sheets prior to attachment to the sides and top of arena walls to avoid direct contamination of the arena walls, which were used in subsequent trials. Barriers of sandpaper and salt paper were attached directly to the arena wall surface. Vaseline was selected as a method of attachment for all barriers as it allows for a smooth seal between the arena wall and the barrier and it does not appear to affect *G. maculosus* behaviour (based on observations by the authors). A single adult *G. maculosus* was placed in the centre of each arena. Direct observations of escaping behaviour were then undertaken every ten minutes for two hours. Individuals were deemed to have escaped when the entire body of the slug was outside the arena and clear of the barrier, at which point the time of escape was recorded. If after two hours an individual had not escaped, the arenas were covered with a large plastic container (sealed) and left for 24 hours at which stage containers were opened and any individuals that had left the arenas were noted.

Starvation

While feeding behaviour in *G. maculosus* can be easily identified by a hunched position coupled with the head and tentacles protruding slightly from beneath the mantle (Simroth 1891), the camera resolution did not permit identification of the exact position of the head and tentacles during recordings. Therefore, it was not possible to determine whether the slugs were simply resting on food or actively feeding during the recorded trials. Consequently, this behaviour was examined by direct observation, under dark conditions, within the previously mentioned wooden chambers prior to the commencement of recorded trials to assess the proportion of time that individuals were feeding when in contact with food. Slugs selected for direct observations were divided into three groups, each consisting of 24 individuals. Each group was allocated a starvation regime of 0, 24 and 48 hours prior to the start of observations. Individual adult *G. maculosus* specimens were thereafter placed in the centre of a standard 9cm Petri dish containing a damp layer of filter paper and a slice of carrot. Feeding behaviour was then observed every ten minutes over two hours.

2.4. Feeding behaviour

Using the optimum experimental conditions (see below results and discussion), food choice experiments were undertaken to establish *G. maculosus* food preferences. The food options presented consisted of nine lichen, three liverwort and two moss species each of which was selected because they are found in the forest and open habitats where *G. maculosus* occurs and are known to be eaten by the species (Reich *et al.* 2012) (Table 1). Lichen and bryophyte samples were identified using keys (Dobson 2000; Atherton *et. al.* 2010) and later verified by experts. Using these reference species, fresh samples were identified and collected from conifer plantations for use in feeding experiments.

Table 1 Lichen[a], liverwort[b] and moss[b] species upon which *Geomalacus maculosus* feeds (Reich *et al*., 2012) with their microhabitat associations and habitat types.
[a]Dobson 1992 [b]Atherton *et al.* 2010

	Microhabitat associations	**Habitats**	**Morphology**
Lichens			
Cladonia fimbriata (L.) Fr.	Rotten wood, earth, sand dunes amongst mosses.	Open & Closed	Fruticose
Usnea cornuta Körb.	Tree trunks, on rocks in woodland	Closed	Fruticose
Parmelia saxatilis (L.) Ach	Trees, walls, rocks	Open & Closed	Foliose
Cladonia coniocraea (Flörke) Spreng	Rotting wood in forests	Closed	Fruticose
Parmotrema perlatum (Huds.) M. Choisy	Trees, bushes and acidic rocks	Closed	Foliose
Cladonia squamosa (Scop.) Hoffm.	Rotting wood and acidic peaty soils	Open & Closed	Fruticose
Lepraria incana (L.) Ach	Trees, mosses and acidic rocks	Closed	Leprose
Cladonia portentosa (Dufour) Coem.	Heaths, peaty moorland, dunes.	Open	Fruticose
Cladonia uncialis (L.) F. H. Wigg.	Peat bogs and open acidic heaths	Open	Fruticose
Liverworts			
Frullania dilatata (L.) Dumort.	Well lit trees and shrubs, rock and turf in coastal areas	Open & Closed	-
Saccogyna viticulosa (L.) Dumort.	Woodland banks and damp rock faces	Closed	-
Metzgeria furcata (L.) Dumort.	Bark on wide range of trees and shrubs, occasional on rocks	Closed	-
Mosses			
Campylopus introflexus (Hedw.)Brid	Pioneer species of bare peat	Open	-
Pleurozium schreberi (Brid.) Mitt.	Heathland, bogs, and heathy woods	Open & Closed	-

Food options were presented in the arena as two discs of food (2cm diameter) placed 13cm apart. Discs were placed either in the top left (food zone L) or top right (food zone R) of the arena with a 2cm space between the food zone and the arena wall to permit the slug to move around the food (Fig 1). To account for any preference for a particular side of the arena, positions of food items were alternated between arenas in each trial.

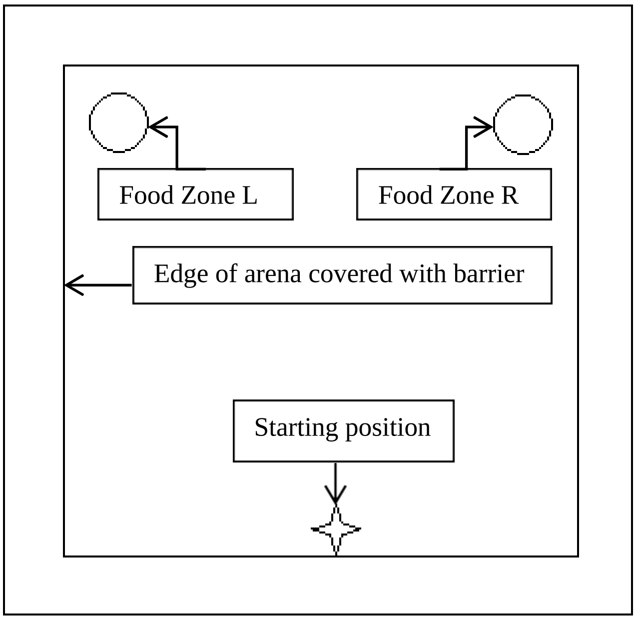

Fig. 1 Layout of arenas for *Geomalacus maculosus* food choice assessment

Following Reich *et al.* (2012) trial duration was two hours with eight slugs recorded simultaneously. A choice was considered to have been made when the centre point of the slug was within a designated feeding zone, thereby making contact with the food. A choice comparison trial was considered complete if all eight individuals made contact with at least one of the food items. If fewer than eight individuals in a trial made contact with a food option, that choice trial was repeated with an additional eight individuals. Following this second trial, even if fewer than eight individuals overall made a choice, the choice test was considered to be complete. Once a test subject made a choice, it was not used in further feeding trials. When an individual did not make contact with a food option, the recording of that individual was excluded from the analysis since all food options tested are known to be fed on by *G. maculosus* (Reich *et al*. 2012). However, these non-responders were subsequently tested in subsequent food preference trials after 24 hours.

The emphasis in this study was on the food choices of the forest morphs since food selection by these slugs has important implications regarding the impacts of commercial forestry practices on this protected species. Since *G. maculosus* has been found by Reich *et al.* (2012) to feed predominantly on lichens, a thorough comparison of nine lichen species was undertaken using the forest morph. In addition, all three liverwort and both moss species found by Reich *et al*. (2012) to be suitable food sources for *G. maculosus* were compared to the top ranking lichen species. Finally, the feeding preference of the open morph was also assessed with the top, middle and bottom ranked lichen species to determine whether open morph preferences were different to those of the forest morph.

2.4.1. Data analysis

Forest morph feeding preferences based on time spent by a slug in contact with individual food choices (cumulative duration) were determined as follows: For each trial, e.g. lichen species A and lichen species B, cumulative duration was calculated as a percentage of the total time spent in contact with food, i.e. lichen species A and B combined (total cumulative duration). This was repeated for species A (reference species) with each lichen species, i.e. species C, D, E, F etc (test species). Once all paired lichen combinations with reference species A were complete, mean percentage of total cumulative duration for reference species A was calculated. The same calculation was applied to all other lichen species tested, using each species in turn as reference species. To determine the rankings for open morph feeding preferences, the cumulative duration rankings derived for forest morphs of *G. maculosus*, the top, middle and bottom choices (*C. fimbriata*, *P. perlatum*, and *C. uncialis* respectively) were compared using open morphs of *G. maculosus*.

All analyses were carried out using SPSS version 21. Where data were normally distributed, an Independent Samples T-test was used to assess the differences between groups. Where data were found to be non-normally distributed, Mann Whitney U-tests were used to compare differences between two groups; where more than two groups were assessed, the Kruskal Wallis H-test was used followed by a Dunn's *post hoc* test to compare pair-wise differences.

2.5. Results

2.5.1. Optimisation of experimental conditions

Containment

As part of the optimisation of experimental conditions the effectiveness of five different barriers were assessed. No slugs escaped when using salt paper, birch tar oil or Insect-A-Slip with copper tape being less effective, containing only 40% of slugs, and all slugs escaping when sandpaper was used (Fig. 2).

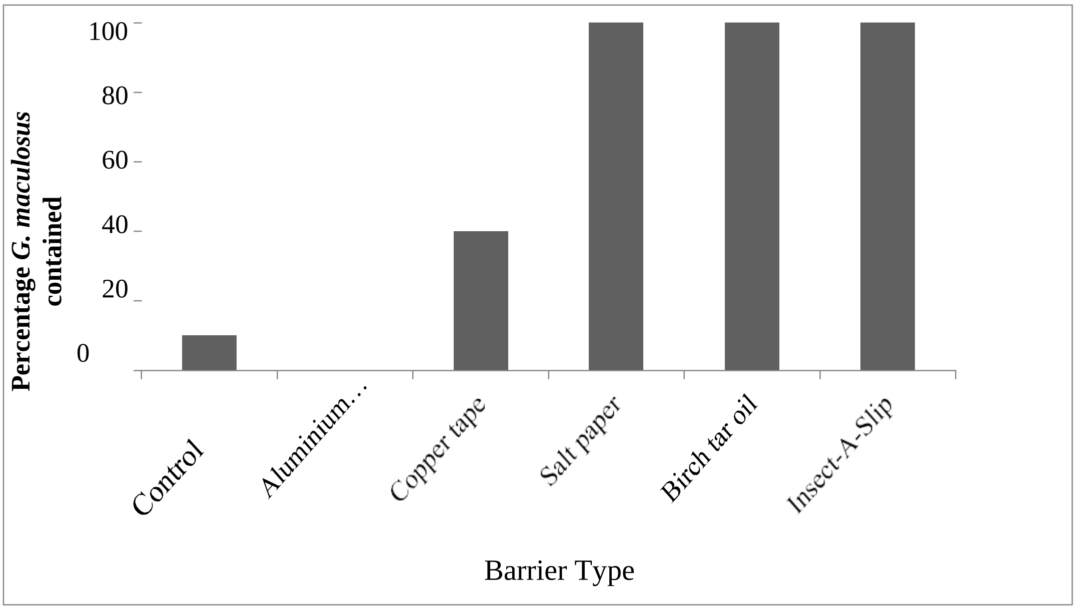

Fig. 2 Percentage of *Geomalacus maculosus* individuals (N = 10 per group) contained over 24 hours in arenas with different barriers

Starvation

As the feeding trials were limited to two hours (Reich *et al*., 2012), it was important to determine the optimal starvation time prior to the trials to maximise the chances of a test slug making a choice. Fifty-eight percent of slugs which had access to food and were not starved prior to the observational trials made contact with the food compared to just 34% and 13% of those which were not fed prior to the trials for 24 and 48 hours respectively. In addition, median percentage time spent actively feeding was greater for those slugs which were not starved 95% compared to

50% and 33% for those starved for 24 and 48-hours respectively (Fig. 3). Significant differences were found in the percentage time spent actively feeding between slugs that were not starved and those which were starved for 24-hours (P = 0.029, Kruskal Wallis followed by Dunn's Post Hoc). Based on the above results, slugs used in subsequent feeding trials were not starved prior to testing. Given that 95% (median) of the time spent in contact with the food source was spent eating, hereafter when individuals are in contact with a food source it is assumed that feeding is taking place, and so the term feeding preference is utilized.

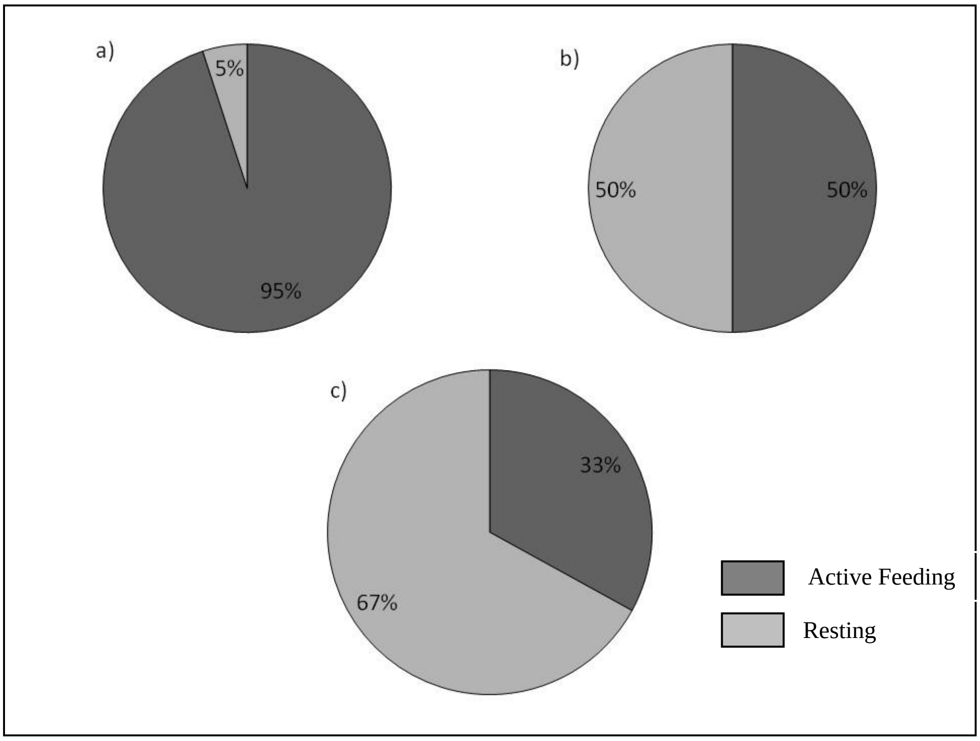

Fig. 3 Median percentage time (N* = 10, 7 and 3 for 0 hours, 24 hours and 48 hours respectively) spent by *Geomalacus maculosus* actively feeding and/or resting when in contact with carrot without any starvation period (a) and after 24 and 48 hours without food (b and c respectively). * Refers to numbers of slugs that made contact with the food source.

2.5.2. Feeding behaviour

Food choice analysis was undertaken for 400 trials where slugs made a choice. To ascertain whether the side of the arena in which the food option was placed had any influence on food choice, differences in latency to first and cumulative duration (regardless of food options presented) to food zone L and food zone R were assessed. The median latency to first and cumulative duration of individuals in contact with food regardless of side was 36.8 mins and 0.6 mins respectively. The median latency to first and cumulative duration of individuals making contact with food zone L was 39 mins and 0.5 mins respectively and with food zone R was 35.2 mins and 0.6 mins respectively. No significant difference was found in latency to first between food zone L and food zone R (P = 0.422, Mann-Whitney U-Test). In addition, there was no significant difference in total cumulative duration in food zones based on the side on which the food option was offered (P = 0.114, Mann-Whitney U-Test). Slugs made contact with both food options in 160 trials (40%) in total. To determine whether the food item chosen by *G. maculosus* (as determined by cumulative duration) was influenced by the food item contacted first, cumulative durations for the first food choice and for the second food choice (regardless of food options presented) were assessed. The median cumulative duration spent on food choices was 1.2 mins and 0.9 mins for choices 1 and 2 respectively, with no significant difference between the two (P = 0.128, Mann-Whitney U-Test).

2.5.3. Food preference: Forest morph

Overall, mean percentage of total cumulative duration ranged from 37% for *Cladonia uncialis* (L.) F. H. Wigg to 67% for *Cladonia fimbriata* (L.) Fr (Fig. 4). Individual comparisons showed a large amount of variation when test species were compared (Table 2 columns). However, *C. fimbriata* had the greatest mean percentage of total cumulative duration in comparisons with three reference species, (*Usnea cornuta* Körb, *C. coniocraea* (Flörke) Spreng, *Parmotrema perlatum* (Huds.) M. Choisy, making it the top preference more often than the other eight lichen species. This was followed by *P. perlatum* which ranked first with two reference species (*Cladonia squamosa* (Scop.) Hoffm, *C. uncialis*) (Table 2 rows).

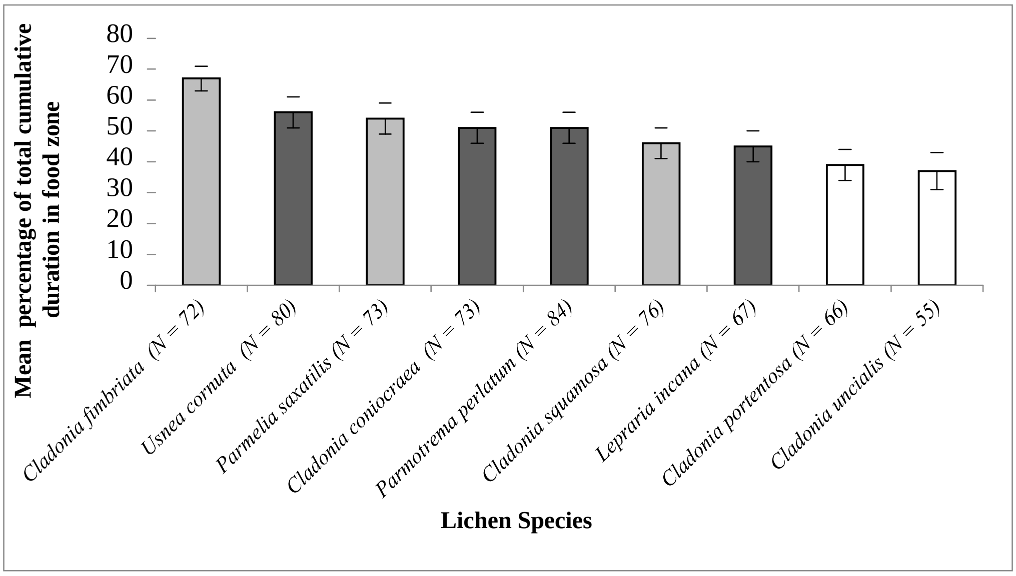

Fig. 4 Mean percentage (± SE) of total cumulative duration spent by *Geomalacus maculosus* forest morph in contact with each lichen species across all lichen species comparisons. Dark grey bars indicate lichen species found in closed habitats, light grey indicates species found in open / closed habitats and white indicates species found in open habitats only (Dobson 1992)

Table 2 Mean percentage (± SE) cumulative duration of *Geomalacus maculosus* forest morphs spent in contact with a test species (left-hand column) placed simultaneously in a feeding preference trial with a reference species (top row). Rows indicate percentage cumulative duration of a test species against all reference species. Numbers in bold indicate the highest ranking test species against a reference species

	Reference Species								
Test Species	***Cladonia fimbriata***	***Usnea cornuta***	***Parmelia saxatilis***	***Cladonia coniocraea***	***Parmotrema perlatum***	***Cladonia squamosa***	***Lepraria incana***	***Cladonia portentosa***	***Cladonia uncialis***
Cladonia fimbriata	-	**69 ± 9**	50 ± 15	**74 ± 11**	**75 ± 10**	80 ± 7	57 ± 13	78 ± 11	43 ± 16
Usnea cornuta	31 ± 9	-	51 ± 13	62 ± 12	71 ± 12	40 ± 13	71 ± 14	**100 ± 0**	47 ± 17
Parmelia saxatilis	50 ± 16	49 ± 13	-	63 ± 10	54 ± 13	58 ± 14	44 ± 11	59 ± 12	60 ± 24
Cladonia coniocraea	26 ± 11	38 ± 12	37 ± 10	-	36 ± 16	54 ± 12	**74 ± 14**	76 ± 14	71 ± 13
Parmotrema perlatum	25 ± 10	29 ± 12	46 ± 13	64 ± 16	-	**81 ± 10**	32 ± 16	50 ± 17	**77 ± 9**
Cladonia squamosa	20 ± 7	60 ± 13	42 ± 14	46 ± 12	19 ± 10	-	67 ± 14	60 ± 16	65 ± 16
Lepraria incana	43 ± 13	29 ± 14	**56 ± 11**	26 ± 14	68 ± 16	33 ± 14	-	42 ± 13	61 ± 31
Cladonia portentosa	22 ± 11	0 ± 0	41 ± 12	24 ± 14	50 ± 17	40 ± 16	58 ± 13	-	68 ± 18
Cladonia uncialis	**57 ± 16**	53 ± 17	40 ± 49	29 ± 13	23 ± 9	35 ± 16	39 ± 31	32 ± 18	-

Table 3 Percentage of choices by *Geomalacus maculosus* forest morph (in trials where only one lichen species was selected) for test species (left-hand column) in a feeding preference trial with reference species (top row). Rows indicate percentage choice of a species against all reference species. Numbers in bold indicate the highest ranking test species against a reference species

		Reference Species								
		Cladonia fimbriata	***Usnea cornuta***	***Parmelia saxatilis***	***Cladonia coniocraea***	***Parmotrema perlatum***	***Cladonia squamosa***	***Lepraria incana***	***Cladonia portentosa***	***Cladonia uncialis***
Test Species	*Cladonia fimbriata*	-	**80**	57	**100**	**83**	**100**	40	**100**	0
	Usnea cornuta	20	-	60	50	**83**	25	**83**	**100**	50
	Parmelia saxatilis	43	40	-	**100**	80	67	33	60	33
	Cladonia coniocraea	0	50	0	-	37	67	75	75	75
	Parmotrema perlatum	17	17	20	63	-	**100**	29	50	**100**
	Cladonia squamosa	0	75	33	33	0	-	71	60	66
	Lepraria incana	60	17	**67**	25	71	29	-	20	50
	Cladonia portentosa	0	0	40	25	50	40	80	-	67
	Cladonia uncialis	**100**	50	33	25	0	34	50	33	-

Similar variance was found with mean (± SE) latency to first which ranged from 39.2 mins (± 4.5 mins) for *P. perlatum* to 51 mins (± 6.1 mins) for *C. uncialis* (Fig. 5).

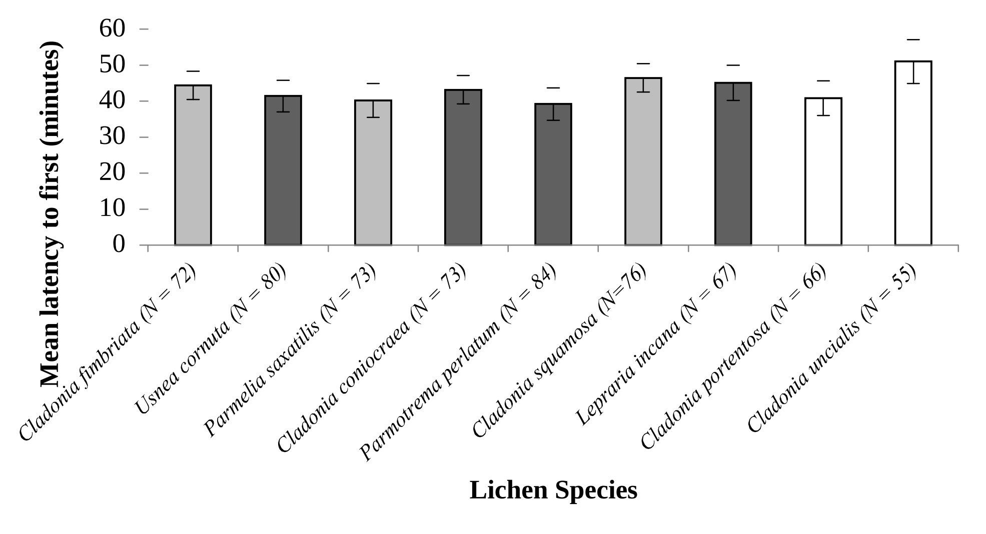

Fig. 5 Mean (± SE) latency to first (minutes) by *Geomalacus maculosus* forest morph to make contact with each lichen species across all lichen species comparisons. Dark grey bars indicate species found in closed habitats, light grey indicates species found in open / closed habitats and white indicates species found in open habitats only (Dobson 1992)

There was no correlation between mean latency to first for each of the lichen species and the mean percentage of total cumulative duration in contact with each species (P = 0.252, R_s = -0.427 Spearman's Correlation). To establish further rankings, trials where individuals made contact with only one food option (240 or 60%) were used to calculate the percentage number of times a particular lichen species was chosen out of all possible choice tests where this species was an option. Overall percentages ranged from 41% for both *C. uncialis* and *Lepraria incana* (L.) Ach to 62% for *C. fimbriata* (Fig. 6). Preferences for lichen species again depended on which lichen species were being offered simultaneously. *Cladonia fimbriata* was once again the greatest or joint greatest percentage choice in comparisons, this time with five species, (*U. cornuta, C. coniocraea, P. perlatum, C. squamosa, Cladonia portentosa*), making it the top preference more often than the other eight lichen species used (Table 3 rows).

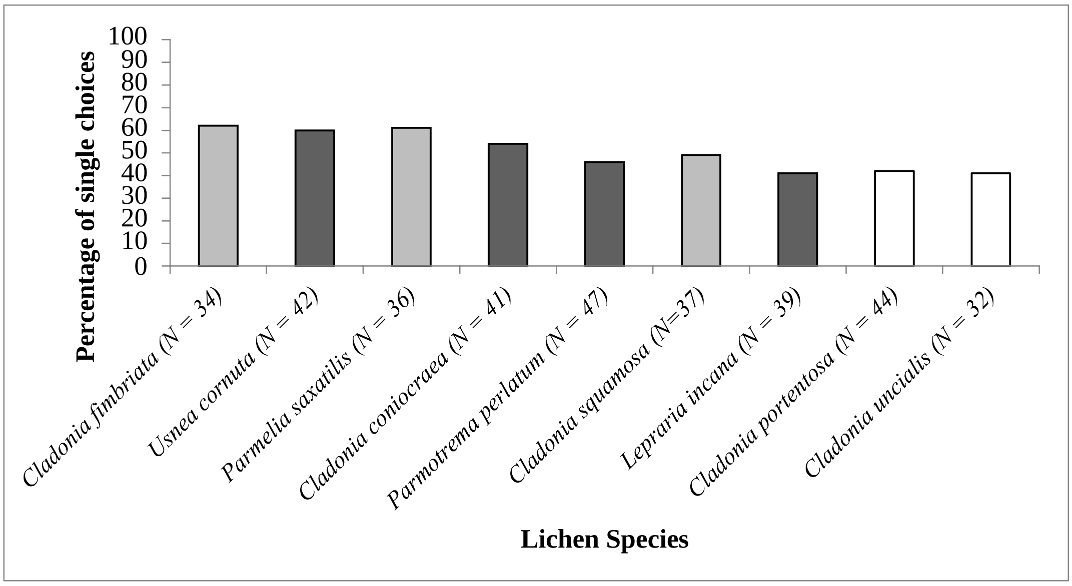

Fig. 6 Percentage of choices made for each lichen species in *Geomalacus maculosus* feeding trials where only one food option was selected. Dark grey bars indicate species found in closed habitats, light grey indicates species found in open /closed habitats and white indicates species found in open habitats (Dobson 1992)

As *C. fimbriata* was the overall top ranking species it was used in comparisons to obtain the preference rankings of the two species of moss and three species of liverworts known to be eaten by *G. maculosus* (Reich *et al*., 2012) using mean percentage of total cumulative duration spent in contact with food. Out of the five species, the liverwort *Frullania dilatata* (L.) Dumort. had the greatest mean (± SE) percentage with 63% (± 18%), followed by the mosses *Pleurozium schreberi* (Brid.) Mitt (62% ± 13%), *Camoylopus introflexus* (Hedw.) Brid (48% ± 12%), and *Metzgeria. furcata* (L.) Dumort (47% ± 15%), and the liverwort *Saccogyna viticulosa* (L.) Dumort (44% ± 12%) (Fig. 7). Mean percentage of the cumulative duration spent in contact with *C. fimbriata* over all five of the comparisons with liverworts and lichens was 50% (± 6%).

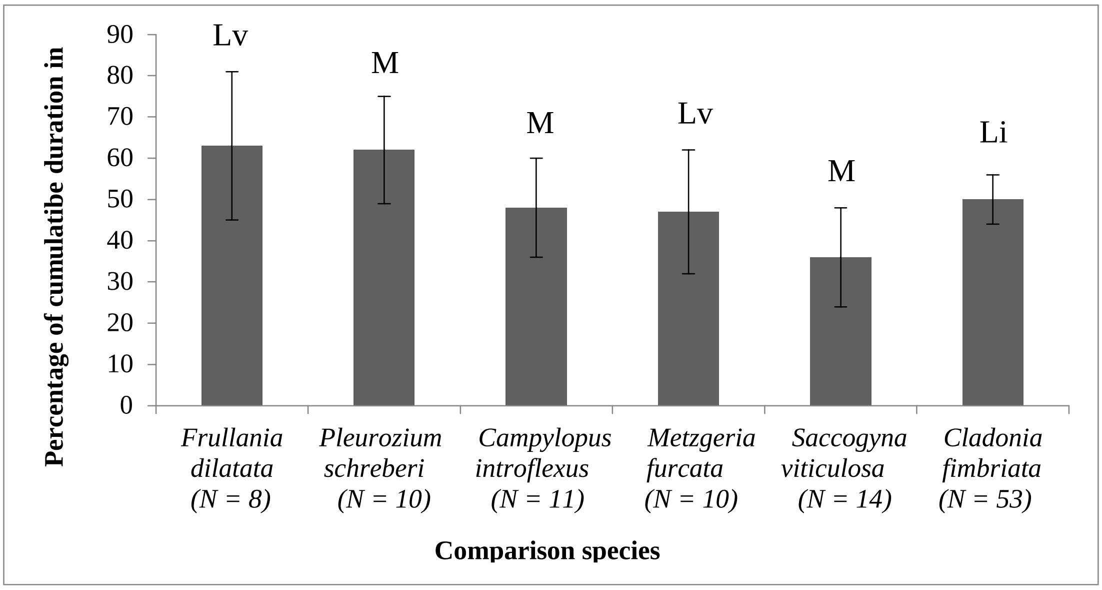

Fig. 7 Mean percentage (± SE) of total cumulative duration of time spent by *Geomalacus maculosus* forest morph in contact with each liverwort (Lv) and moss (M) species in comparison to the lichen *C. fimbriata* (Li)

2.5.4. Food preferences: Open morph

The top ranked choice for open morphs (Fig. 8), based on cumulative duration of contact with food (± SE), was, as in the forest morphs, *C. fimbriata* (67% ± 11%) followed by *P. perlatum* (55% ± 11%) and *C. uncialis* (24% ± 8%). Given that the choices made by open morphs showed the same trends as forest morphs, preferences for moss and liverwort species were not assessed for the open morph.

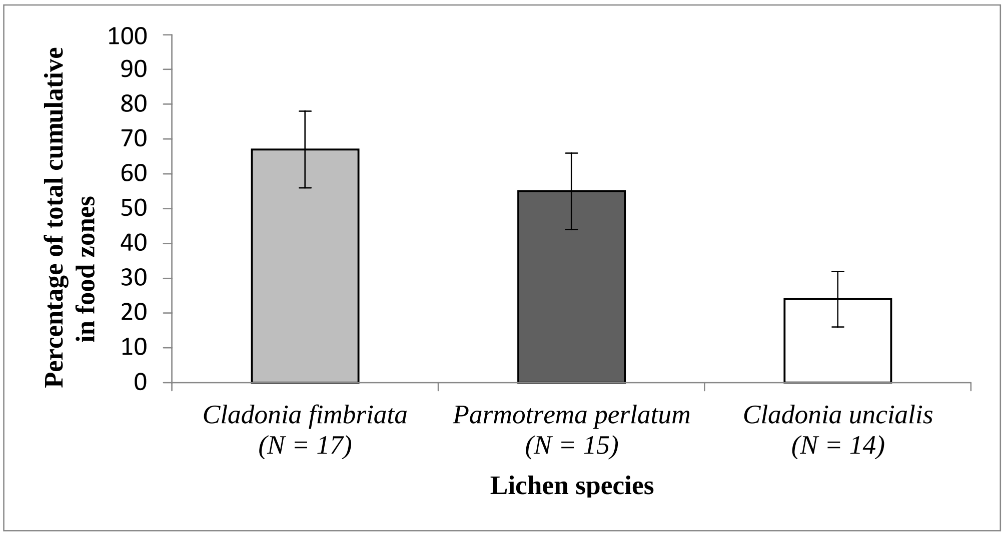

Fig. 8 Mean percentage (± SE) of total cumulative duration spent by *Geomalacus maculosus* open morph in contact with each species across three lichen species comparisons selected based on forest morph rankings. Dark grey bars indicate species found in closed habitats, light grey indicates species found in open /closed habitats and white indicates species found in open habitats (Dobson 1992)

2.5.5. Behavioural differences between colour morphs

In terms of general movement parameters, the mean velocity (± SE) for forest and open morphs was 0.083 cm/s (± 0.008 cm/s) and 0.09 cm/s (± 0.008 cm/s) respectively with no significant difference between the two (P = 0.285, Independent Samples T-test). The mean distance (± SE) moved over two hours for forest and open morphs was 6.7m (± 2.9m) and 5.61m (± 3.6m) respectively again with no significant difference between the two morphs (P = 0.265, Independent Samples T-test). However, the mean meander (± SE) open morphs (942.9 deg/cm (± 79.8 deg/cm) was significantly greater (P = 0.008, Independent samples T-test) than that for forest morphs at 835 deg/cm (± 88.6 deg/cm).

To assess the differences in feeding behaviour between forest and open morphs, outcomes from trials using the same three lichen species (*C. fimbriata*, *P. perlatum*, and *C. uncialis* respectively) were compared. No significant differences were found between forest and open morphs in the cumulative duration spent in contact with *C. fimbriata*, *P. perlatum*, or *C. uncialis* (P = 0.657, P = 0.963, P = 0.193 respectively, Mann-Whitney U-Test). The median frequency of individuals making contact with food zones was 5.5 occasions for forest morphs and 3 occasions in open morphs, with no significant difference between the two (*P* = 0.801, Mann-Whitney U-Test).

2.6. Discussion

2.6.1. Optimisation of experimental conditions

The optimal set-up for the feeding behaviour studies was determined through trials which found that the most effective barriers were those of salt paper, birch tar oil and Insect-A-Slip, all of which were 100% effective in retaining *G. maculosus* within the arenas. These barriers proved to be more effective than copper tape and aluminium oxide sand paper. Given that olfaction plays a role in slug foraging behaviour (Sahley *et al.* 1981), it was decided not to use birch tar oil as its strong smell could influence slug feeding behaviour. In addition, when some slugs made contact with Insect-A-Slip they began secreting dark yellow/orange mucus instead of the usual clear yellow tinged mucus normally secreted by *G. maculosus* (Platts & Speight 1988). These effects have not been reported in cases where Insect-A-Slip has been used successfully as a containment method in other terrestrial gastropod studies (Schüder *et al.* 2004; Armsworth *et al.* 2005; Rae *et al.* 2009). While such changes in mucus colouration have not been recorded previously for *G. maculosus*, changes in mucus colour in other slug species (example *Deroceras reticulatum*) is known to be a symptom of distress (Mc Donnell *et al.* 2009). Salt impregnated blotting paper was, therefore, selected as the barrier for this study due to its effectiveness as a barrier, ease of application, cost effectiveness and the apparent absence of visible effects on slug behaviour.

Following the assessment of different starvation regimes, it was found that individuals which were not food deprived were most likely to make contact with a food option in addition to being least likely to remain in contact with food without actively feeding. This was counter intuitive and may have been a result of the short time period in which the trial was carried out. Although Mølgaard (1986) did not specifically examine different lengths of starvation, he noted that while a level of hunger is necessary to encourage feeding in test subjects, test subjects “must not be so hungry that they will eat anything immediately”. As such, the decision not to deprive individuals of food prior to the choice tests may have ultimately prevented bias in this study. However, further work is required to establish the best pre-treatment for future studies of *G. maculosus* using feeding trials of different durations. The use of ten minute intervals for the observation of feeding behavior may have resulted in incidences where feeding was taking place but was not observed, therefore limiting the data collected. Further research looking at pre-treatment in *G. maculosus* should take place on a continual basis to avoid this limitation.

2.6.2. Feeding behaviour

Despite optimisation tests showing that no starvation prior to food choice trials yielded the greatest feeding responses, a large proportion (54%) of slugs did not make contact with the food options provided in the tests. This is likely a reflection of the inevitable variability within the groups of slugs selected for these trials as Hanley (1995) has reported that even where individuals underwent the same pre-treatment prior to use in experiments, there was a great deal of variation in feeding responses by molluscs. In the majority of cases reasons for this variation is unknown (Speiser 2001) although Hanley *et al.* (2003) note that physiological differences and variations in the learning abilities of test subjects may have a part to play.

There was no statistically significant difference in the time spent by individuals between the first or second food option with which slugs made contact. Previous studies on slug feeding behaviour involving *D. reticulatum* (Bailey 1989; Cook *et al.* 1997) have found that the first choice selected tends to be the one on which slugs fed the most. However, Bailey (1989) further noted that while starved individuals exhibited a preference for their first food choice, this preference was not significant in slugs that were not deprived of food, possibly indicating food sampling behaviour. Given that the results of this study were not significant, it is likely that not starving individuals prior to this study may have prevented such bias. In addition to the lack of influence from first food contact, results of this study showed no statistical difference between the sides of the arena that the food options were placed in for either latency to first or cumulative duration, indicating that choices were not biased by placement within the arenas. Given that the choices by *G. maculosus* were made independently of the side of the arena in which the food option was placed, and that individuals were not more likely to simply remain in contact with the first food choice they encountered, it would appear that choices made by individuals were based on the food options themselves.

2.6.3. Food preference

Test subjects made contact with all species of lichen, moss and liverworts that were presented in trials. The feeding preferences of open colour morphs of *G. maculosus* showed the same ranking preferences as the forest colour morphs, with the top ranking choice (based on percentage of total cumulative duration) being *C. fimbriata*, followed by *P. perlatum*, with *C. uncialis* ranking last. When ranking a number of species against each other, comparing them all in a sequence of choice tests is required to establish the true rankings of species (Mølgaard 1986). In this study rankings were established through a number of different assessments: percentage cumulative duration spent in food option zones; latency to first; and trials where only one choice was made. While rankings based on total cumulative duration and on trials where only one food option was chosen both had *C. fimbriata* as the top choice, individuals were quickest in making contact with *P. perlatum* (latency to first). Variation was evident when the rankings were ordered based on reference species used in all cases, e.g. while *C. fimbriata* ranked first when *U. cornuta* was the reference species, it ranked last when *C. uncialis* was the reference species. As *C. fimbriata* ranked top most often, it was used in further trials in this study comparing moss and liverwort species. Here *C. fimbriata* ranked in third place after the liverwort *F. dilatata* and the moss *P. schreberi.* The variation found in this study is in line with Richardson & Whittaker (1982), who noted that the reference material used when ranking species in terms of preference has an influence on the outcome of the order. The influence of options offered to individuals during a choice test may also account for the difference in rankings observed by Reich *et al.* (2012), who found *U. cornuta* ranked first out of the species included in this study, when multiple food options were presented to the slugs in their trials.

Movement is energetically costly for gastropods (Denny 1980) it would be less efficient for an individual to spend time foraging for a particular food source, than to simply sample and consume the variety of food sources available (Cook *et al.* 1997). While *G. maculosus* appears to primarily feed on bryophyte and lichen species in their feeding preferences (Reich *et al.* 2012), the apparent fluidity of their preference rankings is likely to be beneficial in terms of foraging strategy. In addition, it is unclear how long the preference for a particular species is maintained by *G. maculosus.* Cook *et al.* (1997) have shown food preferences exhibited by some species of slugs to last no longer than a few hours. In other cases preferences were only maintained while the food choice was readily available, after which it was outranked by a more available, previously lower ranking species (Bailey 1989). In addition, seasonal differences in the diet of terrestrial gastropods have been found to be related to the availability of food choices (Iglesias & Castillejo 1999). Indeed, tests undertaken in a laboratory setting cannot account for factors encountered in the wild (e.g. competing odours), which can be dependent on the abundance of a food species within a habitat (Barker 2001). While seasonality is unlikely to impact on species availability in lichens, it may have an influence on other food sources like fungi. Although it has been reported that *G. maculosus* feeds on fungi (Platts & Speight 1988) it has never been examined closely. Given that commercial conifer plantations can support a high diversity of macrofungi that is comparable to that of native oak forest (O'Hanlon & Harrington 2012), this may be an additional food source for *G. maculosus* seasonally. It is, therefore, important that species of fungi are examined for preference in *G. maculosus*.

All of the lichen species examined within this study are listed as either common or abundant within their respective habitats in Ireland (Purvis *et al.* 1992), along with the liverworts *F. dilatata*, and *M. furcata,* and the mosses *C. interoflexus* and *P. schreberi*. Given the fluidity of the preferences of *G. maculosus*, measures that promote lichen and bryophyte growth in areas where *G. maculosus* occurs would likely be of benefit to the conservation of the species. The top ranked lichen species, *C. fimbriata* which is described by Dobson (1992) as being "very common on rotting wood, earth and sand dunes, often amongst mosses, even in polluted areas" and was collected in this study from rotting wood on the edges of commercial conifer plantations. Managed forests have much less fallen wood than natural forests (Kirby *et al.* 1998) and measures to increase dead wood in commercial plantations, i.e. through retention of a portion of standing and fallen deadwood (Humphrey & Bailey 2012) is likely encourage the growth of species like *C. fimbriata*, along with other deadwood associated species *C. squamosa*, and *C. coniocraea* (Dobson 1992). The top ranked liverwort species, *F. dilatata* grows in well-lit surroundings (Atherton *et al.* 2010) and so, measures to increase light within commercial conifer plantations would also benefit not only this species but also the lichen *U. cornuta* (Dobson 1992).

Open colour morphs did not show a preference for lichen species restricted to open habitats which suggests that food choices are not influenced by food availability in the natural habitats of the two colour morphs. This is further supported by the fact that in 40% of the trials, contact with both food options was made with a mean contact frequency of 5.5 occasions for forest morphs and three occasions for open colour morphs observed over two hour trials. This indicates that individuals of both colour morphs frequently made contact with the food options available. This also reflects the findings of Dirzo & Harper (1982) who demonstrated that slugs sample food sources to ascertain whether they are distasteful or not and Cook *et al.* (1997) who found that slugs often returned to feed on an initial choice after moving away a short distance. This may also explain the lack of correlation between mean latency to first and mean cumulative duration for lichen species. It is interesting to note that the two lowest ranking species are both found in open habitats; however although reasons for this are unclear. Although Reich *et al.* (2012) found that the two types of crustose lichens were not consumed, there was no appreciable pattern in ranking based on morphology of the species in this study.

While it is uncertain what factors specifically drive choices in *G. maculosus*, slugs are known to avoid species that produce defensive compounds that reduce palatability and/or select plant species with the greatest nutritional content (Lawrey 1989). Lawrey (1989) notes that the avoidance of unpalatable species is the most likely method of selection in lichen herbivores which is supported by mosses being generally considered unpalatable to slugs due to the presence of phenolic compounds (Davidson and Longton, 1987; Davidson *et al*., 1989) and secondary compounds produced by fungal symbionts in lichens (Asplund and Wardle 2013). Although results from this study found the moss *P. schreberi* ranked higher than the top lichen species *C. fimbriata*, this may be due to *P. schreberi* containing relatively low levels of these phenolic compounds (Reich *et al.* 2012) thus increasing its acceptability. The potential effect of these compounds on feeding preference in *G. maculosus* requires further research to fully understand the factors that drive feeding selection in the species. In addition, while both this study and that by Reich *et al.* (2012) focused only on lichen and bryophyte species, as previously mentioned, other food sources available to *G. maculosus* include fungi (Platts & Speight 1988) and further research should investigate fungal species (Reich *et al.* 2012).

2.6.4. Behavioural differences between colour morphs

The mean distance moved over two hours was 6.7m and 5.6m for forest and open morphs respectively with a mean velocity 0.083 cm/s for forest colour morphs and 0.09cm/s for open colour morphs. These figures were high given that the previous estimate for movement by individuals in the wild was 0.55m per day (Mc Donnell *et al.* 2011). While it is probable that the rate of movement determined by Mc Donnell *et al.* (2011), which was not based on direct observation but mark-recapture experiments, was underestimated, the high rate of movement observed in this study may be an overestimation because novel conditions in the experimental areas may have resulted in increased exploratory behaviour. Given the previously mentioned costs of locomotion for slugs (Denny 1980) and that the substrate provided in the arenas was conducive to movement as it was a smooth, flat, damp surface which was free of obstacles, it is likely that the distances and velocities achieved in this study are greater than those in nature. The mean meander, a measure of the sinuosity of a recorded track (Maréchal 2004), was calculated as 835 deg/cm and 942 deg/cm for forest and open morphs respectively with a significant difference between the two. Looping and turning is thought to be associated with exploratory behaviour in slugs to triangulate the direction of olfactory gradients (Rollo &

Wellington 1979). The significant difference between the two colour morphs was surprising given the lack of differences in their feeding behaviours and movement parameters generally. Reasons for this difference are, as yet, unclear. However, lichen cover in commercial forests in Ireland is greatest in the top third of trees (Coote *et al.*, 2007) whereas lichen species found in more open habitats like *C. uncialis* and *C. portentosa* can be scattered across the substrate (Dobson 1992). These differences in food source aggregation may cause the differences in meander since individuals in open habitats could be forced to exhibit increased exploratory behaviour in the wild to make contact with food sources.

2.7. Conclusion

This study has established, for the first time, an effective, reproducible, objective experimental procedure to analyse *G. maculosus* behaviour under dark conditions using Ethovision XT10 software, highlighting the importance of optimisation trials prior to any feeding studies. Results from feeding trials show a large amount of variability in feeding choice by *G. maculosus* individuals indicating that the species is fluid in terms of the preferences exhibited. *G. maculosus*, therefore, appears to be a generalist lichen and bryophyte herbivore as opposed to having a strong preference for a particular species or lichen growth form. Therefore, measures that promote lichen and bryophyte growth through the retention of deadwood and an increase in light availability within mature conifer plantations are likely to benefit populations of the top ranked lichen and bryophyte species. This study also established, for the first time, a lack of differences generally in terms of feeding preferences between colour morphs of *G. maculosus*. This indicates that where mitigation measures are put in place, the benefits for both colour morphs should be equal although more research is required to determine the impacts of these measures in the field. Finally, the quantification of movement parameters for the first time in the species has shown that *G. maculosus* has potential dispersal rates that are much greater than previously realised which has implications for colonisation of new sites. However, further research investigating rates of movement in the field using radio transmitters, as has been used in snails (eg Bailey 1989) is needed to provide a more precise and realistic representation in the field.

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Chapter 3: Monitoring the EU protected Geomalacus maculosus (Kerry Slug): what are the factors affecting catch returns in open and forested habitats?

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Monitoring the EU protected Geomalacus maculosus (Kerry Slug): what are the factors affecting catch returns in open and forested habitats?

3.1. Abstract

Geomalacus maculosus is a slug species protected under EU law with a distribution limited to the west of Ireland and north-west Iberia. The species, originally thought to be limited within Ireland to deciduous woodland and peatland, has been found in a number of commercial conifer plantations since 2010. While forest managers are now required to incorporate the protection of the species where it is present, no clear species monitoring protocols are currently available. This study examines the efficacy of De Sangosse refuge traps across three habitats frequently associated with commercial forest plantations in Ireland and compares them with hand searching, a commonly used method for slug monitoring. Catch data during different seasons and under different weather conditions are also presented. Results indicate that autumn is the optimal time for sampling *G. maculosus* but avoiding extremes of hot or cold weather. While refuge traps placed at 1.5m on trees in mature conifer plantations and directly on exposed rock in blanket peatlands result in significantly greater catches, hand searching is the most successful approach for clear-fell areas. Hand searches in clear-fell preceded by rain are likely to result in greater numbers caught. The results of this study form, for the first time, the basis for *G. maculosus* monitoring guidelines for forestry managers.

3.2. Introduction

The phylum Mollusca, with 85,000 (approx.) described species across aquatic and terrestrial habitats (Chapman, 2009), is one of the most successful animal groups ranked after arthropods and vertebrates (South, 2012). Nevertheless, 42% of all animal extinctions since the 1500s have been molluscan species (Lydeard et al., 2004). The number of molluscan extinctions alone in the last 400 years outweighs that of birds, mammals, reptiles and amphibians put together (Bouchet et al., 1999). Within Ireland, 150 species of native non-marine molluscs have been evaluated for conservation status and *Geomalacus maculosus* Allman is one of six legally protected mollusc species under European legislation (Byrne et al., 2009). Given the restricted distribution of the species to the west of Ireland and north-west Iberia, *G. maculosus* is protected under Appendix II of the Berne Convention and under Annexes II and IV of the European Union Habitats Directive (92/43/EC). Irish populations are considered to be of international importance as the Iberian range of the species has been reported as severely threatened and declining (Platts and Speight, 1988; Byrne et al., 2009) and *G. maculosus* is listed as vulnerable in Spain (Verdú and Galante, 2006).

Platts and Speight (1988) described *G. maculosus* in Ireland as a “handsome” crepuscular slug, coloured either brown with yellow spots or grey/black with white spots. Brown specimens are commonly found in woodlands and grey/black specimens in the more open habitats such as blanket bog and heath (Rowson et al., 2014), although some crossover has been found to occur (Platts and Speight, 1988). Originally discovered in Co. Kerry in the south-west of Ireland by Allman in 1842, the species was subsequently found in 1873 in Portugal and in 1886 in Spain (Platts and Speight, 1988) with recent research by Reich et al., (2015) indicating that Irish populations are genetically close to those in northern Spain. Given that the species is not found in countries such as France and Britain which lie between north-west Iberia and Ireland (i.e. it has a disjunct distribution), *G. maculosus* is referred to as a Lusitanian species, Lusitania being originally a Roman province corresponding to Portugal and parts of Spain today. In Ireland, *G. maculosus* was traditionally considered to be restricted to the southwest of the country and within this distribution only in areas of deciduous woodland, blanket bog, unimproved oligotrophic open moor and on lake shores (Anon, 2010). In 2010, however, it was found breeding in a commercial conifer plantation in Co. Galway (Kearney, 2010) 200km (approx.) north of its previously known distribution. Since then, *G. maculosus* has also been discovered in numerous conifer plantations in the south-west of Ireland (Mc Donnell and Gormally 2011a; Reich et al., 2012).

The Republic of Ireland is one of the least forested countries in Europe with just over 10% of the land under forest in 2012 of which 68% consists of commercial forestry (Department of Agriculture Food and Marine (DAFM, 2015). Over half of the national forest estate is owned by the state and of this 93% is owned by the state sponsored company Coillte (NFI, 2012). *Picea stichensis* (Bong.) Carr. is the predominant tree planted in commercial plantations in Ireland (DAFM, 2015) and the Forest Service aims to increase forest cover in Ireland to 17% by 2030, primarily through increases in commercial forestry cover (Forest Service 2008). Forestry management is considered one of the main threats to *G. maculosus* along with invasive species, agricultural reclamation and habitat fragmentation (NPWS, 2013). Prior to 2011, the recommended method for surveying the species was through hand searching (National Roads Authority, 2009). No effective or repeatable trapping method for *G. maculosus* existed until Mc Donnell and Gormally (2011b) trialled a range of refuge traps and established that De Sangosse refuge traps were the most effective for surveying *G. maculosus*. De Sangosse traps (50cm x 50cm) consist of a layer of recycled absorbent material (approximately 3mm thick) between an upper reflective thermal foil (< 1 mm thick) and a black plastic lower surface (< 1 mm thick) with perforations (< 1mm) spaced 5 mm (approx.) apart. A metal eyelet (25 mm (approx.) OD; 13mm (approx.) ID) in each of the four corners is used to secure the trap. While Reich et al. (2012) used these traps to determine the influence of environmental factors (e.g. temperature) on *G. maculosus* and successfully captured the species for the first time on tree stumps in a forest clear-fell, their data were sourced from a single commercial plantation only.

The presence of *G. maculosus* in commercial conifer plantations in Ireland means that forestry managers are legally obliged to protect the species while undertaking day-to-day forestry practices (e.g. clear-felling). In addition, managers seeking Forest Stewardship Council (FSC) certification (Principle 6) (2016) are required to conserve biodiversity. This requires the development of standardised monitoring protocols so that managers can determine: a) whether *G. maculosus* is present on site; and b) if present, incorporate appropriate management strategies to ensure its protection. However, standardised protocols are not currently available since the optimal positioning of De Sangosse refuge traps in forests and associated habitats such as clear-fell and unplanted areas has not yet been determined. In addition, no comparison to date has been undertaken to compare the effectiveness of using refuge traps with simple hand searching, another commonly used sampling method for *G. maculosus* (NRA, 2009) and other terrestrial gastropods (Hunter 1968). This provided the incentive for this study which for the first time, examines different trapping methods across a range of open and forested sites.

Aims

1. Assess the impact of De Sangosse refuge trap position in forested and open habitats on *G. maculosus* catches and compare these with hand searching.
2. Quantify the effects of seasonal variation on catches to determine the optimum sampling season for site assessment.
3. Determine the influence of temperature and rainfall in forested and open habitats on catches to inform optimum weather conditions during which to undertake sampling.

3.3. Materials and Methods

3.3.1. Study areas

Two studies, one carried out over twelve months (Long-term study) and one over four months (Short-term study) were undertaken in and near commercial conifer plantations within the range of *G. maculosus* in the south-west of Ireland. Four study sites (Fig. 1) were chosen within which were selected:

1. Compartments (forestry management unit) of mature commercial conifers (predominantly *P. stichensis*) planted on peatland in the early 1970s (Coillte, 2015).
2. A compartment, which was clear-felled in 2013 and, at the time of the study, was dominated by *P. sitchensis* tree stumps interspersed with *Digitalis purpurea* L., *Juncus effusus* L. and mosses.
3. An adjacent area of unplanted peatland containing predominantly *Molinia caerulea* (L.) Moench, and *Calluna vulgaris* (L.) Hull.

Another slug species (*Lehmania marginata* Müller) was also found in conifer plantations during the study but catches of this species represented just 4% of the total catch of slugs across all sites.

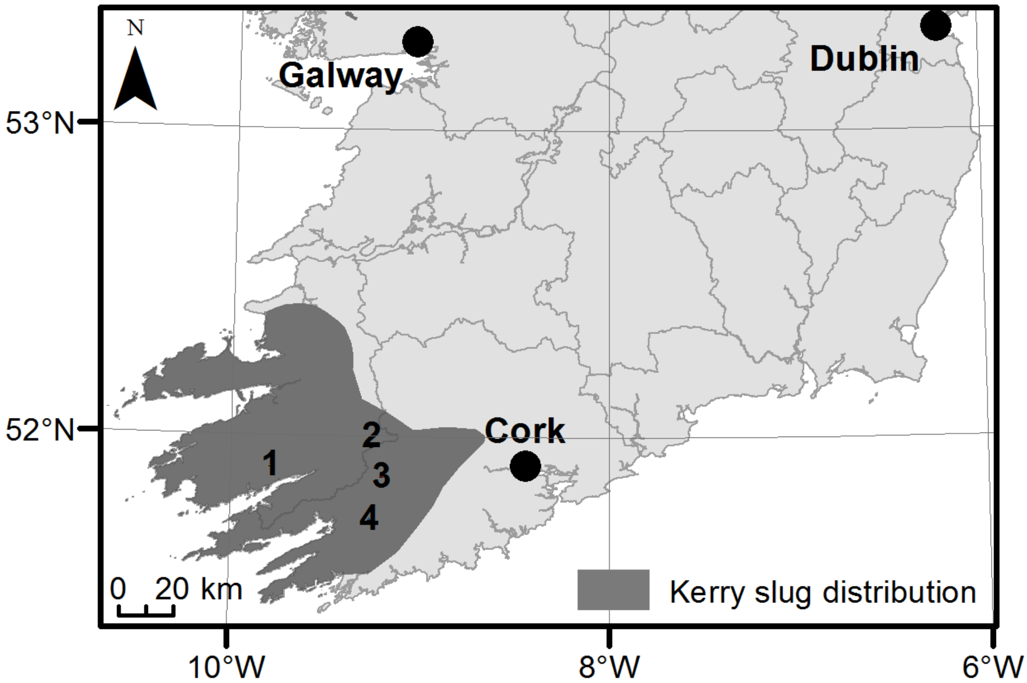

Fig. 1 1 Location (Irish Transverse Mercator coordinates) of the four study sites in the south-west of Ireland: Site 1 (Tooreenafersha), Site 2 (Derrynasaggert), Site 3 (Rathgaskig/Coomlibane) and Site 4 (Barnagowlane) (Kindermann, 2016, created using ArcGIS® software by Esri)).

3.3.2. Long-term Study

The aim of the long-term study was to record catches of *G. maculosus* from a range of habitats over a full calendar year with a view to determining the optimum season for sampling using refuge traps (De Sangosse, Pont du Casse, France, hereafter referred to as "trap"). In each mature compartment, a stand of nine trees in a 3 x 3 grid was selected at least 10m from the edge of the forest. As in Mc Donnell and Gormally (2011b), a single trap was fixed to the north side of each tree (using nails and string) at 1.5m above ground (Fig. 2a). Similarly, in each of the clear-fell compartments, individual traps (secured using nails and string) were placed on the north side and top of 3 x 3 tree stumps (Fig. 2b) situated at least 10m from the compartment edge. At each of the peatland sites, nine traps were secured to rocks as per Mc Donnell and Gormally (2011b) using a high-strength MS polymer adhesive (Tec7®), nails, and string (Fig 2c). In addition, in each mature conifer and clear-fell compartment and in the peatland sections (at a minimum distance of 45m from the tree, stump or rock traps respectively), nine (3 x 3) traps (1.5m apart) were secured (using tent pegs) over vegetation/bare soil on the ground between the traps on trees, tree stumps and rocks. These traps (hereafter referred to as “ground traps”) were deployed because McDonnell and Gormally (2011a) have shown that *G. maculosus* can move between trees.

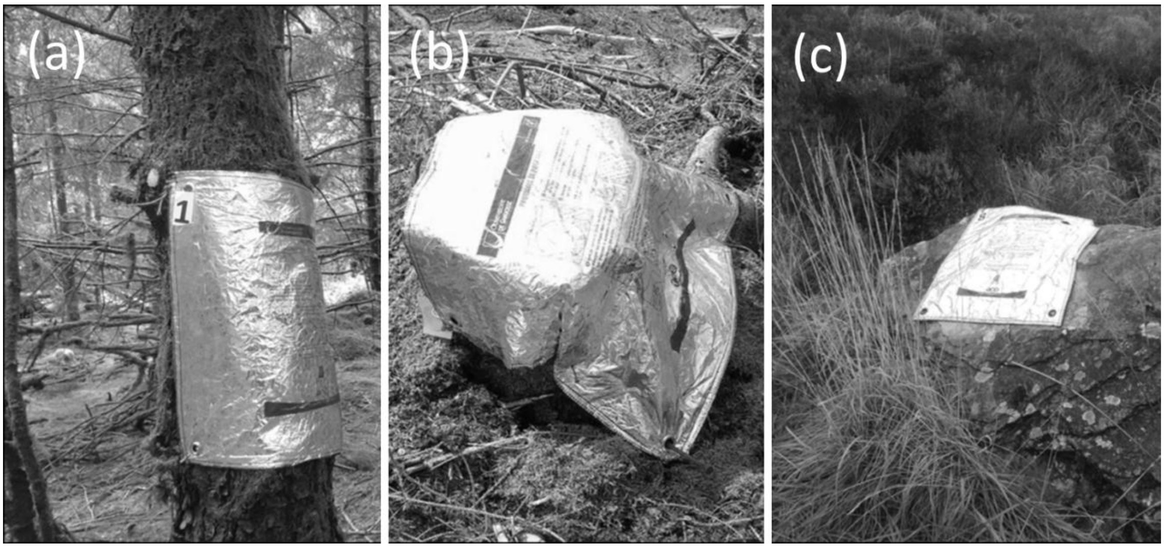

Fig. 2 Traps placed on a tree (a), a tree stump (b) and a rock (c) (G. Kindermann, 2016)

Slug catches under the traps were recorded each day for five consecutive days every month for 12 months from September 2014 to August 2015 and because of this, traps were not baited as in Mc Donnell and Gormally (2011b) since bait degradation would occur between sampling months, thereby influencing the catches on day 1 of each monthly sampling period. The age (i.e. adult or juvenile) and location (i.e. on tree, stump, rock, or ground) of every *G. maculosus* found under the traps were recorded. As the size of *G. maculosus* is difficult to measure and weighing individuals was problematic in the field, slugs greater than 1cm in diameter when rolled into a defensive ball were deemed to be adults.

3.3.3. Short-term study

During the final four months of the long-term study, an additional investigation was undertaken to compare the sampling protocols of Mc Donnell and Gormally (2011b) with previously untried sampling methods. This study was completed at additional locations within each of the four field sites above but using the same protocol regarding distances between traps. The aims were to compare:

a) Efficacy of traps placed on mature trees at 1.5m versus 0.2m above ground

For this study, two additional mature stands of nine trees (3 x 3) were selected within each of the mature compartments included in the long-term study. Traps were placed on the north side of the trees at a standard height of 1.5m (stand 1) and at a height of 0.2m from the base of the tree (stand 2). To avoid any potential bias related to individual trees, traps placed at 0.2m were relocated to 1.5m on the same tree and vice-versa at the end of each sampling week. Sampling regime followed that of the long-term study.

b) Efficacy of traps versus hand searching

Hand searches were also undertaken in the mature and clear-felled compartments and in the adjacent peatland at a distance of 45m from all other trapping locations. Hand searches were completed on nine trees (3 x 3) in the mature compartment, nine stumps (3 x 3) in the clear-fell compartment and over a marked area of similar size (5m x 5m), respectively on the peatland outcrops. Hand searches for both adult and juvenile *G. maculosus* were carried out by two people for five minutes per person in each of the designated areas giving a total of ten minutes searching for each sampling day between June and September 2015. This is equivalent to the minimum amount of time it took to check traps for catches within the compartments. Care was taken not to disturb the habitat too much to make it unsuitable for the slugs in future. Searches involved checking in cracks and ridges, and under lichens and mosses on tree trunks (to a maximum height of approximately 2m), stumps and rocks in addition to examining the areas in between these features.

3.3.4. Temperature and rainfall data collection

TinyTag Plus 2 (TGP-4500) environmental data loggers were used to collect temperature data with readings taken every 20 minutes from 19th of September 2014 to the 31st of August 2015. Each data logger, placed 1m above ground in a Stephenson's Type Screen (ACS-5050, TinyTag), was placed in each mature conifer and clear-fell compartment and in the peatland sections between the groups of traps. The Screen protects TinyTag loggers from direct sunlight and precipitation when monitoring outdoors (TinyTag, 2016). Hourly rainfall data were obtained from the nearest Met Éireann (Irish National Meteorological Service) stations in Cork Airport, Co. Cork, and Valentia, Co. Kerry to allow for an assessment of the influence of rainfall on capture success. These weather stations were selected as Site 1 was nearest (31 km) to Valencia and Sites 2 (53km), 3 (48km), and 4 (53km) were closest to Cork airport.

3.3.5. Statistical analyses

All analyses were undertaken using SPSS version 21. Where the assumptions of normality and homogeneity of variance were violated, Welch's T test or ANOVA was used followed by a Games-Howell *post hoc* test to determine pair-wise differences where more than two groups were examined. Where the assumption of normality was violated but the homogeneity of variance was not, the Kruskal Wallis H test was used followed by a Dunn's *post hoc* test to compare pair-wise differences. Curve estimation was also used to assess the relationship of examined variables to each other. Where linear relationships were found two-tailed Spearman rank correlations were performed. Mean temperature over seasons was calculated by averaging readings taken every twenty minutes from data loggers over the course of the investigation.

3.4. Results

3.4.1. Comparison of trap position and hand searching on *G. maculosus* catches in forested/open habitats (Short-term Study)

Six hundred and fifty-six adult and 63 juvenile (8.8% of total catch) *G. maculosus* were caught on 135 sampling occasions in the mature forest compartments with all individuals caught by hand searching found on tree trunks only (Table 1). Adult / juvenile catches were greatest using traps placed on tree trunks 1.5m above ground (412 / 39), followed by traps placed at 0.2m above ground (219 / 21), hand searching (20 / 3) and traps placed directly on the ground (5 / 0). For adults statistically significant differences were found between all sampling methods ($P < 0.001$, Welch's ANOVA followed by Games-Howell *post-hoc* analysis) except between traps placed directly on the ground and hand searches. For juveniles statistically significant differences were found only between traps placed at 1.5m and hand searching ($P = 0.020$, Welch's ANOVA with Games-Howell *post-hoc* analysis). No juveniles were found beneath ground traps. Where juveniles were caught the percentage of the overall catch consisting of juveniles for individual sampling methods was greatest for hand searching (13% of total catch) compared to traps at 1.5m (8.6% of total catch) or 0.2m above ground (9.5% of total catch).

Table 1 Mature Forest: Total and mean (±SD) catch of adult and juvenile *Geomalacus maculosus* using traps placed on trees 1.5m above ground, 0.2m above ground, directly on the ground and using ten minute hand searches from June to September 2015 (N = 135 sampling occasions).

	1.5m traps	0.2m traps	Ground traps	Hand search
Adult				
Total catch	412	219	5	20
Mean ± SD	3.05 ± 5.00	1.62 ± 3.41	0.05 ± 0.23	0.15 ± 0.38
1.5m trap	-	-	-	-
0.2m trap	**0.033**	-	-	-
Ground traps	**0.000**	**0.000**	-	-
Hand search	**0.000**	**0.000**	0.080	-
Juvenile				
Total catch	39	21	0	3
Mean ± SD	0.34 ± 1.24	0.16 ± 0.67	0	0.02 ± 0.15
1.5m trap	-	-	-	-
0.2m trap	0.081	-	-	-
Ground trap	-	-	-	-
Hand search	**0.020**	0.296	-	-

Adult: Test statistic = 26.635; d.f. = 3; $P < 0.001$, Welch's ANOVA. *P* values given in bold indicate significant differences between trapping methods, Games-Howell multiple comparison test; Juvenile**:** Test statistic = 4.696; d.f. = 2; $P = 0.010$, Welch's ANOVA. *P* values given in bold indicate significant differences between trapping methods, Games-Howell multiple comparison test.

One hundred and forty-four adult and 29 juvenile (16.8% of total catch) *G. maculosus* were caught over 80 sampling occasions in the clear-felled compartments (Table 2). Adult / juvenile catches were greatest using hand searching (99 / 27), followed by traps placed on stumps (36 / 2), and traps placed directly on the ground (9 /0). For adults statistically significant differences were found between all three methods ($P < 0.001$, Welch's ANOVA with Games-Howell *post-hoc* analysis). For juveniles, statistically significant differences were found between hand searches and traps placed on tree stumps ($P = 0.037$, Welch's T-test with Games-Howell *post-hoc* analysis). No juveniles were found beneath ground traps and all adults and juveniles (126 in total) caught by hand searching were found on tree stumps only. Where juveniles were caught the percentage of the overall catch consisting of juveniles for individual sampling methods was greatest for hand searching (21.4% of total catch) compared to tree stump traps

(5.3% of total catch).

Table 2 Clear-felled compartments: Total and mean **(**±SD) catch of adult and juvenile *Geomalacus maculosus* using traps placed on tree stumps, on the ground and using ten minute hand searches from June to September 2015 (N=80 sampling occasions)

	Tree stump traps	Ground traps	Hand search
Adult			
Total catch	36	9	99
Mean ± SD	0.45 ± 0.81	0.11 ± 0.36	1.82 ± 2.82
Tree stump traps	-	-	-
Ground traps	**0.003**	-	-
Hand search	**0.011**	**0.000**	-
Juveniles			
Total catch	2	0	27
Mean ± SD	0.03 ± 0.16	0	0.34 ± 1.31
Tree stump traps	-	-	-
Ground traps	-	-	-
Hand search	**0.037**	-	-

Adult: Test statistic = 14.690; d.f. = 2; $P < 0.001$, Welch's ANOVA. *P* values given in bold indicate significant differences between trapping methods, Games-Howell multiple comparison test; Juvenile: Test statistic = 4.478; d.f. = 1; $P = 0.037$, Welch's T-test. *P* values given in bold indicate significant differences between trapping methods, Games-Howell multiple comparison test.

Forty-four adult and 17 juvenile (27.9% of total catch) *G. maculosus* were caught over 80 sampling occasions on the rock outcrops on the peatland (Table 3). Adult and juvenile catches were greatest using rock traps (42 / 14), followed by hand searching (2 / 3), and none were captured under traps placed directly on the vegetation between the rocks (ground traps). Statistically significant differences were found between rock traps and hand searching for both adults and juveniles ($P = 0.029$, Welch's T-test). All adults and juveniles (5 individuals) caught by hand searching were found on rocks only. Where juveniles were caught the percentage of the overall catch consisting of juveniles for individual sampling methods was greatest by hand searching (60% of total catch) compared to rock traps (25% of total catch).

Table 3 Peatland: Total and mean (±SD) catch of adult and juvenile *Geomalacus maculosus* using refuge traps placed on rocks (rock traps, on vegetation among rocks (ground traps) and using ten minute hand searches from June to September 2015 (N = 80 sampling occasions)

	Rock traps	Ground traps	Hand search
Adult			
Total catch	42	0	2
Mean ± SD	0.53 ± 0.84	0 ± 0	0.03 ± 0.16
Rock traps	-	-	-
Ground traps	**-**	-	-
Hand search	**0.000**	**-**	-
Juveniles			
Total catch	14	0	3
Mean ± SD	0.18 ± 0.50	0 ± 0	0.38 ± 0.25
Rock traps	-	-	-
Ground traps	-	-	-
Hand search	**0.029**	-	-

Adults: Test statistic = 27.288; d.f. = 1 $P < 0.001$ Welch's T test. *P* values given in bold indicate significant differences between trapping methods; Juveniles: Test statistic = 4.890; d.f. = 1 $P = 0.029$ Welch's T test. *P* values given in bold indicate significant differences between trapping methods.

3.4.2. Seasonal variation in *G. maculosus* catches (Long-term study)

Catches are reported as mean number of *G. maculosus* caught per sampling occasion to allow for comparison across the seasons (Table 4). Mean number of adults caught using traps was greatest in the autumn (4.62), followed by spring (2.43) and summer (1.62), with lowest catches occurring in the winter (1.43). Mean number of juvenile caught was also greatest in autumn (0.38), followed by summer (0.36), spring (0.14) and winter (0.12). Autumn catches for both adults and juveniles were significantly greater (P = 0.000; P = 0.001 respectively) than winter and spring catches ($P < 0.001$; P = 0.002 respectively), Welch's ANOVA with Games-Howell post-hoc analysis. Additional significant differences in adult and juvenile catches between seasons can be seen in Table 4. The percentage of the total catch represented by juveniles was greatest in the summer (18.3%) followed by winter (7.9%), autumn (7.7%) and spring (5.4%).

Table 4 Seasonal variation: Adult and juvenile *Geomalacus maculosus* catches in autumn (September 2014 to November 2014, N=230), winter (December 2014 to February 2015, N=245), spring (March to May 2015, N=245) and summer (June to August 2015, N= 225) across all three habitats (mature forest, clear-fell and peatland) using refuge traps.

	Autumn 14	Winter 14/15	Spring 15	Summer 15
Adult				
Total catch	1062	349	596	358
Mean ± SD	4.62 ± 6.53	1.43 ± 2.70	2.43 ± 3.95	1.64 ± 2.97
Autumn	-	-	-	-
Winter	**0.000**	-	-	-
Spring	**0.000**	**0.006**	-	-
Summer	**0.000**	0.0837	0.068	-
Juvenile				
Total catch	88	30	34	80
Mean ± SD	0.38 ± 0.96	0.12 ± 0.44	0.14 ± 0.42	0.36 ± 0.95
Autumn	-	-	-	-
Winter	**0.001**	-	-	-
Spring	**0.002**	0.975	-	-
Summer	0.997	**0.003**	**0.006**	-

Adults: Test statistic = 17.813; d.f. = 3; $P < 0.001$, Welch's ANOVA. P values given in bold indicate significant differences between seasons Games-Howell multiple comparison test. Juveniles: Test statistic = 9.280; d.f. = 3; $P < 0.001$, Welch's ANOVA. P values given in bold indicate significant differences between seasons Games-Howell multiple comparison test

In the mature conifer compartments lowest mean catch in winter corresponded with the lowest average temperatures and second lowest catch success in summer corresponded with the highest mean temperatures (Fig. 3). In both the clear-fell compartments and peatland sections the lower catches generally occurred in winter and spring (peatland) and winter, spring and summer (clear-fell) (Fig. 3).

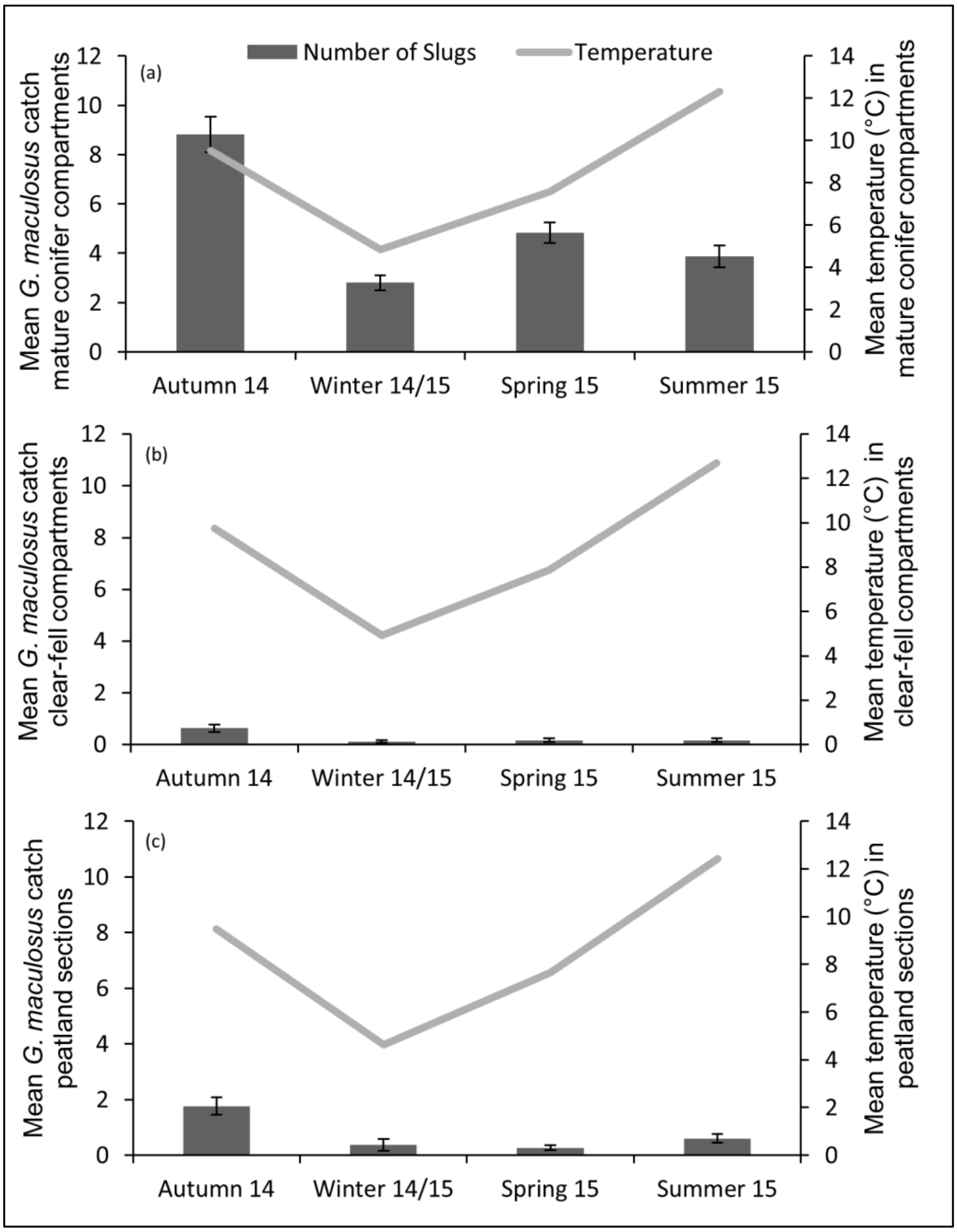

Fig. 3 Mean (±SE) *Geomalacus maculosus* individuals (bar graph) caught (using traps) in mature conifer compartments (a), clear-felled compartments (b), and peatland compartments (c) with mean temperature (°C ± SE) (represented by the line graph) for each season - September 2014 to November 2014 (autumn), December 2014 to February 2015 (winter), March to May 2015 (spring) and June to August 2015 (summer)

3.4.3. *G. maculosus* catches in relation to temperature and rainfall (Long and short term studies)

Significant, but weak, quadratic relationships (Fig. 4) were found between total capture success using refuge traps placed at 1.5m and average temperature during the 24 hour period prior to sampling in mature conifer compartments ($P < 0.001$, $r_s = 0.069$) and in clear-felled compartments ($P < 0.001$, $r_s = 0.053$) (Fig. 4). There was no significant relationship between temperature and capture success in peatland areas ($P = 0.167$, $r_s = 0.020$). Significant, but weak, quadratic relationships were also found between capture success and the temperature reading recorded during the twenty minutes it took to assess traps in mature conifer compartments ($P < 0.001$, $r_s = 0.067$) and in clear-felled compartments ($P = 0.024$, $r_s = 0.029$) (Fig. 4). There was no significant relationship between temperature and capture success in peatland areas (P = 0.072, $r_s = 0.024$).

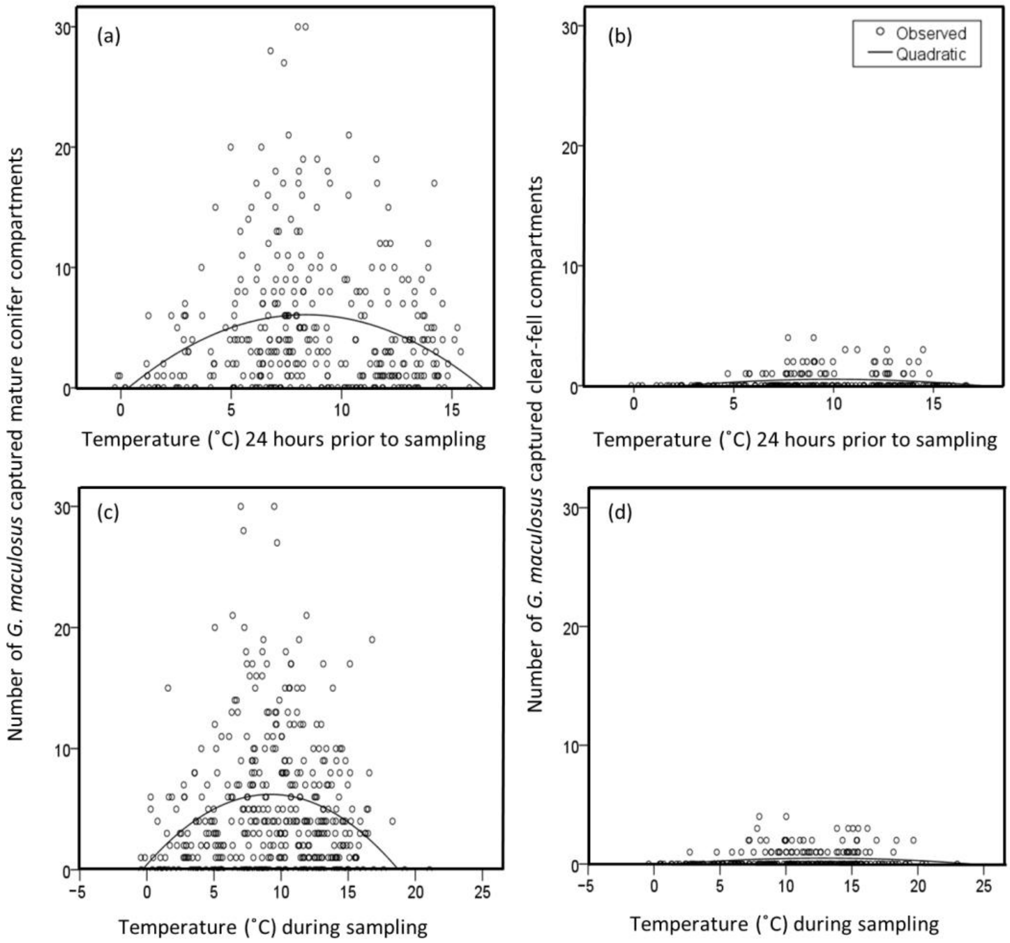

Fig 4 Quadratic relationship (represented by line) between *Geomalacus maculosus* individuals caught (using traps; October 2014 – August 2015) and average temperature (°C) 24 hours prior to sampling in mature conifer compartments (a) and in clear-fell compartments (b). Quadratic relationship (represented by line) between *G. maculosus* individuals caught (using traps; October 2014 – August 2015) and the temperature (°C) recorded during the twenty minutes it took to assess traps in mature conifer compartments (c) and in clear-fell compartments (d).

Significant, but weak, quadratic relationships (Fig. 4) were found between total capture success using refuge traps placed at 1.5m and average temperature during the 24 hour period prior to sampling in mature conifer compartments ($P < 0.001$, $r_s = 0.069$) and in clear-felled compartments ($P < 0.001$, $r_s = 0.053$) (Fig. 4). There was no significant relationship between temperature and capture success in peatland areas ($P = 0.167$, $r_s = 0.020$). Significant, but weak, quadratic relationships were also found between capture success and the temperature reading recorded during the twenty minutes it took to assess traps in mature conifer compartments ($P < 0.001$, $r_s = 0.067$) and in clear-felled compartments ($P = 0.024$, $r_s = 0.029$) (Fig. 4). There was no significant relationship between temperature and capture success in peatland areas (P = 0.072, $r_s = 0.024$).

Significant, but weak, negative Spearman's rank-order correlations were found between individuals caught using hand searches and both the average temperature during the 24 hour period prior to sampling and the temperature during hand searching ($P = 0.038$, $r_s = -0.268$, and $P = 0.012$, $r_s = -0.279$ respectively) in clear fell compartments. No significant correlations were found between hand search catch success and average temperature during the 24 hour period prior to sampling in either mature conifer compartments ($P = 0.689$, $r_s = 0.040$) or peatland sections ($P = 0.651$, $r_s=0.060$). Furthermore, no significant correlations were found between hand search catch success and temperature during hand searching in either mature conifer compartments ($P = 0.689$, $r_s = 0.040$) or peatland sections ($P = 0.651$, $r_s = 0.060$).

A significant, moderate positive Spearman's rank-order correlation was found between individuals caught using hand searches and the average rainfall during the 24 hour period prior to sampling ($P = 0.001$, $r_s = 0.371$) in clear-fell compartments. No significant correlations were found between hand search catch success and average rainfall during the 24 hour period prior to sampling in either mature conifer compartments ($P = 0.368$, $r_s = -0.078$) or peatland sections ($P = 0.226$, $r_s = 0.137$). Additionally, no significant correlations were found between hand search catch success and rainfall during hand searching in either mature conifer compartments ($P = 0.242$, $r_s = -0.101$), clear-fell compartments ($P = 0.487$, $r_s = 0.079$), or peatland sections ($P = 0.334$, $r_s = -0.109$).

3.5. Discussion

3.5.1. Trap position/hand searching and *G. maculosus* catches

Within the mature forest compartments, traps placed at a standard height of 1.5m had greater catch success for adults and juveniles combined (63% of total catch) compared to traps placed at 0.2m (33%), hand searching (3%) and ground traps (< 1%). While Platts and Speight (1988) list the forest floor in deciduous forests as a potential microhabitat for *G. maculosus*, a small study by Mc Donnell and Gormally (2011a) in a native oak-birch-holly woodland found more individuals under identical traps placed at 1.5m on tree trunks than under ground traps albeit made of a range of different materials. It is, therefore, likely that individual trees are an important microhabitat for *G. maculosus* with most slug activity in commercial conifer plantations occurring on trees rather than on the ground between trees. The fact that ground traps in mature plantations resulted in the least number of catches and no slugs were caught on the forest floor during hand searches further strengthens this conclusion. While lichens, the primary food plant of *G. maculosus* (Reich et al., 2012), are more species rich in the upper third of trees in Sitka spruce plantations (Coote et al., 2007), humidity also decreases with increasing elevation on trees (Hosokawa et al., 1964). It is probable that while slugs may forage in the upper parts of the tree, they return to the more humid, shaded conditions found in the lower parts of the trees to avoid desiccation. This being the case, the first trap they would encounter as they move down the tree would be the trap placed at 1.5m where almost twice as many individuals were caught in comparison to catches under traps placed at 0.2m. The likely movement of individuals up and down the tree trunks may have contributed to the relatively poor efficacy of hand searching in the mature conifer compartments simply because, for practical reasons, counts of slugs on tree trunks were limited to a maximum height of 2m.

In clear-felled compartments, hand searching yielded the greatest catches of adults and juveniles combined (73% of total catch) compared to tree stump traps (22%) and ground traps (5%). Allowing for differences in numbers of traps employed and numbers of sampling occasions at the mature forest and clear-felled compartments, catches at the mature forest compartments overall were almost double those at the clear-felled compartments. While this is likely to be a reflection of the actual numbers in each habitat type, another possible reason for the relatively low capture rates using traps at clear-felled compartments, in particular, is that the exposed nature of clear-fell areas often resulted in the area immediately beneath the traps drying out, making them less attractive to slugs wishing to use them as shelters. In comparison, traps deployed on tree trunks in plantations tended to remain damp for longer possibly due to the flow of water down the trunk of trees following rainfall (Ovington, 1954) in conjunction with the more shaded conditions beneath the tree canopy. Given that only 23% of all individuals captured on tree stumps were found beneath traps compared to 77% by hand searching also suggests that traps did not function at an optimal level in this habitat. In addition, the total number of captures using traps in the clear-fell (47) is close to that found beneath traps in the other exposed habitat studied i.e. peatland (56) with exactly the same sampling effort. That no slugs were found between stumps when hand searching could be the result, in some cases, of the presence of *J. effusus* and *D. purpurea* making it difficult to see specimens. Indeed, McDade and Maguire (2005) have noted that when surface conditions are more structurally complex it becomes more difficult to detect slugs using hand searching.

In peatland sections traps placed on rocky outcrops had the greatest catches of adults and juveniles combined (92% of total catch) compared to hand searching (8%), with no individuals found beneath traps placed directly on the ground between rocks. This mirrors the findings by Mc Donnell and Gormally (2011b) who successfully captured *G. maculosus* with traps placed on rocky outcrops in peatland. Individuals captured using hand searching were also found only on rocky outcrops within the hand searching area. It is likely that successful capture of slugs was limited to rocks because of the presence of an abundant source of lichens on which *G. maculosus* feeds (NRA, 2009). The absence of individuals found either by hand searching and under traps placed on the ground between rocks indicates the importance of outcrops as a habitat feature for the species in peatland habitats. Having said that, dense vegetation in peatlands, particularly the presence of *M. caerulea*, may reduce the effectiveness of hand searching. In addition, the absence of catches under ground traps placed between rocks in this study may reflect genuinely low abundances in that *G. maculosus* has only been rarely seen on open peatland vegetation (Mc Donnell, *pers.comm.*). It is, however, possible that higher levels of moisture in peatland vegetation may reduce the attractiveness of the traps as a refuge from desiccation unlike those in the drier conifer compartments.

While overall numbers of juveniles caught were lower than those of the adults, trends observed followed those of the adults in each of the three habitats. Although greatest numbers of juveniles were caught using traps (excepting ground traps) in both mature conifer compartments and peatland, the proportion of juveniles caught in each of the three habitats was consistently greater using hand searching compared to using traps. Rollo and Wellington (1979) found that adults of *Deroceras reticulatum* Müller, four *Arion* species and *Limax maximus* L. tended to be more aggressive than juveniles resulting in juveniles being unable to compete with the larger adult slugs for shelters. In addition, Rollo (1982) in a later study found that juvenile slugs (*Deroceras* species, *Arion species* and *L. maximus*) spent a larger portion of their active period foraging. It is, therefore, possible that a combination of competition for shelter and more time spent foraging resulted in lower proportions of juveniles found under traps. Where there are time constraints and simply presence or absence data are required, initial hand-searching under appropriate weather conditions and during the appropriate season is probably sufficient. Hand searching at night (using torches) could yield interesting results and the effect on catches of searching at different times of the day is worth further investigation. If no specimens are found by hand-searching, traps could be placed subsequently to confirm the presence or absence of the species. Traps are also useful in instances where personnel undertaking hand-searching are inexperienced and in cases where long term monitoring is required. Following further research into the relationship between weight and age categories of *G. maculosus*, weighing of slugs in the field could be used (time permitting) to separate the different age stages and further the understanding of population dynamics in the field.

3.5.2. *Geomalacus maculosus* catches – in relation to temperature and rainfall

While *G. maculosus* was collected year round, results of the long-term study indicate that capture success varies across the seasons. Capture success for both adults and juveniles was greatest during the autumn months and least in winter. After autumn, spring and summer catches were the next highest for adults and juveniles respectively. The results suggest that *G. maculosus* monitoring surveys and / or relocation prior to clear-felling should be undertaken during autumn to ensure optimal catch success. The second peak in juvenile catches in summer is likely to be the result of egg laying by adults in the spring (Wisniewski, 2000). Summer surveys would therefore provide useful information on the health of the population by indicating the extent of breeding and recruitment by juveniles. Further research whereby populations are monitored over a number of years (ideally with different weather patterns) would further refine optimum sampling seasons for the species.

Significant but weak quadratic relationships were detected between temperatures during the 24-hour period prior to and at the time of sampling with capture success using traps in both mature conifer and clear-felled compartments. No significant relationship was found in the peatland sections where trap catches were overall substantially less. The quadratic nature of the relationships suggests that both low and high temperatures have a negative effect on numbers of individuals found beneath traps in mature conifer/ clear-felled compartments. It is likely that the oceanic nature of climate in Ireland with its relatively small temperature range (Met Éireann, 2016) may have contributed to the weak relationship between temperatures and slug catches recorded between October 2014 and August 2015. Nevertheless, catches at each of the three sites were lowest in winter (corresponding to the lowest mean temperatures) and although catches were next lowest in summer (highest mean temperatures) at the mature conifer plantations, this was not the case for the clear-fell and peatland habitats where numbers of catches were substantially lower. It is interesting to note that in clear-fell compartments, where hand searching was most successful, there was a negative correlation between numbers of individuals caught by hand searching and average temperatures prior to and during sampling. Coupled with this was the positive correlation between individuals caught using hand searches in clear-fells and the average rainfall during the 24 hour period prior to sampling. Given that hand searching has been reported as being highly dependent on weather (Bruelheide and Scheidel, 1999), there are clearly a number of factors at play relating to the attractiveness of traps coupled with levels of slug activity under different weather conditions.

As previously mentioned, it is possible that at higher air temperatures the surface beneath the traps dries out, particularly in clear-felled compartments, making them less attractive to *G. maculosus* thereby resulting in lower catches. Terrestrial slugs are known to be extremely susceptible to dehydration (Cameron, 1970), and seek to avoid exposure to unfavourable conditions as a means of protecting themselves (Rollo, 1982). Slugs in general are also known to move down through the soil profile to avoid freezing temperatures (Cook, 2004). Indeed, the authors have observed *G. maculosus* sheltering below ground and under the moss cover at the bases of trees, stumps and rocks during warm and dry weather, as well as during cold weather. With regard to rainfall, it is interesting to note that no significant relationship was found between rainfall at the time of sampling and hand search capture success in any of the habitats. This finding is somewhat surprising given that *G. maculosus* is reported to be only diurnally active during or after rain (Taylor 1906; Platts and Speight, 1988). Given that rainfall data were sourced from weather stations more than 30km from the sites, they may not have reflected local variation in rainfall accurately. In addition, Ovington (1954) found that duration and intensity of rainfall are the most important factors dictating the amount of rainfall that reaches the ground in conifer plantations. It has been widely reported that temperature and rainfall are important factors influencing slug activity in general (Barnes and Weill, 1945; Webley et al., 1964; Young et al., 1991; Shirley et al., 2001; Choi et al,. 2006) and this is, to some extent, reflects the results of this study. Although logistics in this study did not permit the recording of weekly catch data, future studies incorporating on-site weather data, particularly rainfall measurements in addition to weekly catch data would further refine the relationship between weather conditions and catch success.

The results of this study clearly indicate for the first time that approaches to monitoring *G. maculosus* needs to take into account the habitat under investigation. Of the sampling strategies investigated in this study, traps placed at a height of 1.5m on trees in mature conifer plantations will likely result in optimal numbers of catches of *G. maculosus*. In clear-fell areas, hand searching under suitable weather conditions, preferably when rain has fallen in the previous twenty-four hours, is recommended. For peatlands, traps should be placed on exposed rock. Overall, autumn is the preferred time of sampling for adult slugs, while summer sampling is recommended if breeding and recruitment studies are required. Sampling during extremes of hot and cold weather should be avoided as results are likely to give an underestimation of slug densities, which could lead to the implementation of poor management decisions. While the results of this study form the basis for guidelines to forestry managers who are legally obliged to protect *G. maculosus* when undertaking routine forestry practices, further work regarding the presence of the species in the upper canopy is required. In addition, measuring humidity and temperature beneath traps using probes in conjunction with numbers of slug catches will further refine how best to maximise the use of trap data for the protection of *G. maculosus* in the future.

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Chapter 4: Implications of commercial forestry practices on the EU-protected *Geomalacus maculosus* (Kerry Slug)

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***Key Message* Mature conifer stands, particularly trees of greater circumference, result in greater numbers of *Geomalacus maculosus* captures than adjacent clear-felled stands and adjacent peatland with Before-After-Control-Impact-Paired analysis indicating lower catches of *G. maculosus* post-felling.**

Context The discovery of EU protected *G. maculosus* in commercial plantations requires an understanding of the implications of forestry practices on the species within the context of sustainable forest management.

Aims 1. Compare *G. maculosus* captures across mature planted conifer, previously clear-felled stands and adjacent peatland habitats. 2. Assess the suitability, for forest managers, of population estimate models for *G. maculosus*. 3. Assess implications of felling by comparing relative abundances of *G. maculosus* directly before and after a clear-felling event.

Methods *G. maculosus* catches were compared across mature conifer stands, clear-felled stands and adjacent peatlands using metric traps and hand-searching. Capture-mark-recapture studies were undertaken to estimate population sizes. A BACIP (Before-After–Controlled-Impact-Paired) analysis was undertaken in one forest stand to determine impacts of a clear-felling event.

Results Mean catches of *G. maculosus* adults in the mature forest stands were over 10 and 11 times greater than mean catches on peatland and existing clear-fell stands respectively. The Schnabel model for estimating population size was most suited for mature forest stands but could not be utilised for other habitats. BACIP analysis showed a significant impact of clear-felling with a 95% reduction in *G. maculosus* mean catches after a clear-felling event where none of the individuals marked prior to felling were recaptured compared to 21% recapture rates at the control site. Greater tree circumference in mature conifer stands correlated with greater catches.

Conclusions Guidelines are needed to ensure the protection of *G. maculosus* in commercial forestry. Interventions could include stand retention and/or translocation of the protected species.

4.1 Introduction

The Kerry Slug (*Geomalacus maculosus*) has a disjunct distribution and is referred to as a Lusitanean species in that it is restricted solely to western Ireland and northern Iberia (Scharff, 1983; Patrão 2015). Since the species, which is protected under the European Union Habitats Directive (92/43/EC) and the Wildlife Act in Ireland, is listed as severely threatened in Iberia (Platts and Speight 1988; Byrne et al. 2009), Irish populations of the species are considered to be of international importance. In Ireland, *G. maculosus* was originally considered to be associated with deciduous woodland and peatlands (such as blanket bog and unimproved oligotrophic open moor) in the south-west of the country (Anon 2010) where it is known to take refuge in rock crevices, soil or bark when not active (Platts and Speight 1998). Consequently, these habitats have been the focus of conservation efforts for the species (Anon 2010). However, in recent years Kearney (2010) discovered the species breeding in a commercial conifer plantation in Oughterard (Co. Galway) 200km (approx.) north of its previously known distribution since when it has also been found in numerous conifer plantations in the south-west of Ireland (Mc Donnell and Gormally 2011; Reich et al. 2012). Although there is no empirical evidence, to date, regarding how *G. maculosus* became established in commercial conifer plantations, it is possible that as planted trees in the south-west of Ireland matured, they were colonised by the slug from surrounding peatlands in which the species was naturally present. It has also been hypothesised by Reich et al. (2012) that the population in Oughterard was introduced by forestry machinery. Although *G. maculosus* is known to eat lichens and mosses on blanket bogs, it also eats lichens, mosses and liverworts commonly found growing on mature conifers in commercial plantations (Reich et al. 2012). The maximum height at which Kerry Slugs are found on tree trunks has, to date, never been assessed and there is little information regarding its use of microhabitats within the tree canopy. However, it is likely that the species occurs in the upper reaches of mature conifer trees where lichens also proliferate. *Geomalacus maculosus* is rarely seen or trapped on the ground between trees (Johnston et al. 2016) but it is found beneath mosses at the base of mature conifers in unsuitable weather conditions during which time the species is generally absent from the portion of tree trunks visible from ground level (*pers. obs.*). Regardless of how the slug originally colonised commercial conifer plantations, the presence of this legally protected species presents considerable challenges to foresters in Ireland regarding its protection particularly during clear-felling operations.

Clear-felling, the norm for harvesting commercial conifer plantation stands in Ireland, results in a rapid transformation of a forested landscape into an open one. It can impact forest fauna with the process of harvesting itself causing considerable disturbance to ecosystems and changes to the physical environment (Larsen 1995). The low mobility of slugs (Strayer et al. 1986) and their susceptibility to dehydration (Prior 1985) mean that changes in microclimate can also have an adverse effect on populations in disturbed areas. In addition, slug assemblages have been found to be sensitive to forestry management (Nystrand and Granström 2000; Kappes 2006; Rancka et al. 2015). While Strayer et al. (1986) suggest that disturbances in New England forests could reduce gastropod densities with some local species extinction, their studies indicate that gastropod communities can recover quickly, at least from small areas of forest disturbance within forest patches of differing ages. Indeed, it has been noted that the impacts of forestry on biodiversity depends on the ecological context in which the forestry is found (Carnus et al. 2006; Bremer & Farley 2010). However, Platts and Speight (1988) noted that forestry operations in Portugal appear to have eradicated *G. maculosus* from sites in which it was previously found. For these reasons, the presence of this protected species in commercial conifer plantations in Ireland is of concern. Listed among current threats to the species are forest planting on open ground, forest replanting and forestry clearance (NPWS 2013). Nevertheless, the National Parks and Wildlife Service (NPWS), in its 2013 Article 17 report to the EU on the conservation status of Irish species and habitats, states that *G. maculosus* is "resilient" to clear-felling in that the latter has a short-term albeit negative impact. However, this statement, primarily based on localised studies (Mc Donnell and Gormally 2011; Reich et al. 2012), is qualified by the recommendation that more data are required regarding the temporal occupation of woodland by the species in addition to its responses to forestry operations (NPWS 2013). The absence of comprehensive population estimates for *G. maculosus* is also highlighted (NPWS 2013).

Only one investigative study, to date, has carried out a preliminary investigation of *G. maculosus* catches in clear-fell and mature conifer stands (Reich et al. 2012). The study, undertaken in a single plantation, recorded significantly lower catches of *G. maculosus* in clear-fell stands (felled one year prior to the study) than in mature plantations. Since no data regarding *G. maculosus* catches prior to felling were available for the study, Reich et al. (2012) recommended that a before-after-control-impact assessment of the species be undertaken in future investigations given forestry manager obligations to protect *G. maculosus*. This is particularly urgent since current forestry guidelines for commercial forests in Ireland (published prior to the discovery of *G. maculosus* in commercial conifer plantations) do not list the potential impact of forestry practices on the species (Forest Service 2009).

In the Republic of Ireland the state sponsored body Coillte (The Irish Forestry Board Limited) is a commercial company owning approximately 54% of the national forest estate (DAFM 2016). It currently holds Forest Stewardship Council (FSC) certification which requires that its forests be managed with consideration for ecosystems and biodiversity (Principle 6, FSC 2016). In this study, a review of 45 Coillte forestry management plans (2011 – 2015) for areas that overlap with known *G. maculosus* distribution revealed that only one plan (Killarney Forest KY13) made specific reference to *G. maculosus* (Coillte 2016). Given the current gaps in the knowledge and Ireland's obligations under the EU Habitats Directive coupled with the commitment of Coillte to FSC certification, further study is urgently required. To address these gaps, this study aims to:

1. Compare *G. maculosus* captures across mature planted conifer, previously clear-felled stands and adjacent peatland habitats

2. Determine the suitability, for forest managers, of population estimate models for *G. maculosus*

3. Assess the implications of felling by comparing relative abundances of *G. maculosus* directly before and after a clear-felling event.

4.2 Materials and Methods

4.2.1 Study areas

Four study sites (1-4 consisting of commercial forestry plantations owned by Coillte with adjacent peatland areas, were chosen in 2014 within the distribution range of *G. maculosus* in the south-west of Ireland. Within the four sites were selected:

- Two / three stands (Table1) of mature conifer plantation (predominantly P. sitchensis) planted by Coillte on peatland in the early 1970s (Coillte, 2014). These mature plantations (hereafter referred to as MP) were of felling age with Coillte forest stands being on average 19ha in size

Originally two stands (a & b) were selected with stand "a" acting as control and stand "b" scheduled for felling within the lifetime of the project (July 2014 to October 2015). Due to changes in the felling schedule, which were outside the control of this project, the impact assessment using a Before-After–Controlled-Impact-Paired (BACIP) analysis was limited to just one of the four planned MP stands scheduled for felling (i.e. stand 2b). Two additional stands (2c and 3c) were included in the study as back-ups in the event of further changes to the felling schedules. However, none of the remaining or additional sites were felled in sufficient time to allow a before and after comparison and these data were subsequently incorporated in the MP dataset (total number of MP stands = 10).

- One previously clear-felled stand (hereafter referred to as PCF) which was felled in 2013, prior to the start of the project and, at the time of the study, was dominated by *P. sitchensis* tree stumps interspersed with, *inter alia*, *Digitalis purpurea* L., *Juncus effusus* L. and mosses (total number of PCF stands = 4).

- One adjacent area of peatland (hereafter referred to as PL) containing, predominantly *Molinia caerulea* (L.) Moench and *Calluna vulgaris* (L.) Hull (total number of PL sites = 4).

Table 1 Management history of mature plantation (MP) stands (a, b and c) at four sites[1]

Site	Stand	Number of thinnings	Years since last thinning	Age of stand	Yield class
1	a	2	10	45	16
1	b	3	10	44	14
2	a	4	4	44	16
2	b[2]	3	3	43	12
2	c	3	3	43	12
3	a	3	6	43	18
3	b	3	7	43	18
3	c	3	6	43	16
4	a	1	8	43	16
4	b	1	9	44	12

[1]Source: Coillte 2014; a, b and c refer to stands designated as controls (a); stands designated for felling during the study (b); and additional stands as “back-ups” in the event of unpredicted changes to felling schedules (c).

[2] The sole forest stand which was subjected to a clear-felling event during the course of this study

4.2.2 Sampling design

Two sampling methods (details given below) were utilised in this study, namely sampling using traps (July 2014 - November 2015) and searching by hand (June – September 2015). For the trapping method, a sample of nine trees, at least 10m from the edge of the forest was selected in each mature forest stand. A single refuge trap (De Sangosse, Pont du Casse, France, hereafter referred to as “trap”) was fixed to the north side of each tree (using nails and string) at 1.5m above ground level after Mc Donnell and Gormally (2011). The traps consist of three layers: a perforated plastic layer, a padded fabric layer to retain moisture, and a metallic foil layer (Johnston et al. 2016). While Johnston *et al.* (2016) demonstrated that traps placed on trees at 1.5m result in greater catches than traps placed near the base of trees, it was not possible (for reasons of health and safety in combination with time constraints) to place traps further up the trees. In clear-fell stands, individual traps (secured using nails and string) were placed on the north side and top of tree stumps situated at least 10m from the stand edge. At peatland sites, nine traps were placed on rocks using methods described by Mc Donnell and Gormally (2011) for *G. maculosus* sampling on rock outcrops in peatland. In addition, in each habitat (at a minimum distance of 45m from the tree, stump or rock traps), nine traps (1.5m apart) were secured (using tent pegs) over vegetation/bare soil on the ground between trees, tree stumps and rocks. These traps (hereafter referred to as "ground traps") were deployed because McDonnell and Gormally (2011) showed that *G. maculosus* can move between trees and along the forest floor. While protocols using traps follow those of Mc Donnell and Gormally (2011), additional sampling methods (i.e. hand searching, described in section 2.4) were undertaken over four months (June to September 2015) to allow for any possible variation in trapping efficiency across habitats. Shortly before the tree felling event took place during the course of this study, traps were removed for health and safety reasons. These traps were then replaced on the remaining stumps, in the newly clear-felled stand (hereafter referred to as NCF) following the removal of logs from the site.

4.3 Mark-recapture studies

Once a month, over a 16 month period, slugs were marked every day over five consecutive days following Reich et al. (2015). Kendall and Bjorkland (2001) recommend monthly sampling with each monthly sample consisting of five consecutive daily samples to ensure a robust design. This decreases bias and allows for a more efficient estimate of population dynamics. These data are hereafter referred to as "sampling weeks" and "sampling days". On each of the sampling days, all of the traps were checked at every site and in every habitat. For the purposes of this study, slugs greater than 1cm in diameter when rolled into a defensive ball are referred to as "adults" (adapted from Reich et al. 2015) and hence large enough to be tagged. Smaller slugs (too small to tag effectively) were considered sub-adults and are referred to as "juveniles" hereafter. Confirmation by dissection to determine sexual maturity was not an option in this live population study and since humidity levels are known to affect the outcomes of weight measurements for slugs (A O Hanlon, *pers. comm.*), these could not be used as an effective determinant of maturity in the field.

The marking strategy for this project was based on that developed by Mc Donnell and Gormally (2011). Visible Implant Elastomer (VIE) (Northwest Marine Technology, Shaw Island, Washington) in nine different colours was used to mark adult slugs. VIE is a medical-grade, silicone based material which is injected as a liquid and cures into a pliable, biocompatible solid (Northwest Marine 2015). Marks for the different months were distinguished from each other based on different colours and locations of tags. For the initial nine months, from July 2014 to March 2015, tags were inserted into the left hand side of the foot from the head down to the tail. For the final seven months, from April to October 2015, the tags were placed into the right hand side of the foot from the tail and to the head. The colour location was reversed for slugs caught in ground traps to distinguish them from those caught in tree traps. To check for the presence of tags in captured individuals, each slug was lightly pressed against a clear piece of plastic and a torch emitting a deep violet light (405 nm) (Northwest Marine Technology, Shaw Island, Washington), was then shone over the individual. The torch is designed to cause VIE in red, orange, blue, yellow, green, and pink to fluoresce, making tags easier to observe, particularly in poor light conditions. Every adult *G. maculosus* found was checked for any previous tags, recorded and marked with the relevant colour for the sampling month. Any juvenile slugs caught were also recorded to provide information on juvenile activity levels but these were not tagged due to their small size. Slugs were then returned to the relevant trap. Damaged traps were replaced as required.

4.2.4 Hand searching

Given that Johnston et al. (2016) found that hand searching was more effective than traps in clear-fell stands, hand searches were also undertaken for a limited period (June and September, 2015) at mature forest / clear-fell stands and peatland sites at a distance of 45m from all other trapping locations. Hand searches were completed on nine trees in mature forest stands, nine stumps in the clear-fell stands and over a marked area of similar size (5m x 5m) in peatland. Hand searches for both adult and juvenile *G. maculosus* were undertaken by two people for five minutes per person in each of the designated areas, giving a total of ten minutes searching for each sampling day. Searches consisted of examining tree trunks (to a maximum height of approximately 2m), tree stumps and rocks in addition to examining the areas in between these features, thereby surveying all likely refuges in each habitat. Where *G. maculosus* was found during hand searching the individuals were not tagged.

Tree circumference at breast height (1.5m) and at the base of trees was recorded using a flexible tape measure. Percentage cover of moss from ground level to 1.5m on tree trunks was also recorded. Data regarding MP management such as year of planting were provided by Coillte (2014) (Table 1).

4.2.5 Data analysis

All analyses were undertaken using SPSS version 21 except for population estimates (Jolly-Seber and

Schnabel models) which were calculated using Excel through formulae described by Krebs (1999) and Greenwood (1996). The Jolly-Seber model allows for an “open” population where the number of animals varies (due to immigration, emigration, birth and death) while the Schnabel estimate provides a “closed” population estimate which assumes that the number of animals does not vary during the period of study (Krebs, 1999). In the comparisons of habitats, and direct comparisons between control and impact stands, where assumptions of normality and homogeneity of variance were violated, Welch’s T-test or ANOVA was used followed by a Games-Howell *post hoc* test to determine pair-wise differences where more than two groups were examined. Correlations were determined using Spearman’s rank correlation. The Before-After-Control-Impact-Paired (BACIP) analysis (Smith 2002) was carried out using an Independent samples T-test on the differences between control and impact sites, before and after the impact (i.e. clear-felling).

4.3Results

4.3.1 Comparison of *G. maculosus* catches in mature plantation (MP) stands, previously clear-felled (PCF) stands and adjacent peatland (PL)

Catches are reported as mean catch per sampling day to allow for comparison across the different habitat types over the 16 months of sampling (Table 2). The stand subjected to a clear-felling event during the course of this study (Site 2b) was not included in these analyses. The mean number of adult *G. maculosus* catches per sampling day using traps was greatest in MPs (5.23), followed by PLs (0.50) and PCFs (0.47). Significant differences were found between MPs and PCFs and between MPs and PLs (P < 0.001 and P < 0.001 respectively, Welch’s ANOVA followed by Games-Howell *post-hoc* analysis). The mean number of juvenile catches, while considerably less than those for adults, was also greatest in MPs (0.47), followed by PLs (0.24) and PCFs (0.047). Significant differences were found between all three habitats (Table 2).

Both with and without the addition of hand search data, the mean number of adult *G. maculosus* caught (June – September 2015) was still greatest in mature forest stands, followed by PCFs and PLs with significant differences between MPs and PCF/PL (Table 3). However, numbers of adult specimens found in PCFs when hand searching was included was 3.8 times greater than where hand searching was not employed with a significant difference found between the two sampling strategies (P < 0.001, Welch’s T-test). The mean number of juveniles was also greatest in the MPs but there was an 18 fold increase in the mean number of juvenile specimens found in the PCFs when hand searching data were included (Table 3) with a significant difference found between the data including and excluding hand searches (P = 0.015, Welch's T-test). No significant difference, however, was found between data including and excluding hand searches for the MPs and PLs for both adults (P = 0.766, P = 0.890 respectively, Mann-Whitney U-test) and juveniles (P = 0.881, P = 0. 953 respectively, Mann-Whitney U-test).

Table 2 Comparison of *G. maculosus* catches across mature plantation (MP), previously clear-felled forest (PCF) and adjacent peatland (PL) using traps (July 2014 – October 2015) placed on tree trunks, stumps and rocks, respectively, in addition to ground traps[1].

	MP	**PCF**	**PL**
No. of sampling days (N)	585	320	305
Mean no. of adults / day (± SD)[2]	5.23 (± 5.80)	0.47 (± 0.90)	0.50 (± 1.14)
P values			
MP	-	-	-
PCF	**0.000**	-	-
PL	**0.000**	0.957	-
Mean no. of juveniles / day (± SD)[3]	0.47 (± 1.10)	0.047 (± 0.23)	0.24 (± 0.77)
P values			
MP	**-**	**-**	**-**
PCF	**0.000**	**-**	**-**
PL	**0.001**	**0.000**	**-**

Adult: Test statistic = 190.449; df = 2; P < 0.001, Welch's ANOVA. P values given in bold indicate significant differences between habitats, Games-Howell multiple comparison test; **Juvenile:** Test statistic = 45.091; df = 2; P = < 0.001, Welch's ANOVA. P values given in bold indicate significant differences between habitats, Games-Howell multiple comparison test.

[1]Data from stand 2b which was subjected to a clearfelling event during the course of the study are not included in this table. [2] Individuals > 1cm (diam.) when rolled into a defensive ball. [3] Individuals < 1cm (diam.) when rolled into a defensive ball.

Table 3 Comparison of *G. maculosus* catches across mature plantation (MP), previously clearfelled forest (PCF) and adjacent peatland (PL) using traps only (**Tr**) and traps in combination with hand searching (**Tr&Hs)** (June – September 2015) with traps placed on tree trunks, stumps and rocks respectively, in addition to ground traps[1]

	MP		**PCF**		**PL**	
	Tr	Tr&Hs	Tr	Tr&Hs	Tr	Tr&Hs
No. of sampling days (N)	135	135	80	80	80	80
Mean no. of adults (± SD)[2]	3.68 (± 4.06)	3.93(± 4.24)	0.56 (± 0.9)	2.13 (± 3.17)	0.53 (± 0.84)	0.58 (± 0.94)
P values						
MP	-	-	-	-	-	-
PCF	**0.000**	**0.001**	-	-	-	-
PL	**0.000**	**0.000**	0.960	**0.000**	-	-
Mean no. of juveniles (± SD)[3]	0.71 (± 1.22)	0.73 (± 1.24)	0.02 (± 0.14)	0.36 (± 1.31)	0.16 (± 0.48)	0.21 (± 0.54)
P values						
MP	**-**	**-**	**-**	**-**	**-**	**-**
PCF	**0.000**	**0.115**	**-**	**-**	**-**	**-**
PL	**0.000**	**0.000**	**0.026**	0.614	**-**	**-**

Adult: Test statistic Tr = 38.744, Test statistic Tr&Hs = 44.477; df = 2; $P < 0.001$, Welch's ANOVA. *P* values given in bold indicate significant differences between habitats, Games-Howell multiple comparison test; **Juvenile:** Test statistic Tr = 23.891, Test statistic Tr&Hs = 8.749; df = 2; $P = < 0.001$, Welch's ANOVA. *P* values given in bold indicate significant differences between habitats, Games-Howell multiple comparison test.

[1]Data from Site 2b which was subjected to a clearfelling event during the course of the study are not included in this table. [2]Individuals > 1cm (diam.) when rolled into a defensive ball. [3] Individuals < 1cm (diam.) when rolled into a defensive ball.

4.3.2 Population estimates

Jolly-Seber estimates could not be calculated for any sampling weeks in PCFs and PLs due to low numbers of recaptures (estimates are only considered to be accurate when the number of recaptured animals over the sampling week is greater than ten (Greenwood 1996)). Within the MPs, estimates using the Jolly-Seber method could only be calculated for six sampling weeks (out of a total possible 98) due to either low recaptures (less than ten) or a failure of the Jolly-Seber goodness of fit test (Sutherland 1996). Of these six estimates, only those at two MP stands, 2a and 3c (density of 0.7 individuals/m^2 and 1 individuals/m^2 respectively), could be calculated during April (2015) when the overall goodness of fit was satisfactory. As with the Jolly-Seber estimates, due to low capture numbers, population estimates using the Schnabel model could not be calculated for PCF and PL habitats. However, the Schnabel model was found to be a good fit in MPs for 33 sampling weeks (out of a total possible 135). Estimates could not, however, be calculated for two of the MP stands (2c and 4b), due to low capture numbers (Fig. 1). For the same reasons estimates could not be calculated in July 2014, January 2015, June 2015 or September 2015 for the remaining eight stands (Fig. 2). Mean (± SD) Schnabel population estimates in the mature plantations ranged from 9.61 (± 4.63) individuals/ m^2 to 23.49 (± 12.19) individuals/ m^2 with mean number of individuals captured (excluding recaptures) over the sampling week, and mean total catch per sampling day following similar patterns (Fig. 1). Discounting occasions where estimates could not be calculated, the mean (± SD) Schnabel population density estimate per m^2 (Fig. 2) ranged from 24.4 individuals/m^2 (± 12.7) in August 2014 (week 2) to 4.5 individuals/m^2 (± 0) in February 2015 (week 8). Significant positive Spearman's rank correlations were found in MPs between Schnabel population estimates and mean total catch of *G. maculosus* per sampling day ($P < 0.004$, $r_s = 0.490$) (Fig. 3a) and between Schnabel population estimates and numbers of captures (excluding recaptures) during sampling weeks ($P < 0.001$, $r_s = 0.891$) (Fig. 3b). When captures for each of the sampling days (1 to 5) were averaged for the MPs over the length of the study, the mean percentage of marked individuals in each catch increased over time so that by day five a mean of 60% of captures consisted of marked individuals (Appendix 1). Overall, the average percentage (± SD) of unmarked individuals was 25% (± 10%) of the catch in MPs, 59% (± 34%) in PCFs, and 54% (± 21%) in PLs.

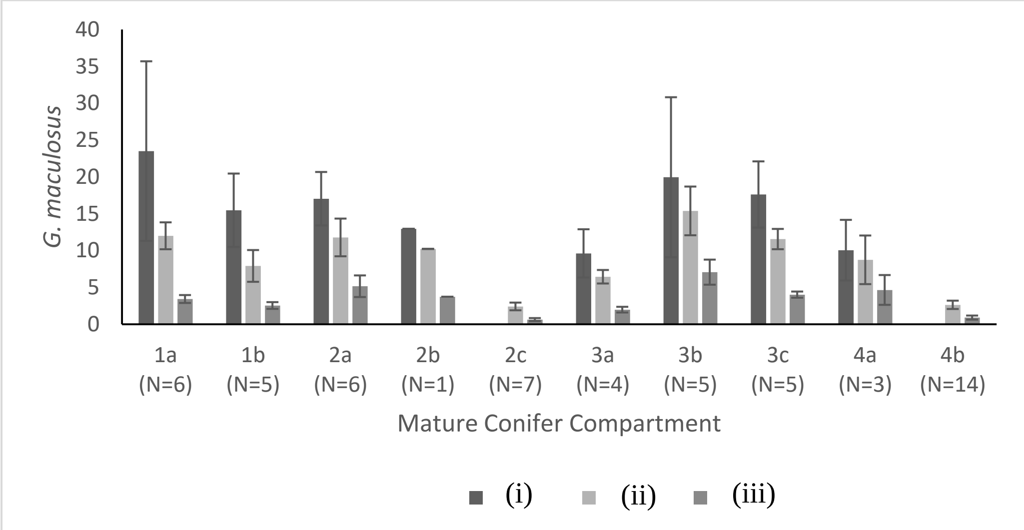


Fig. 1 Mean (± SE) *G. maculosus* in mature plantations (MP) over sixteen months (July 2014 – October 2015): (i) Schnabel population estimate (mean number / m^2)[1]; (ii) number of individuals captured (excluding recaptures) over sampling week (mean / sampling week / m^2); (iii) total catch (mean / sampling day / m^2). N = number of months; error bars = SE.

[1]Population estimates for compartments 2c and 4b could not be calculated due to low capture numbers and lack of fit.

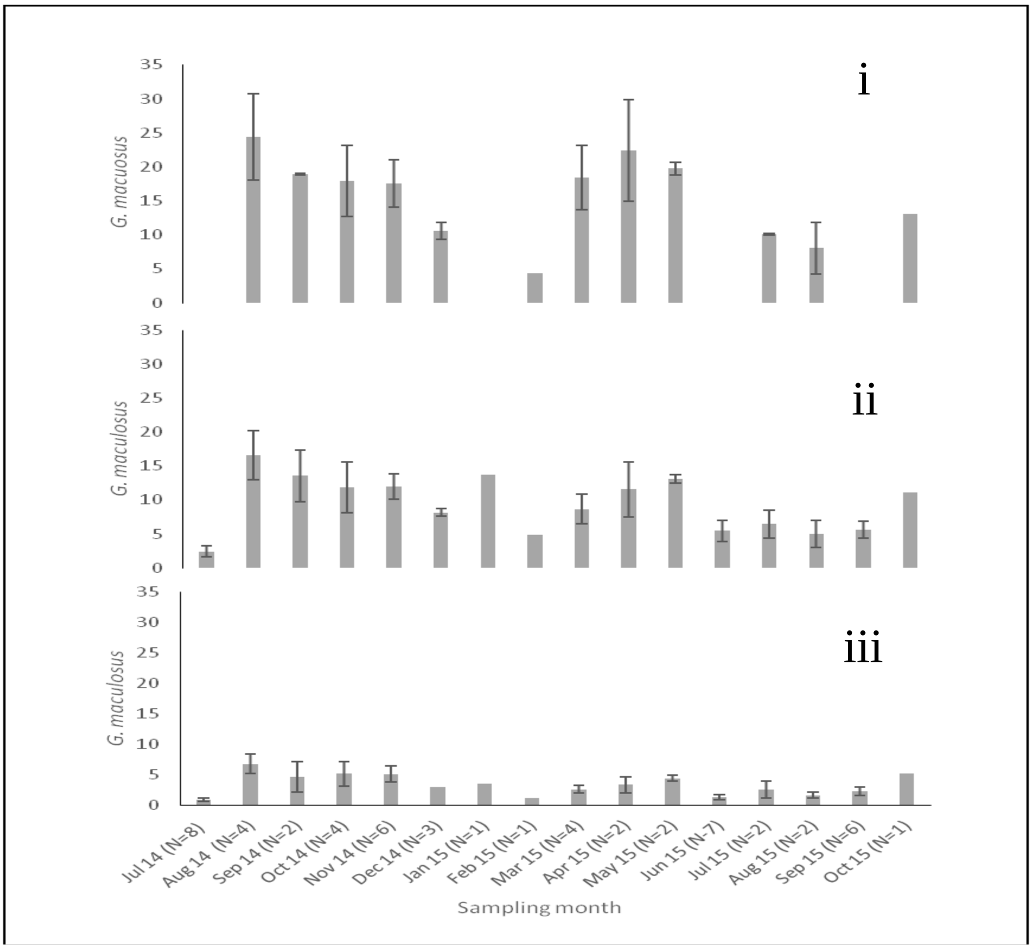


Fig 2 Mean (± SE) *G. maculosus* in mature plantations (MP) on each sampling month (July 2014 – October 2015): (i) Schnabel population estimate (mean / m^2)1; (ii) number of individuals captured (excluding recaptures) over sampling week (mean / m^2); (iii) total catch (mean per sampling day) / m^2. N = number of stands; error bars = SE.

[1]Population estimates for July 2014, January, June and September 2015 could not be calculated due to low

capture numbers and violation of the goodness of fit.

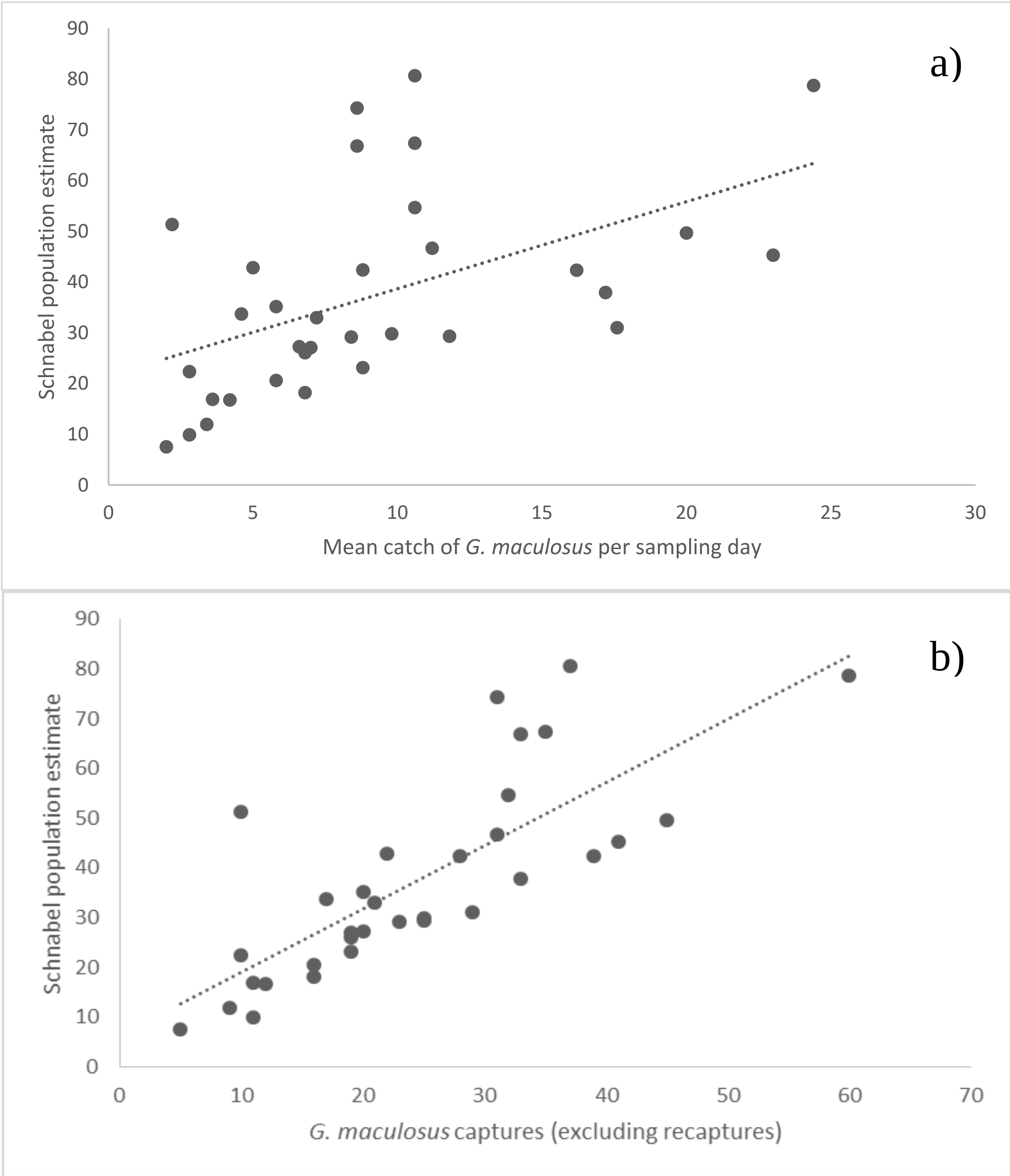


Fig. 3 Relationship between Schnabel population estimates (July 2014 - October 2015) and: (a) mean total catch of *G. maculosus* per sampling day in eight mature plantations (MP; N=33); (b) *G. maculosus* captures (excluding recaptures) per sampling month in eight mature plantations (MP; N=33).

4.3. Before and After Impact Assessment- Paired (BACIP)

As population estimates could not be calculated in NCF due to low numbers, BACIP analysis was carried out using total catches per sampling day to allow for comparison post-impact (i.e. after clear-felling). Mean number of individuals per sampling day (± SD) caught over two sampling weeks (i.e. ten sampling days) before felling in the control and impact stands (2a and 2b respectively) were 6.9 (± 8.7) and 6.3 (± 4.3) respectively. No significant difference was found between the control and impact stands prior to felling ($P = 0.848$, Welch's T-test). Felling and forwarding was undertaken over five months (Fig. 4) during which time, for health and safety reasons, no sampling took place. The traps were replaced in February (2015) onto the remaining stumps of the trees which were sampled prior to clear-felling and two weeks later the first catches (post clear-felling) were recorded. The mean number of individuals per sampling day (± SD) over the eight months following trap replacement in the impact stand was 0.3 (± 0.6), while the corresponding months in the control stand had a mean (± SD) of 6.2 (± 5.1) with a significant difference found between the two stands ($P < 0.001$, Welch's T-Test). None of the individuals captured over two sampling weeks in the impact stand prior to felling were recaptured during eight sampling weeks post-felling in contrast to a 21% recapture rate at the control stand over the same timeframe. The other three MP stands sampled (1a, 4a, 4b) during the same period yielded a mean recapture rate of 38% (± 15% SD). In addition, in post impact sampling weeks, numbers were consistently lower in the impact stand than those in the control stand, even when 10 minute hand searches were included (Fig. 4). A BACIP analysis (Smith 2002) confirmed a significant impact from the felling event using data from catches from traps only, but also when hand searching data were included with that from the traps ($P = 0.015$ and $P = 0.014$ respectively, Independent Samples T-test).

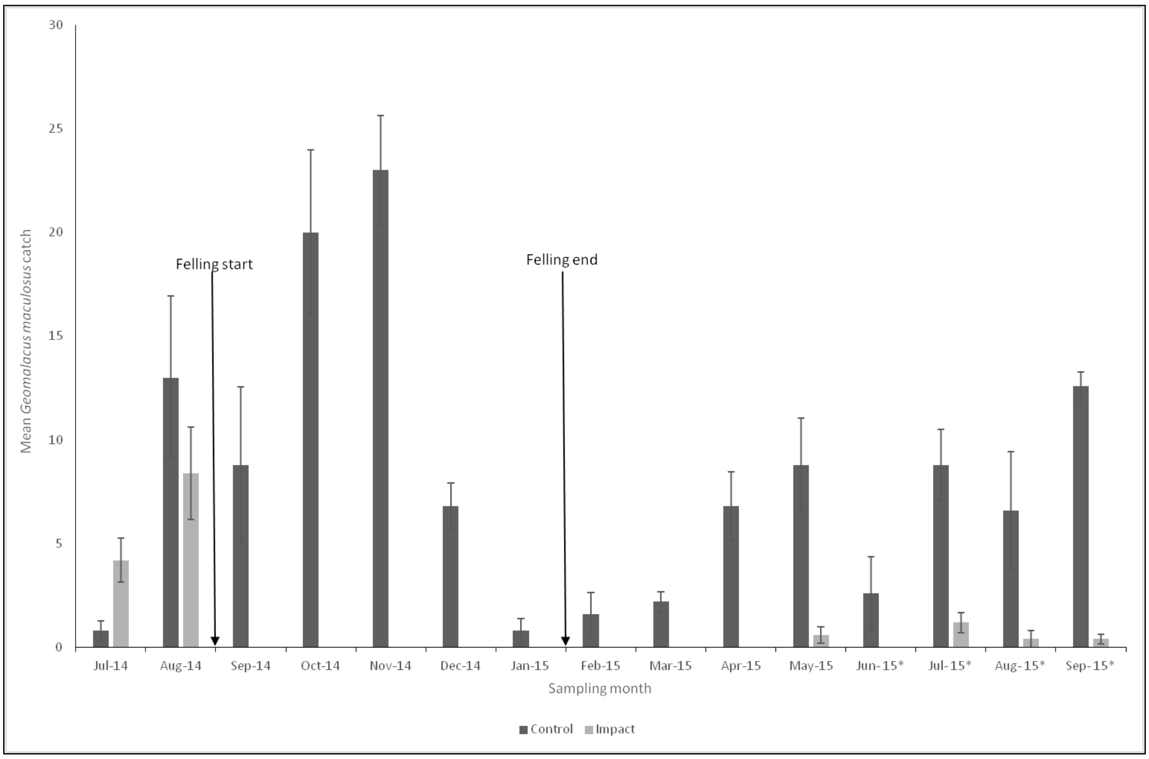


Fig 4 Mean (± SE) number of catches of adult *G. maculosus* per sampling day in the control and impact stands over 15 months between July 2014 and September 2015. Traps were removed from the impact site during felling for health and safety reasons. *Months where hand searching data were included.

4.3.4 Stand characteristics

Significant, moderate, positive Spearman's rank correlations were found between average catch of adult *G. maculosus* per tree and circumference at the base of the tree (P < 0.001, r_s = 0.369), as well as circumference at breast height (P = 0.015, r_s = 0.286). No significant correlation was found between average catch of adult *G. maculosus* per tree and the percentage moss cover (P = -0.58, r_s = 0.626). No correlations were found between average sampling week catch of adult *G. maculosus* per MP and the stand age (P = 0.474, r_s = -0.257).

4.4 Discussion

4.4.1 Comparison of *G. maculosus* catches in mature plantation (MP) stand, previously clear-felled (PCF) stands and adjacent peatland (PL).

Given that PL has been considered historically as a natural habitat for *G. maculosus* (Platts and Speight 1988), it is surprising to find greater catches in MP and PCF stands. While this is likely to reflect actual numbers found within the stands, it is important to consider trapping efficiency across the three habitat types. Johnston *et al.* (2016) found that the area under traps on tree stumps in PCF stands tends to be drier in comparison to those in MPs and PL. The shape of tree stumps means that it is not possible to attach traps as tightly to the stump surface and sides as it is on the tree trunks and rocks found in MPs and PLs respectively. It is likely that runoff from rainfall in MPs and PLs enters the narrow space between the trap and the surface to which it is attached thereby maintaining damp conditions under the traps. In MPs this is likely to be further influenced by tree shading. Clearly, drier traps would be less attractive to slugs seeking to avoid dehydration and the greater numbers of slugs captured in the PCF stands, in particular, when hand-searching was incorporated in the sampling methodology, supports this hypothesis. This emphasises the importance of undertaking hand searching in addition to traps, particularly in clear-felled areas, when assessing sites for *G. maculosus* as suggested by Johnston et al. (2016). Given the consistently greater numbers of *G. maculosus* found in MPs, conservation efforts should focus more on commercial forestry to ensure adequate future protection of the species. Although Johnston et al. (2016) hypothesised that *G. maculosus* moves up the tree to forage, no study has to date examined the distribution of the species higher in the canopy. It is, therefore, still unknown how much of the tree and associated microhabitats is used by the species. The impact of this on trapping efficiency and density estimates, while outside the scope of this study, requires further investigation.

4.4.2 Population estimates

Krebs (1999) describes the Jolly-Seber model as a method of population estimation for open populations, which allows for births, deaths, immigration and emigration. As the Jolly-Seber method is generally unreliable without at least ten recaptures (Greenwood 1996), its use was limited in this study because many sampling occasions, particularly those in PCF, NCF and PL habitats, had to be eliminated due to failure to meet this requirement. In addition, the method requires that there is some permanent emigration (Sutherland 1996) but high recapture rates in MP stands (when taken over the entire length of the study) violated this assumption. The continual recapturing of individuals over several succeeding months in the mature plantations suggests that the dispersal rate of the individuals was relatively low, likely due to movement predominantly occurring up and down the tree as opposed to between trees. Lack of movement between trees, at least at ground level, is supported by low numbers of catches found under traps placed on the forest floor (Johnston et al. 2016).

Since the Schnabel population estimate assumes that a population size is constant, with no limit to the maximum number of recaptures (Alcoy 2013), it was more appropriate for this study. Despite this, it was still not possible to obtain population estimates in PCF, NCF or PL habitats as either there were no recaptures to calculate the estimate, or the Schnabel goodness of fit test was violated. The Schnabel estimates in the MPs, however, correlate with both the mean total catch per sampling day and the number of captures (excluding recaptures) over the sampling week. This indicates that despite the shortcomings of the method, the estimates reflected the actual numbers of individuals caught during sampling. Activity in terrestrial gastropods is associated with a number of environmental factors (Young and Port 1989), and (apart from July 2014 at the start of the study) greater proportions of new *G. maculosus* individuals were present in the warmer months from April to August than in colder months. When the proportion of unmarked individuals was averaged over sampling days, 60% of the catch on the last sampling day (day 5) consisted of recaptured individuals entering the traps. It is likely that individuals that were previously deemed too small to tag may have entered the appropriate size class in later months, which may have contributed to the percentage of unmarked individuals. Nevertheless, the majority of individuals were caught over five sampling days in MP stands, and the Schnabel population estimates calculated correlates with the mean total catch per day of *G. maculosus* adults. Since, calculating population estimates using mark-recapture is labour intensive and requires specialist training and equipment, it is unlikely that this will be adopted by foresters in conservation strategies for the species. However, the use of mean total catch per day as a proxy for foresters undertaking surveys to estimate population sizes of *G. maculosus* in mature conifer plantations, at least in the south-west of Ireland, may provide a more feasible solution.

4.4.3 Before and After Impact Assessment- Paired (BACIP)

Prior to the onset of felling there were no significant differences in catch between the control and impact stands. At the impact stand, however, BACIP analysis found a significant difference in mean numbers before and after felling, both including and excluding additional hand search data post-impact. No individuals were caught in the impact stand for the first three months post trap replacement. While mean adult catch increased over the period from March to September,, catches were consistently less for each month at the impact stand during this period. The mean catch per sampling day at the impact stand dropped from 6.3 (± 4.3) to 0.3 (± 0.6), a reduction of 95%, while in the control stand the mean catch changed from 6.9 (± 8.7) to 6.2 (± 5.1), a reduction of only 10%. Even the addition of hand searching within the impact stand on 20 sampling days between June and September (2015) resulted in only two additional individuals (during the August 2015 sampling week) being captured. Significant differences were also found in a direct comparison of the control and impact stands post-felling, while no significant differences were observed prior to felling. It is important to note that, due to the timing of felling operations, only one stand of mature conifers was felled with sufficient time to allow sampling before and after the impact event. Future long-term studies are required to determine the rate of recolonisation of a clear-felled site in conjunction with an assessment of those factors which influence this. Nevertheless, these results are consistent with the findings of Strayer et al. (1986) who reported that disturbances (including clear-felling) may reduce densities of gastropods. The presence of *G. maculosus* in the impact stand, albeit in low numbers in the months following trap replacement, indicates that the species is present post-felling. However, none of the individuals that were tagged prior to the impact were recaptured post-felling, unlike the control site where 21% of the catch within the same timeframe consisted of recaptures. The relatively large numbers of slugs found under traps on trees compared to ground traps in mature plantations (Johnston et al. 2016) suggests that most slug activity takes place on the trees. It is, therefore, perhaps not surprising that none of the individuals found prior to clear-felling are present post-clear-felling. While it is likely that those few individuals found within the impact stands post-felling had colonized from nearby habitats, the effect of surrounding stands harbouring *G. maculosus* on the colonisation of clear-felled stands requires further investigation particularly given the low dispersal ability of *G. maculosus* within habitats (McDonnell and Gormally 2011).

4.4.4 Stand characteristics

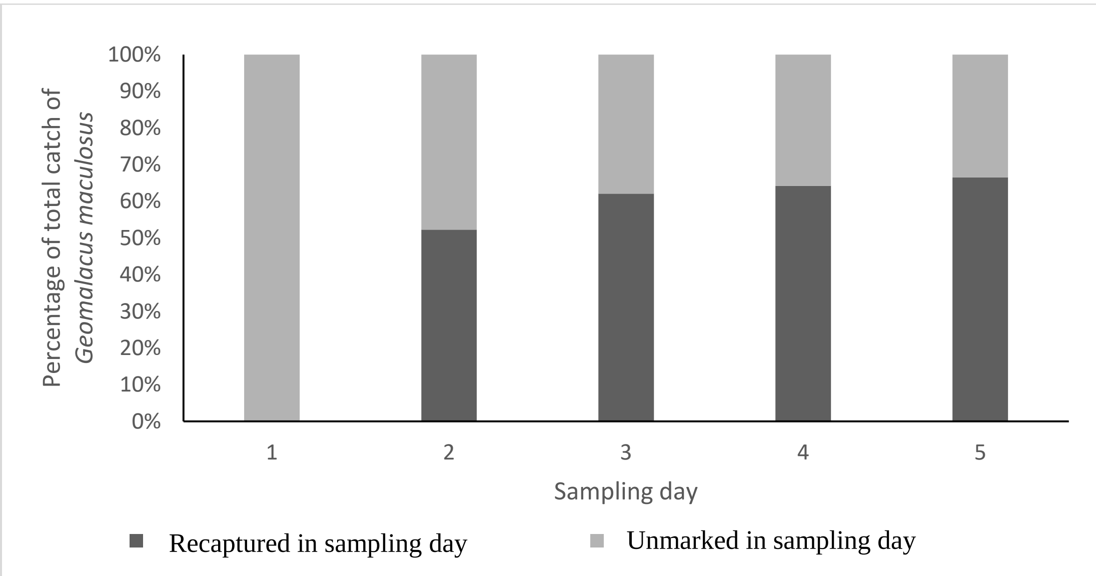
Significant, moderate, positive correlations were found between average catch of adult *G. maculosus* per tree in the MP stands and circumference at both the base of the tree and at breast height. This correlation mirrors that found by Reich et al. (2012) who hypothesised that this was due to the association of greater bryophyte cover on older trees with larger circumference at breast height. However, unlike Reich et al. (2012), species catch across a range of MP stands does not correlate with percentage moss cover. As terrestrial gastropods are known to avoid exposure to unfavourable conditions (Rollo 1982) and evade cold temperatures by moving below ground (Cook 2004), it is likely that *G. maculosus* makes use of the base of the tree as a refuge during non-optimal weather conditions. Indeed, the authors have observed *G. maculosus* sheltering at the base of trees throughout the study period. This suggests that while *G. maculosus* makes use of bryophytes as a source of both food and shelter (Platts and Speight 1988), the association with larger tree circumference, particularly at the base of the tree, may be of greater importance for the species. Overall, these results indicate that greater catches of *G. maculosus* are associated with higher quality stands of commercial forestry. The lack of correlation in this study between *G maculosus* captures and MP stand age is unsurprising as all of the stands in this study were planted within five years of each other, and as such, the differences in stand composition caused by this would be minimal. However, this result highlights that the quality of forestry stands is likely to have more influence on slug numbers than simply the age of the stands. The influence of surrounding habitat types on catches within plantations, particularly PCF stands, has yet to be examined fully.

4.4.5 Conclusions

Of the three habitats investigated in this study the greatest number of *G. maculosus* captures occurred in MPs, highlighting the need for their protection where they occur in commercial forestry. The high number of recaptures in conjunction with few individuals found on the ground between the trees as reported by Johnston et al. (2016) appears to indicate that populations of *G. maculosus* within MPs are largely confined to the trees. In addition, the greater catches of *G. maculosus* associated with higher quality stands of commercial forestry is not compatible with current forestry practices in Ireland where clear-felling and removal of trees is the norm. BACIP analysis shows a significant impact of clear-felling on *G. maculosus* captures with a 95% reduction post-felling. Current legislation under the Habitats Directive requires

Member States to prohibit, among other factors, the "deterioration or destruction of breeding sites or resting places" of an animal species listed in Annex IV in their natural range, of which *G. maculosus* is one (EC 2007), and under Irish legislation the species is protected wherever it occurs. The results of this study, when taken into account in the context of these legislative obligations, indicate the need for practical mitigation measures. Two possible measures include retention of small stands of forestry (Raivio et al. 2001) and translocation of species (Germano et al. 2015). While a short-term study undertaken in Co. Galway by Reich et al. (2012) demonstrates the possible benefits of retaining 3m stumps post-clear-felling, the long-term benefits to *G. maculosus* has not yet been assessed. In addition, translocation has never been examined in *G. maculosus* and therefore it is not possible to speculate on this as a measure without further research into both the carrying capacity of forests, inter / intra-specific competition and the ability of *G. maculosus* to adapt to new areas. Given these findings, further research is urgently required to determine practical mitigation measures to protect the species where it occurs in commercial forestry.

Appendix



Mean percentage of total catch of *G. maculosus* per sampling day (N=117) consisting of individuals recaptured at least once during the sampling week in mature plantations (MP) (July 2014 - October 2015)

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Chapter 5: General Discussion

1. General Discussion

Geomalacus maculosus is a protected species under the EU Habitats Directive. This requires Member States to designate Special Areas of Conservation for the species (Annex II) and protect it where it occurs within its natural distribution (Annex IV). In Ireland, the protection of the species is currently supported by the publications: *Forestry & Kerry Slug Guidelines* (Forest Service 2009); and *Ecological Surveying Techniques for Protected Flora and Fauna during the Planning of National Road Schemes* (NRA 2009). However, both guidelines fail to recognise that commercial forestry can provide a viable habitat for *G. maculosus* in addition to which they do not include the most recent information on the distribution of the species in Ireland. Of even greater concern is that, at the time of writing, only one of 45 Coillte management plans (2011 – 2015) for areas that overlap with known *G. maculosus* distribution makes reference to the species. This is despite the fact that *G. maculosus* had already been discovered in commercial conifer plantations in the west (Kearney, 2010) and south-west (Mc Donnell and Gormally, 2011) of Ireland and that forestry is listed as a threat to the species (NPWS 2010). With a view to updating these guidelines a number of research gaps had first to be addressed including: determining the food preferences (if any) of the slug; devising a national monitoring strategy for the species by assessing the efficacy of survey methodologies; and quantifying the impacts (if any) of commercial forest management practices on the species. Ireland's legal obligations to protect the species coupled with the virtual absence of practical measures to protect it in commercial forestry, provided the incentive for this work, the aims of which were to:

1. Quantify, for the first time using behavioural software, the food preferences (if any) of *G. maculosus* which first required the establishment of an experimental procedure for the species.
2. Compare the efficacy of two sampling methods (De Sangosse refuge traps and hand searching) across three habitats commonly found in commercial forestry in Ireland and establish optimal surveying times for a proposed national monitoring programme.

3. Evaluate the usefulness of employing capture-mark-recapture for assessing population sizes in addition to comparing *G. maculosus* captures rates in mature, clearfell and unplanted forest. The impacts of clear-felling were also examined at a site which was clear-felled during the course of the study.

5.1. Key findings

1. *Geomalacus maculosus* exhibits diverse feeding preferences and while there are no differences between the two commonly occurring colour morphs, differences in sinuosity could reflect food availability

Results indicate that salt paper, birch tar oil and Insect-A-Slip (Fluon) are all effective barriers for *G. maculosus* in the laboratory although the impact of Fluon on *G. maculosus* mucus production has not, to the best of the author's knowledge, been recorded previously for other slug species where it has successfully been utilised in the containment of terrestrial gastropods (Symondson 1993; Schüder *et al.,* 2004; Ribadula Nogueira, 2011). Salt impregnated blotting paper was chosen for the behavioural trials since it was easy to apply and replace; retained all individuals within the arena over the 24-hour trial duration; did not appear to impact on behaviour in test subjects; and was cost effective. The examination of the pre-treatment of test subjects prior to use in feeding choice trials found that individuals which were not food deprived were most likely to make contact with a food option and were most likely to actively feed when in contact with food. These findings informed the final behavioural analysis set-up and starvation regime for this study.

Results of the feeding behaviour trials found that feeding preferences exhibited by *G. maculosus* did not appear to be influenced by the habitat type (open or closed) from which the individuals came as both colour morphs showed the same preferences for food species. Of the lichen species presented to *G. maculosus*, *Cladonia fimbriata* was found to be the most preferred species overall based on mean percentage of total cumulative duration and on percentage of choices (where only one of the two food items was selected).

This was followed by *Usnea cornuta* and *Parmelia saxatilis* as the next most favoured choices. The time taken for *G. maculosus* to reach its first choice (mean latency to first) also reflects this in that the above three species are among the top four lichens for which it took least time for the slugs to reach. Interestingly *P. perlatum*, which had the shortest mean latency to first, ranks fifth in mean percentage of total cumulative duration, suggesting that while slugs were attracted to it in the first instance, they spent less time in contact with it than the other three highly ranked species. Out of the five species of bryophytes compared using *C. fimbriata* as a reference species, the liverwort *Frullania dilatata* had the greatest mean (± SE) percentage of total cumulative duration with, followed by the moss *Pleurozium schreberi*. The high percentage of total cumulative duration of these two species (63% and 62% respectively) show that individuals spent more time in contact with these species than in contact with the top lichen choice, *C. fimbriata*. Therefore, given that food choice by *G. maculosus* varies considerably at the level of individual choices, *G. maculosus* appears to be a generalist herbivore of lichen and bryophyte species and it is not restricted to any one of the food options investigated in this study. The generalist nature of *G. maculosus* may be part of the reason why the species has survived well in mature conifer plantations. Six species examined in this study are solely associated with forested habitats, three with peatland habitats, and the remaining five occur in both forested and peatland habitats. Given that most of these species are considered common species, within their habitat associations in Ireland, (Dobson 1992) it seems unlikely that food resources are a limiting factor to the distribution of *G. maculosus* generally. Furthermore, *G. maculosus* has been observed to feed on fungi and algae (Platts and Speight 1988). These have not been investigated as food sources in *G. maculosus* in Ireland to date but future research should aim to assess feeding preferences in *G. maculosus* for these species with a view to establishing their importance as food items.

The establishment of an efficient, automated method for analysing *G. maculosus* behaviour permitted, for the first time, an examination of *G. maculosus* mobility. The mean distance moved over two hours was 6.7m and 5.61m for forest and open colour morphs respectively, while the mean velocity was 0.083 cm/s and 0.09 cm/s respectively. If slugs were to continue this rate of movement in a straight line for 24 hours, a maximum distance of 80.4m could be covered. While this is unlikely to reflect the situation in the wild given that slugs do not move constantly at consistent speed in addition to which they meander, it does inform the potential of the species to migrate to suitable habitats in which the slug is currently absent. This deserves further investigation as the distribution of the species is monitored in future years with the potential to develop models to predict its spread in Ireland in the future. While the higher rate of movement found in this study indicates that *G. maculosus* has a greater potential for dispersal than previously thought, the estimates provided by this study are likely to be an over estimation. The only other estimate regarding distances moved by *G. maculosus* (0.55m per day) was calculated by Mc Donnell *et al.* (2011) through a mark-recapture study. Given that the surface provided in the arena was conducive to locomotion and movement in slugs is costly in terms of energy expended (Denny 1980), it is likely that distances travelled in nature fall somewhere between the two estimates above. While the analysis of movement parameters in *G. maculosus* is useful, estimates taken in the field would provide a more accurate representation. Studies regarding movement in the field have investigated rates of movement successfully in snails using radio transmitters (eg. Bailey 1989) however this has not been carried out to date with slugs. A similar approach would provide a far more accurate representation of dispersal rates of *G. maculosus* in the wild.

While there was no significant difference between the average distance moved and velocities of colour morphs, the mean meander, a measure of sinuosity of a recorded track (Maréchal 2004), was found to be significantly higher in open morphs than forest morphs. Learned behaviour associated with feeding is known to occur in slugs (Gelperin 1975; Sahley *et. al* 1981), and so the differences found may be a reflection of foraging behaviours learned in the wild. Although further research is required to establish clear reasons for this, one possibility may be the differences in food source aggregation between the two habitat types. Lichen cover in commercial forests in Ireland is greatest in the top third of trees (Coote *et al.*, 2007) and given that individual commercial conifer trees can support large numbers of *G. maculosus*, slugs moving up and down the trees would locate a ready food source without expending too much energy. In contrast, the distribution of the lichen species (given as food choices to slugs in this study) can sometimes be scattered across the substrate (Dobson 1992). These differences could result in open morphs having an increased exploratory behaviour to help them locate and make contact with food sources although this would need to be substantiated by further work.

2. Monitoring protocols for *G. maculosus* abundances require careful consideration of time of year in which sampling is undertaken, weather conditions and the type of habitat being sampled

The investigation of trapping efficacy in different habitats indicates that trapping needs to be tailored according to the habitat under investigation. Refuge traps were found to catch more slugs (in comparison to hand searching) in mature conifer and peatland compartments when placed at 1.5m on tree trunks and on rocky outcrops respectively. Given that slugs appear to spend most time on trees in mature conifer plantations, as evidenced by the low number of slug captures on traps placed on the ground in the forests, slugs moving up and down the a mature conifer tree are likely to encounter a refuge trap given the relatively small surface area of the tree trunk. It is therefore not surprising that catches using refuge traps on trees were greater than those found using hand searching, or that traps placed at 1.5m were more effective than when placed at 0.2m. In peatland habitats, the only individuals found were located on rocky outcrops, and no individuals were found on ground between the outcrops either through trapping or hand searching. This may be due to dense vegetation present in peatlands reducing the effectiveness of hand searching, as when surface conditions are more structurally complex it becomes more difficult to detect slugs using hand searching (McDade and Maguire 2005). The increased moisture in the peatland vegetation may also have reduced the attractiveness of ground traps as individuals did not have a need to shelter beneath them due to sufficient levels of humidity. It is also likely that the capture of *G. maculosus* was limited to rocky outcrops because of the presence of an abundant source of lichens on which to feed (NRA, 2009). Refuge traps were not found to be efficient in trapping *G. maculosus* within previously clear-felled compartments. A large proportion (77%) of *G. maculosus* caught during the short-term study was collected using hand searches. None of the individuals that were found during hand searches in the clear-fell were found on the ground between stumps. This may be, again, due to vegetation between stumps making it difficult to see *G. maculosus*. Surveys conducted in mature conifer compartments and peatland habitats should, therefore, make use of refuge traps, while hand searching should be carried out in previously clear-felled compartments. These findings highlight the importance of taking into account the habitat in which a survey is taking place when surveying for *G. maculosus*, as utilising inappropriate methods could result in an underestimation of catch.

While *G. maculosus* was successfully captured year round, catch success varied over the course of the year. Greatest capture success for *G. maculosus* was in autumn, with highest catch success for both adults and juvenile *G. maculosus* occurring August to November. This may vary to some extent from year to year in that a very dry late Summer / Autumn or unseasonably cold conditions, could reduce the efficacy of refuges traps (see below). Indeed, August 2015 was below average in terms of average temperature, with Valencia weather station reporting the coldest August in 29 years (Met Eireann 2015) which may have caused the catch to be less than what was caught the previous year. Given that this is not a regular occurrence in Ireland, surveys to confirm the presence of *G. maculosus* or to collect individuals should probably take place during this period. A second peak in capture rates of juveniles was found in summer which is likely to be the result of egg laying by adults in the spring (Wisniewski, 2000). Summer surveys could, therefore, be utilised to gather information on the extent of breeding and recruitment of juveniles to a population, indicating the health of a population. It was also established that survey days should not take place at low or high temperatures to ensure that *G. maculosus* was found beneath traps rather than sheltering at the base of the trees to avoid unsuitable conditions. Although *G. maculosus* has been reported to only be active by day during or directly after rain (Taylor 1906; Platts and

Speight, 1988), no significant relationship was found between rainfall at the time of sampling and hand search capture success in any of the habitats. However, a positive correlation was found between individuals caught using hand searches in previously clear-felled compartments and the average rainfall during the 24 hour period prior to sampling. These results, although somewhat limited by the distance between the site and the weather stations from which meteorological data were extracted, highlight the importance of weather and seasonality in surveying for *G. maculosus*. Surveys, both trapping and hand searching, that are carried out in unsuitable conditions may result in an underestimation of catch.

Given the above findings, foresters who aim to carry out surveys for *G. maculosus* should do so in Autumn months for optimal catch conditions. As trapping efficacy varies between habitat types, it is important that this be considered in survey technique. In all three habitats, an initial hand search may be utilised to confirm the presence of *G. maculosus*. Following this, if presence is not confirmed, refuge traps should be placed at 1.5m on tree trunks in mature conifer compartments and on rock outcrops in peatland habitats. Within mature conifer compartments greater numbers of *G. maculosus* are found at the edges (O'Callaghan 2015) and so traps should be placed close to edges if looking to confirm presence or absence. These traps should be left for at least a two-week period before rechecking to confirm that *G. maculosus* is not present. Given that hand searching is considered most efficient in previously clear-felled compartments, a second hand search should instead take place to confirm that the species is not present. Monitoring in mature conifer compartments and peatland habitats may be carried out using refuge traps only. Where monitoring occurs in clear-felled compartments, a timed hand searches may allow for a reproducible method of survey to be carried out.

3. **Even when data from hand searches and refuge traps were combined, mature commercial conifer compartments had greater catches of *G. maculosus* than previously clear-felled or unplanted peatland compartments.**

Once the limitations of using refuge traps and hand searching in isolation were determined, the data were combined to compare overall catches for mature conifer plantations, clear-felled sites and unplanted peatland sites respectively. The number of *G. maculosus* caught was greater in the mature conifer stands compared to clear-fell stands and unplanted peatlands. More individuals were caught using refuge traps in peatlands than in clear-fells. However, this pattern was reversed when numbers from hand searching were included, resulting in significantly higher numbers in clear-fell than caught in peatlands. The higher numbers in the previously clear-felled stands when compared to peatlands was surprising as peatland is considered a natural habitat for *G. maculosus* (Platts and Speight 1988). While the former may be part of the remnant population from the forest retained post felling, given the lack of recaptured individuals found post felling (see below), it appears more likely (at least in part) that *G. maculosus* recolonises recently clear-felled areas from surrounding areas with the remaining stumps providing food sources for slugs following colonization by lichen species associated with deadwood (see below). Overall, the results found are of great significance in relation to the protection of *G. maculosus* in forestry habitats, showing the potentially important role mature conifer plantations play in sustaining high numbers of *G. maculosu*s in comparison to the more traditionally recognised habitat for the species, namely peatland. Numbers of *G. maculosus* caught in mature plantations were almost double those captured in clear-fell and close to seven times those caught in unplanted peatland. Notwithstanding the possible limitations of the sampling methods, it is probable, based on this study that a far greater proportion of the Irish *G. maculosus* population is found in commercial conifer plantations than was previously realised. Given the implications of this finding for commercial forestry management, it is important that populations in commercial forestry associated habitats are considered in the conservation of the species.

4. Estimation of population sizes of *G. maculosus* is limited by lack of recaptures and/or goodness of fit.

The capture-mark-recapture (CMR) portion of this study was carried out over 16 months in mature conifer and previously clear-felled stands in addition to peatland habitats. The CMR study enabled an assessment of two different population models for *G. maculosus* i.e. the Jolly-Seber and the Schnabel methods. The Jolly-Seber method is described by Krebs (1998) as being an open population estimate. Open populations are more realistic in an ecological sense as they make the presumption that immigration and emigration take place, which is not the case in closed populations, as estimated by the Schnabel method (Krebs 1998). The Jolly-Seber method allowed for population estimates for the mature plantations to be calculated on just two (out of 98) occasions, while the Schnabel method allowed for 33 (out of 135) estimates. As the Jolly-Seber method requires at least 10 recaptures over the primary sampling period (Greenwood 1996), clear-fell and peatland habitats had to be eliminated due to the low recapture rates. In mature conifer stands, even on sampling weeks where the minimum number of recaptures was achieved, the goodness of fit often still failed as the method requires there to be some permanent emigration (Sutherland 1996) which high recapture rates in this study violated. The continual recapturing of individuals over several succeeding months in the mature plantations suggests that the dispersal rate of the individuals was relatively low, likely due to movement predominantly occurring up and down the tree as opposed to between trees. Therefore, only two population estimates using this model could be made where the minimum number of recaptures was made and the goodness of fit satisfied. The Schnabel model of population estimates appeared more suited to

G. maculosus populations in mature conifer stands as 33 population estimates were calculated in mature conifer stands over the course of the study. The correlation found between mean catch per day and these estimates indicates that they were reflective of the catch. However, there were still limitations to this method and estimates could not be calculated for all sampling periods. Low capture numbers in clear-fell and peatland habitats again excluded them from this analysis

Overall the CMR methodology was a labour intensive process to undertake. In addition, this method requires training and specialist equipment that would not be readily available to foresters. Given these restrictions, it is impractical for this method to be utilised by foresters who wish to assess population numbers within forested stands. Over the five secondary periods, most of the catch on the final day consisted of individuals that were already tagged in addition to which, Schnabel population estimates correlated with the mean total catch of adult *G. maculosus*. Mean catch could, therefore, be utilised by foresters as a proxy for *G. maculosus* population estimates. The use of this approach would allow for better monitoring of *G. maculosus* populations in at risk areas.

5. Before-After-Control-Impact (BACIP) demonstrates significantly reduced numbers of *G. maculosus* post-felling

The BACIP study showed significantly lower numbers of *G. maculosus* post clear-felling, in addition to which none of the individuals which had be tagged prior to clearfelling were subsequently recaptured during eight months of sampling post-felling. This is in comparison to a 21% recapture rate at the control stand over the same timeframe. In addition, the mean number of individuals per secondary period (± SD) over the eight months following trap replacement in the clear-felled stand was only 0.3 (± 0.6) compared to 6.2 (± 5.1) at the control stand. It is possible that at least some of the low numbers of slugs found post clearfelling migrated from surrounding areas since no marked animals were found after clear-felling. However, further work using tagged animals is required to substantiate this hypothesis. The original plan was to study a number of sites which were to be clear-felled within the timeframe of this study. Due to circumstances beyond our control, the clear-felling schedule did not take place as planned but the future investigation of additional sites is strongly recommended.

Clear-felling in the impact site was carried out during autumn months, which was also the season with highest catch success. This may have resulted in greater mortality if a higher proportion of individuals were actively foraging, and therefore moving up and down the trees. Ideally, felling should have taken place during the winter or summer months, as overall, catches of *G. maculosus* were found to be lowest in winter and summer for adults, the seasons with the lowest and highest temperatures respectively. However, this may not be suitable as felling in unsuitable weather conditions, like those often found in winter, may increase environmental impacts of the operation (DAFM 2000) Both adult and juvenile slugs have been observed, during the course of this study, to hide beneath the surface of the moss at the bases of rocks, trees and stumps in both very warm and very cold conditions. It is possible that this behaviour, given that terrestrial slugs protect themselves through avoidance of adverse conditions (Rollo 1982), is the result of *G. maculosus* retreating from unsuitable humidity or temperature conditions. Following a normal harvesting operations, low stumps between 5 and 10cm in height usually remain, with the full trunk of the tree removed (Forest Service 2000). Therefore if slugs are sheltering at the base of the tree during felling operations the felling machinery may avoid damaging individuals.

While a large proportion of the reduction in slug numbers post-felling may have been caused by the physical removal of habitat, impacts on the food species of *G. maculosus* may have further exacerbated the impacts. The top four out of the nine lichen species investigated in the previously mentioned feeding trials are associated solely with forest habitats (Dobson 1992). Further, the top ranked liverwort (*F. dilatata*) and moss (*P. schreberi*) are also associated with forested habitats (Atherton *et al.* 2010). Given that clear-felling operations involve the removal of the trunk of the tree often only leaving brashing and stumps behind (Forest Service 2000), lichen and bryophyte species associated with trees would be adversely impacted by felling operations due to changes from a forested to an essentially open landscape. Therefore, any remnant population which may have survived clear-felling activity in addition to subsequent log removal, would find itself contending with a potentially reduced food supply post-felling. On the other hand, lichen species such as *C. fimbriata*, *C. coniocraea* and *C. squamosa,* which are associated with rotting wood *(Dobson 1992),* may benefit from the presence of rotting stumps

/deadwood left behind by felling operations but it could take some time for these lichens to colonise a newly clear-felled site. The moss *C. interoflexus* which has a high ecological tolerance (Klinck 2010) and is known to be a coloniser of bare peat (Atherton *et al.* 2010), would also benefit from the exposure of bare peat following clear-felling thereby providing a food source for *G. maculosus* once established. More research is, however, required to determine the length of time between felling operations and the subsequent colonisation by these food species.

6. Commercial conifer stand characteristics i.e. tree circumference result in greater numbers of *G. maculosus* catches

Positive associations were found between the average catch of *G. maculosus* per tree in mature conifer stands and the circumference of both at breast height and the base of the tree. Reich *et al.* (2012) posited that this was a product of the bryophyte cover found on trees, with older, larger trees providing better cover. However, this study did not find a correlation between bryophyte cover and average number of *G. maculosus* per tree. Nevertheless, it is likely that the negative associations with catch success at both low and high temperatures, and the propensity of terrestrial gastropods to avoid rather than endure exposure to unfavourable conditions (Rollo 1982; Cook 2004), mean that the larger the base of the tree, the more surface area slugs have to use as a refuge. This may also explain why the correlation between slug numbers and circumference at the base of the tree was stronger than that at breast height. Horgan *et al.* (2003) define yield as the “maximum potential volume of wood of a given species that a site can produce per ha per annum”, and it is calculated based on age and size (Edwards and Christie 1981). This means that taller, faster growing stands of conifers will have a higher yield class and so these trees are expected to have a greater circumference. In addition, in general, the higher the yield class, the earlier the stand is thinned (Horgan *et al.* 2003) and the greater the intensity of thinning (Teagasc 2016) which, in turn, may result in more light and rainfall reaching the forest floor (Kerr and Hauf, 2011). This could result in changes in species assemblages of lichens, one of the food sources for *G. maculosus* (Fletcher 1999; Reich et al. 2012).

The association found between *G. maculosus* and larger stands of mature conifers has implications for forestry within the south- west of Ireland. *Geomalacus. maculosus* has been found in conifer plantations throughout the south-west of Ireland in what is traditionally known as its natural distribution range (Mc Donnell *et al.* 2011; Reich *et al.* 2012). The presence of juveniles in all four of the conifer plantation sites that were examined for this study suggests that populations within all of the mature conifer plantations are healthy. However, the previously mentioned BACIP study findings indicate that there is a significant impact on populations of *G. maculosus* caused by clear-felling. Under the current legislation regarding the protection of *G. maculosus*, EU Member States are obliged to protect the species where it occurs within its natural range, including protecting its breeding and resting places as habitats (EEC 2007). Given that the stands with the highest yield class have the highest level of forest productivity, they are also the most valuable in terms of timber production. These stands are, therefore, more desirable for clear-felling and replanting and so there are likely to be conflicts of interest as conifer plantations are felled in the future in the south-west of Ireland. In addition, given the Irish government's commitments to increasing forestry cover in Ireland by 7% (Forest Service 2008), areas where these conflicts occur are expected to increase in the coming years. Therefore, it is imperative that mitigation measures are put in place to ensure that the obligations under the Habitats Directive for the species are met.

5.2. Implications for mitigation measures

The findings of this study indicate that not only does there appear to be an association between populations of *G. maculosus* and higher quality stands of mature conifers, but there is an adverse impact caused by clear-felling on the species. Therefore it is imperative that mitigation measures be established as a way of protecting the species where these conflicts arise.

To date, the only study which has examined potential mitigation measures for the protection of *G. maculosus* in commercial conifer plantations is that of Reich *et al.* (2012). The study examined a small stand of 3m tall conifer tree stumps. These stumps were retained by the NPWS as a trial method for mitigating damage on the species during a clear-felling operation to create buffer zones for the protection of the EU protected freshwater pearl mussel (*Margaritifera margaritifera*). While there were higher numbers of *G. maculosus* found in the stand section with retained stumps than in nearby traditional clear-fell, there were no data on catch numbers prior to felling the stand to establish how much the process reduced impact on the *G. maculosus.* In addition, it is unclear how long the measures will remain effective. The stumps have been observed to be degrading and losing the bark, and the original bryophyte and lichen cover that would make them attractive to *G. maculosus* (I. Reich *pers. comm*.). While a number of the food species that *G. maculosus* has been found to consume are associated with deadwood species, including the top ranked lichen species *C. fimbriata*, this is a relatively ephemeral microhabitat and so the longevity of such a measure is uncertain.

The retention of small stands of live forestry is often used as a method for conserving biodiversity (Raivio *et al.* 2001). Perhans *et al.* (2009) found that while bryophytes decreased in both abundance and species richness within the retention areas, lichens showed no significant changes. The retention of trees is costly (Raivio *et al.* 2001) and in addition to this retention patches show higher levels of tree mortality (Gustafsson *et al.* 2010) and so the long term feasibility would have to be established. The translocation of species is another method that is often used to mitigate damage to populations. This measure is considered successful if the translocation of the species in question results in a self-sustaining population (Griffith *et al.* 1989). Griffith *et al.* (1989) found that better habitat quality was associated with greater success, and that success was also associated with finding middle ground between the minimum number of individuals required for successful release, and the point at which additional individuals was no longer beneficial. As this has never been examined in *G. maculosus* it is not possible to speculate on this as a method for mitigating effects on the species without further research into both the carrying capacity of woodlands and the ability of *G. maculosus* to adapt to new locations.

Continuous cover forestry involves maintaining tree cover to prevent *inter alia* soil exposure (Troup 1927) and involves a variety of silvicultural methods (York 1998). Often used as a means to enhance biodiversity (Mason 2007), continuous cover forestry is desirable in protected areas (Ní Dubháin *et al.* 2010). The system allows for the development of an understory to take place in stands of commercial conifers through the prescribed removal of single or small groups of trees (Ní Dubháin *et al.* 2010). Crown thinning is a method by which the transformation of a traditional single aged stand of conifer forestry can be converted to continuous cover by favouring an early selection of dominant and co-dominant trees and removing competing stems, and is best utilised in young stands of forestry (Mason and Kerr 2004). The conversion of stands of traditional forestry would allow the creation of gaps in the canopy, with variation in light levels across this gap (Ní Dubháin *et al.* 2010). This could potentially increase the diversity of lichens and bryophytes found within stands, promoting species associated with light conditions that would not normally be present in the lower light conditions associated with intact stands of conifers. The use of low impact silvicultural systems like continuous cover forestry as a method of reducing impact on *G. maculosus* where it occurs in the west of Ireland does, however, have a number of limitations. The higher cost (Kluender *et. al.* 1998) and increased labour intensity (Buongiorno 2001) mean that it is a less desirable option for foresters. As well as this, the conversion to continuous cover forestry is unsuitable in areas prone to windthrow. In addition, no study has to date been undertaken that examines the impacts of transforming traditional conifer stands into continuous cover on *G. maculosus*, and so the benefits to such a system are, as yet, unclear.

Limitations imposed on felling operations in commercial forestry known to contain *G. maculosus* may provide a further method for protecting the species. Restrictions on the timing of felling operations has been utilised in Ireland to mitigate impacts on other protected species. *Margaritifera margaritifera* (Freshwater pearl mussel) felling guidance restricts felling operations from November to March, as ground conditions tend to be wetter during this period, which can increase impacts on the species caused by run-off (Forest Service 2008). Restrictions to felling in *G. maculosus* should prevent felling during the periods in which *G. maculosus* is most active. The seasonal variation in *G. maculosus* catch in the study sites showed greatest catch success in autumn and spring for adults, and autumn and summer for juveniles. Therefore, restriction to felling during winter may reduce damage to *G. maculosus*. However, no study to date has examined if such a practice would reduce the level of impact, and confirmed that *G. maculosus* is indeed restricted to the base of the tree in unsuitable conditions. Furthermore, where overlaps occur between *G. maculosus* habitat and river catchments associated with *M. margaritifera* felling would then be restricted almost entirely for the entire year.

5.3. Recommendations

Based on the findings of this study recommendations are as follows:

Presence / absence surveys for *G. maculosus* should be carried out during autumn months when the species was observed to be most active. Initial hand searches should be carried out to confirm the presence of *G. maculosus* in all habitats. Where presence is not confirmed through hand searching, refuge traps should be placed at 1.5m on tree trunks in mature conifer stands, and on rock outcrops in peatland habitats to remain for at least two weeks before checking to confirm presence or absence. In clear-felled stands, a second hand search should instead be carried out to confirm initial assessment, taking care not to disturb bark and bryophytes if subsequent monitoring is to take place. Where monitoring is required in mature conifer stands nine refuge traps should be placed at 1.5m on separate trees in mature conifer stands. After an initial two weeks to allow slugs to locate traps, counts should be taken at regular intervals for five consecutive days and the mean catch per day calculated as a proxy for population density estimates.

Existing management approaches that may prove beneficial to the protection of *G. maculosus* within commercial forestry stands. based on the findings of this study a the following recommendations can be put forward as possible mitigation approaches, however, further studies are required. While all potential measures have limitations, a combination of uses may reduce this. If felling is to occur in stands where the presence of *G. maculosus* has been confirmed, operations could be restricted to winter months when slug activity is lowest due to colder temperatures, if further research confirms the restriction of *G. maculosus* to the tree base. Where these stands overlap with *M. margaritifera* river catchments, it is recommended that conversion to low impact continuous cover forestry take place through crown-felling as restrictions on clear-felling would therefore encompass the full year, greatly hampering traditional forestry management practices. Where no overlap is present with *M. margaritifera,* translocation of *G. maculosus* collected via refuge trap during the autumn months prior to felling in winter may further reduce the effects of clear-felling. The collected specimens should then be transferred to a nearby stand of conifers that is known to contain populations of *G. maculosus* but will not be impacted by impending harvesting activities. Following these measures it is recommended that a proportion of deadwood retained within the conifer stands be preserved to increase food sources for the remnant *G. maculosus* population.

5.4. Conclusions

G. maculosus is not restricted to a single food option, feeding on all species provided and showing plasticity in its food preferences, indicating that the species is a generalist herbivore of lichen and liverwort species. While refuge traps were most effective in mature plantation and peatland habitats, hand searches are more efficient for *G. maculosus* in previously clear-felled stands. Surveys to confirm presence of or to collect *G. maculosus* should be carried out between August and November when catch success is highest. Greatest numbers of *G. maculosus* were caught in mature conifer stands and previously clear-felled stands compared to peatland habitats, highlighting the importance of considering forestry habitats in the conservation of the species. The positive correlations with yield class and tree circumference indicates that *G. maclosus* has an association with higher quality, more productive stands of mature conifer stands. A significant, negative, impact was found through the BACIP study which examined the impacts of clear-felling. While only one site was felled within the study, the results showing significantly lower numbers of *G. maculosus* in the previously clear-felled stands when compared to mature conifer stands supports this. While a number of mitigation measures may be suitable for *G. maculosus* further research is urgently needed to assess these to ascertain the practicalities and impacts they may have for the adequate conservation of the species where it occurs in commercial forestry.

5.5. Opportunities for further research

While this study addressed important aspects of the conservation of *G. maculosus*, there are a number of limitations to this study that need to be considered, and areas where further research is required.

5.5.1. Assessment of food choice, feeding behaviour and general behaviour:

- While the results of the optimisation trials regarding starvation proved useful for this study, it is unclear whether these results would remain the same given longer time in the feeding trials. Further research is required to test this.
- A number of the potential food species that were assessed as food items by Reich *et al.* (2012) could not be found within the current study sites. Furthermore, there is a larger suite of food options, including these species and others such as fungi, available to *G. maculosus.* Further research should address these gaps and allow for a more in depth analysis of other food options available to *G. maculosus.*
- A large proportion of trials involved individuals that did not make contact with a food source. This caused a reduction in the numbers of replicates available and increased dramatically the numbers of trials required to determine preferences. Future researchers examining feeding in *G. maculosus* should run additional preliminary examinations of test subjects prior to the start of the trial to ascertain that the individual is likely to eat. This would allow for greater numbers of useful replicates to be run. Greater replicates may also help to reduce the variation found between individuals.
- Given the difficulties in differentiating adult and juvenile slugs in the field, future research that examines the relationship between slug size and the level of maturity is needed. This would provide a more precise reference point by which to differentiate the age stages and allow for more accurate assessment of differences in their behavior.
- Further research investigating feeding preferences with juveniles would

provide further insight into the ecology of the species at different stages. In addition, an assessment of the impacts of competition on food selection would provide for a more realistic interpretation of food choices made in the wild. While this study provided an assessment of movement parameters, given that this was conducted in a laboratory this probably is an over estimation of distances travelled by the species in the wild. Future research investigating the dispersal ability of *G. maculosus* should make use of tracking devices in the wild to establish an accurate estimate.

5.5.2. Improving guidance on trapping:

- The assessment of different trapping methods provided information on optimal trapping methods in three different habitats. However, while hand searches were carried out, these were not undertaken at night for health & safety reasons as recommended by current guidelines (NRA 2009). While *G. maculosus* is frequently active during daylight hours, further research should investigate refuge traps in comparison to night hand searches.
- While the investigation into different trapping methods was valuable, the study period was limited to four months long. Given the seasonal variation seen by *G. maculosus* over the longterm study, further research investigating the efficacy of hand searches over a longer period is required
- Although relationships between temperature and catch success could successfully be established due to placement of data loggers at each site, examination of relationships between other weather variables was limited due to the distances from weather stations. Future studies investigating seasonal variation and weather influences on the species should make use of accurate readings taken close to the study site to increase information on optimal trapping conditions.

5.5.3. Assessment of forestry management

- The investigation of the impacts of clear-felling showed, for the first time, a direct impact on *G. maculosus.* However, the conclusions that can be drawn from this study are limited due to only one stand being felled. Further BACIP studies should be carried out to establish the extent of this impact, and whether felling at different times of the year may reduce this impact.

- Further investigations should also be carried out to ascertain impacts of other forestry management practices including replanting and thinning on the species, along with the effects of regeneration of ground vegetation on populations in previously clear-felled stands. In addition, studies investigating presence in younger conifer stands should also be carried out to determine the point at which populations are negatively influenced by canopy cover given that Reich *et al.* (2012) did not find the species in such stands.
- Further studies examining population sizes in deciduous forests and comparing these to populations in coniferous plantations would provide valuable information on differences between natural forests and commercial forestry. If population sizes were similar across the two forest types this may provide an option for mitigation through the replanting of deciduous trees.
- Given the lack of studies investigating mitigation measures to reduce impacts of commercial forestry on the species, research examining these different practices is urgently required.

5.6. References

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