Is it all about the ants?

What are the factors influencing the presence of *Plebejus argus* (the Silver-studded Blue butterfly) on Studland Peninsula?

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Abstract

The nationally scarce butterfly, *Plebejus argus* (silver-studded blue) was identified as a priority species requiring special conservation measures within the UK Biodiversity Action Plan in 1994 and this status was retained by the UK Post 2010 Diversity Framework (2012). The species has undergone a major decline throughout most of its range in the UK and its small populations are particularly vulnerable to local extinction. Current research ascertains that early successional vegetation, with a high abundance of host plants and the presence of the symbiotic *Lasius* ant species, is vital for the longevity of *P. argus* colonies which are increasingly threatened by fragmented and isolated habitats.

This project forms an investigation into the spatial occurrence of the heathland species, *Plebejus a argus* across Studland Peninsula, a 350-hectare area of dune, lowland heath, salt marsh, mire and open water habitat on the south-eastern side of Poole Harbour. At present, records from Butterfly Conservation transects document the presence of *P. argus* at Plateau Heath, on the oldest part of the peninsula, but there is no evidence of populations on the eastern side of the peninsula, despite apparently similar terrestrial components. This study investigates the distribution of *P. argus* across the peninsula and assesses the factors driving this distribution pattern. Results show a significant, positive association between *P. argus* with the host plant *Erica tetralix* (cross-leaved heath), short grass (2-5 cm) and the presence of the black ant *Lasius niger*, the latter which is distributed primarily on the western side of the peninsula despite the availability of suitable habitat conditions to the east. This research investigates the distribution of six heathland ants found at Studland and considers whether interspecific competition between *Lasius niger* with the red wood ant, *Formica rufa*, could be influencing the distribution of this myrmecophilous butterfly.

1 Introduction

1.1 The Background to the project

1.1.1 The Cyril Diver Project

This study of the habitat requirements of *P. argus* at Studland was inspired and funded by the National Trust's Cyril Diver Project which ran from 2013-2015. In the 1930s, Captain Cyril Diver (first Director-General of the Nature Conservancy) carried out an extensive, detailed survey of South Haven Peninsula's diverse habitats and its associated floral and faunal communities alerting the scientific world to Studland's immense ecological value, particularly its rare habitats and species. Diver's meticulous records are considered to have been instrumental in protecting the area from the development which has taken place in many areas around Poole Harbour, just across the water. Today Studland Heath has many conservation designations reflecting the importance of the area. Designations include Studland and Godlingston Heath Site of Special Scientific Interest (SSSI), Poole Harbour SSSI, Dorset Heaths and Studland Dunes Special Area of Conservation (SAC) and Dorset Heaths RAMSAR and Special Protection Area (Peters et al. 2011).

In 2013, The National Trust set up The Cyril Diver Project to build on Diver's legacy by carrying out a new survey of the peninsula over 80 years later. The project has recruited over 90 volunteers from a variety of backgrounds (many within conservation) whose survey results have highlighted the ways in which Studland has changed since the 1930s. This research on *P. argus* was inspired by the discovery of Diver's original map (Figure 1) which illustrated the butterfly's 1930s distribution presenting a very different spatial occurrence from the transect records which have been collected annually by Butterfly Conservation on Studland Heath since 1976.

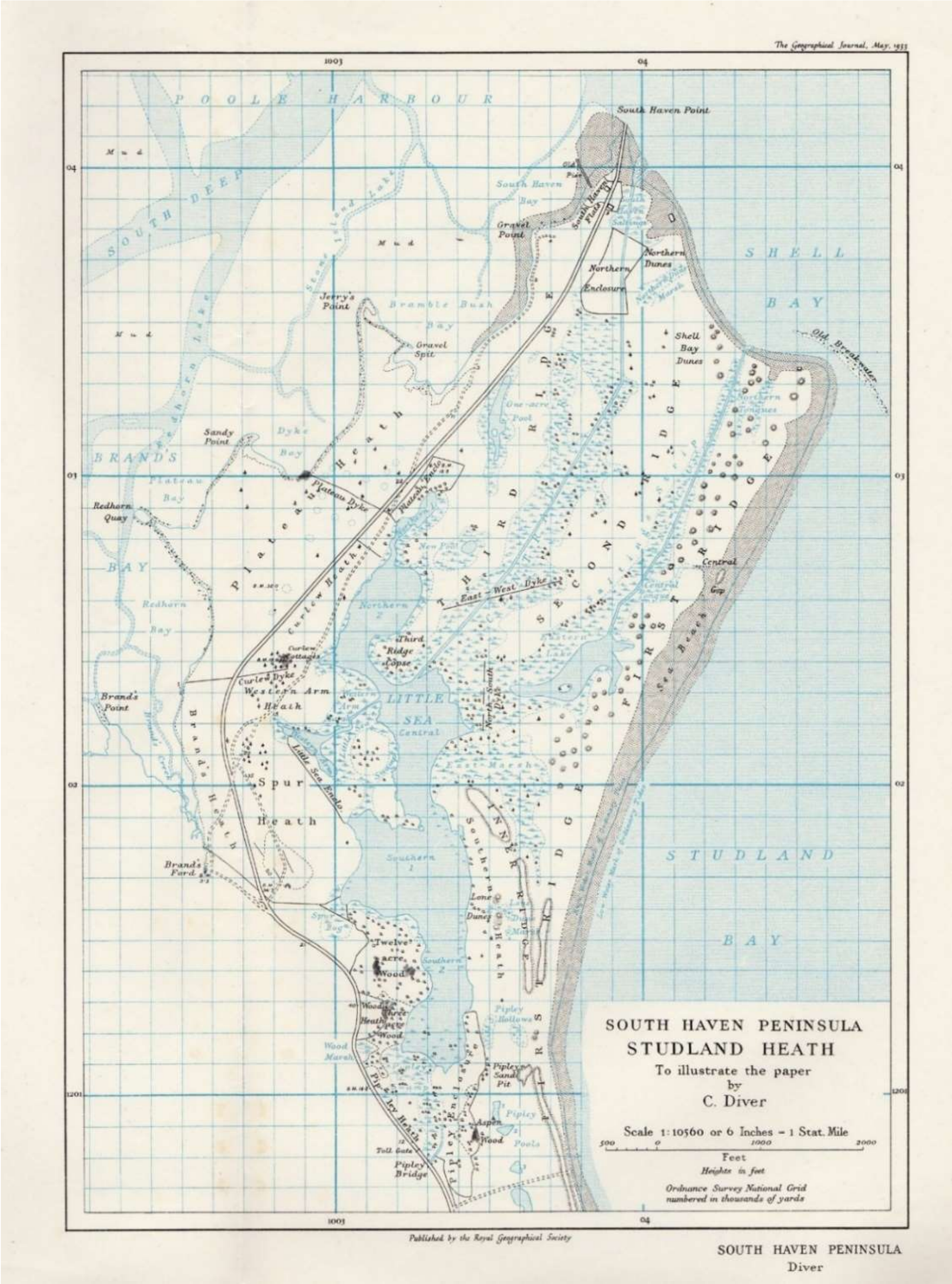

Figure 1 : The distribution of *P. argus* across Studland peninsula in the 1930s (Diver 1934)

Archive map from Dorset History Centre, Dorchester

1.1.2 The geology of Studland Peninsula

South Haven Peninsula (known locally as Studland Peninsula) in Dorset (OS grid reference SZ031851), a narrow, 3.6 km, low lying promontory on the southeast shore of Poole Harbour, was formed from clays, silt, sand and gravels initially laid down during the Tertiary period, 66 -2.5 million years ago. Over the past 400 years, the eastern boundary of the peninsula has extended rapidly due to sand accretion sourced from the carbonate-free sands of the Bagshot Beds of Bournemouth cliffs (Carey 1938, West 2015) which has been controlled by wave action and by the process of longshore drift. The progradation of the beach has resulted in the formation of four, parallel dune ridges, created roughly one hundred years apart and separated from one other by dune slacks, marsh land and scattered pools. The freshwater lake, Little Sea, which was cut off from the sea in the late C19th and is now an oligotrophic-mesotrophic lake (Edwards 2006), divides the dune ridges on the east from the Tertiary plateau to the west. While the main part of the dunes has seen extensive sand accretion, there has been some erosion at the southern extremity where land has been lost (West 2015). The low pH of the Bagshot source sand (Brown 2014, West 2015) and the minimal shell content (Carey 1938, Brown 2014) has resulted in an acidic dune system which has a direct impact on the vegetation and organisms which the peninsula supports. Much of the soil on the site has been described as deep, stoneless, acidic humose sandy soil, affected by groundwater, belonging to the Sollom 2 Association (Peters et al. 2011).

1.1.3 The habitats at Studland Heath

Studland Heath has particular value due to its areas of lowland heathland, a priority habitat recognised by the UK Biodiversity Action Plan initially in 1994 and by the UK Post 2010 Diversity Framework (2012). Lowland heathland, which is generally below 300 metres in the UK, has been defined as an open landscape with low-nutrient, impoverished, acidic and shallow soil dominated by *Ericaceous/Ulex* minor communities (JNCC 2003). It has been estimated that 80% of lowland heathland has been lost nationally in the last two hundred years (Price 2003) while 86% has been lost in south east Dorset alone since the 1800s, causing a consequent decline in species which are dependent upon this increasingly rare habitat.

Studland Heath is an important lowland heathland site on the South Haven Peninsula. The habitat includes areas of dry acidic heath, damp, humid and wet heath in addition to mires and scattered areas of open water. The dry heaths, primarily on the dune ridges formed over free-draining sandy soils have been recorded as a H2a *Calluna vulgaris – Ulex minor* sub-community (Edwards 2006). *C. vulgaris* dominates, forming extensive dense cover (especially on First and Second Ridge, Brown 2014) while *Erica cinerea* is also present with locally abundant bryophyte cover (Edwards 2006). In the transitional areas, where dry and humid heath graduate to wet heath, a H2c *C. vulgaris – Ulex minor* heath, *Molinia caerulea* sub-community has been recorded; *E. cinerea* gives way to *Erica tetralix* with locally frequent pockets of *M. caerulea* and a continual presence of *Agrostis curtisii* and *Ulex minor*. The wettest heathland areas on Godlingston Heath and Plateau Heath, have been recorded as a M16a *E. tetralix – Sphagnum compactum* typical sub community with dominance by *E. tetralix* and frequent cover of *M. caerulea* and *C. vulgaris*. Bryophytes are locally abundant and frequent cover of *Trichophorum cespitosum*, *S. compactum* and *S. tenellum* are found (Edwards 2006). Figure 2 shows the diverse range of habitats found on the peninsula.

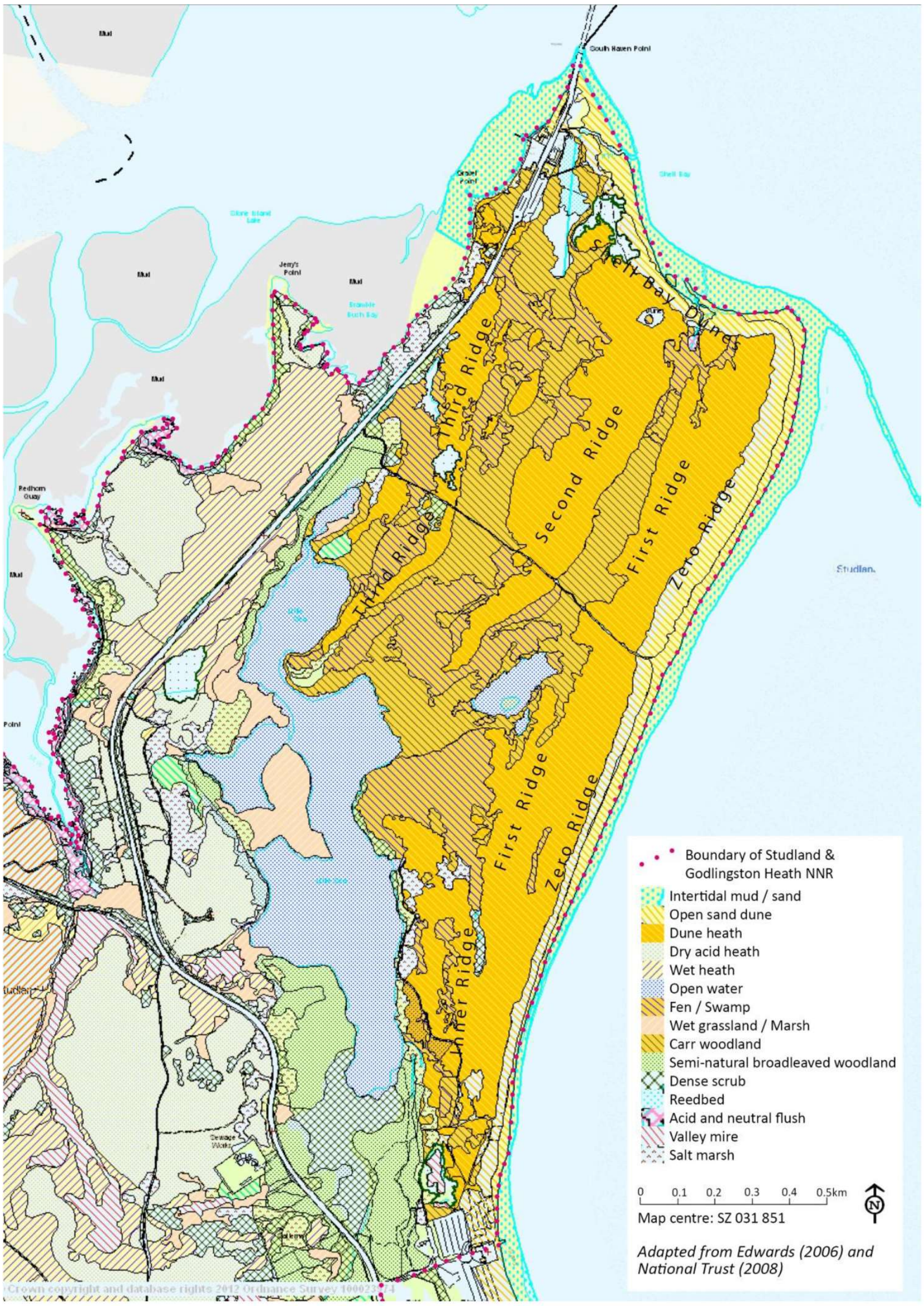

Figure 2 : The range of habitats on Studland Peninsula (National Trust 2015)

1.1.4 **Studland Heath and *Plebejus argus* L. (Lepidoptera: Lycaenidae)**

Diver's maps and notes show the presence of *Plebejus argus* (silver-studded blue butterfly) on the peninsula in the 1930s and Butterfly Conservation transect records show a continual presence at Studland Heath since 1976 (Fox et al. 2015). The Genus

Plebejus, within the Lycaenidae family (subfamily Polyommatinae), is considered to have four subspecies (Ravenscroft & Warren 1996) namely *P. argus (*Linnaeus 1758*),* the most widespread lowland species; *P. argus cretaceus* (Tutt 1909) found in the south of England; *P. argus masseyi* (Tutt 1909) recorded in N.W. England and *P. argus caernensis* (Thompson 1937) found in Wales (Emmet & Heath 1990). This classification is not, however, accepted by all lepidopterists in the UK (Thomas 1983 cited by Emmet & Heath 1990) but it is the premise used in this research as it is in keeping with current British usage (Emmet & Heath 1990). It is the lowland subspecies *P.a.argus* which is currently found at Studland.

P. argus has been identified as a nationally scarce butterfly in the UK requiring special conservation measures within the UK Biodiversity Action Plan (1994) retained by the UK Post 2010 Biodiversity Framework. During the C20th, *P. argus* declined by up to 80% in many areas in the UK (Ravenscroft & Warren 1996) and is now believed to be extinct in Scotland and Northern England with rare sightings in central and eastern England. Abundant populations in high densities are still widespread in heathland areas in Hampshire, Dorset and Wales (Thomas 1985, Lewis et al. 1997) however, and over the last ten years, a 19% increase in occurrence has been shown nationwide (Fox et al. 2015) possibly due to the conservation management of heathland areas since the formation of the initial Biodiversity Action Plan (1994). The next section outlines the main threats to *P. argus* and sets out the research question and objectives underpinning this investigation.

2 The aims of the research and research questions

2.1 The threats to *P. argus* population persistence

The main threats to the long-term persistence of *P. argus* populations in the UK have been identified as primarily the conversion of heathland for agricultural, forestry or commercial use and the loss of early successional habitat through the demise of traditional heathland management techniques (Asher at al. 2001, de Whalley et al. 2006, Fox et al. 2015). It is estimated that over 60% of lowland heath in the UK has been lost (de Whalley et al. 2006) through conversion in the last century and that this has been exacerbated by the neglect of existing heathland as traditional practices such as livestock grazing, turf and furze burning and cutting have ceased allowing succession to the climax community to occur.

In addition, the population structure of *P. argus* renders it particularly vulnerable to extinction due to both stochastic and deterministic processes (Seymour et al. 2003). The species lives in high densities in small populations on, often scant patches of land. As *P. argus* use only early successional vegetation, there is a constant need to colonise new areas of suitable habitat (Thomas 1985). While occasional dispersal occurs between small, local populations allowing genetic flow and recolonization of extant colonies, generally the species is highly sedentary rarely flying more than ten metres during the imago stage (Emmet & Heath 1990). Although a few colony members have been noted, through mark and recapture schemes, to fly up to 1.5 km to a new site, this is considered rare (Asher et al. 2001). This weak dispersal capability means that *P. argus* requires either a large area of suitable habitat or a small, closely-linked network of areas if the delicate balance between local extinctions and re-colonisations is to be maintained (Brookes et al. 1997, Thomas 1998). If suitable habitat becomes fragmented, the species becomes confined in small isolated clusters where dispersal cannot occur leading to local extinction and the loss of genetic flow. Brookes et al. (1997) suggest that this can then lead to loss of fitness for remnant populations as genetic variation is reduced endangering the viability of the species in the long-term.

2.2 Gaps in Current Research on the habitat requirements of *P. argus*

There are many management strategies in place across British nature reserves which aim to protect threatened *P. argus* populations. The suspension of vegetation succession to a climax community has been achieved through the creation of disturbance in the form of livestock grazing and vegetation burning while the removal of trees and shrubs, which are encroaching onto open heathland areas, ensures that early successional stages are constantly available for the butterfly.

While there has been considerable study of the vegetation requirements of *P. argus* populations in both heathland and calcareous areas, there has been less consideration of the myrmecophilous nature of the butterfly and how this might affect habitat requirements. *P. argus* has a symbiotic, highly evolved, relationship with *Lasius* spp. (Thomas 2007) and several investigations have shown that *P. argus* will deposit ovum only in areas where *Lasius* spp. pheromones can be detected (Thomas 1985, Seymour et al. 2003, Dennis & Sparks 2006). Several studies claim that obligate myrmecophilous lycaenids, like *P. argus*, are completely dependent on their association with ants for survival and that the presence of host ant nests is a key factor in butterfly distribution (Mouquet et al. 2005 cited by Fiedler 2006). This investigation aims to look at this gap in current research. The National Trust's ecological records of Studland's biodiversity in the 1930s have enabled a comparison to be made, not only of the distribution of *P. argus* on Studland Heath since Diver's records but also of ant distribution on the peninsula. Ant community dynamics are likely to change as succession moves towards a climax community and this could potentially have an impact on the distribution of *P. argus* on the peninsula.

2.3 Aim and research questions

The aim of the project is to investigate the current distribution of the heathland subspecies, *Plebejus a. argus* (referred to as *P. argus* in this research) across Studland Peninsula. Current records (The Cyril Diver Project 2013-5, Living Record 2014) indicate that there are populations at the southern end of Second Ridge and on Plateau Heath but there is no evidence of *P. argus* habitation on the eastern side of the peninsula, despite apparently similar terrestrial components. This project seeks to establish the current distribution and abundance of *P. argus* across the peninsula and will identify possible reasons for absence of the species in areas which are presently unpopulated. Research on ant distributions on Studland's dune/heathland ridges, carried out by The Cyril Diver Project, has indicated that the distribution of *Lasius* spp. appears to have moved in a northerly/western direction since Diver's research in the 1930s. This has possibly occurred because of the eastward colonisation of the ridges by *Formica rufa* (red wood ant) which has extended its range as the dunes have succumbed to vegetation succession. It is possible that the contraction in the distribution of *Lasius* spp. has had an impact on the range of *P. argus* populations but other factors, such as changes in substrate composition and in habitat components (possibly through the intensification of non-livestock grazing) could also be relevant. It is hoped that results from the project will be beneficial to the conservation of *P. argus* by revealing the key habitat requirements for the heath dwelling butterfly and that this will influence future management strategies on the peninsula.

The research seeks to answer the following questions:

1) What is the current distribution of *P. argus* across the peninsula?
2) What factors are driving *P. argus* distribution?
3) Has there been a change in distribution since Diver's records in the 1930s?

To address the three research questions, several actions were carried out. A six week survey (June 10th - July 29th 2015) of *P. argus* abundance was carried out on the peninsula to ascertain the current distribution of the butterfly. The factors driving this distribution were determined by the cross-referencing of *P. argus* numbers with results from vegetation surveys and soil analysis taken at 40 sample points across Studland and, as *P. argus* is a *myrmecophilous* butterfly, records of ant species and abundance were also taken for each sample point. To determine whether there has been a change in distribution, Diver's original map of *P. argus* distribution in the 1930s was compared with the distribution of the butterfly found in this research. Although these two distribution maps depict two snapshots in time of *P. argus* spatial occurrence and do not show how the butterfly fared in the period between Diver's map and 2015, it is possible to see that the distribution has changed allowing for discussion of possible reasons for this occurrence. The methods influencing these actions are explained in more detail in the Methodology (section 4).

Before the results of the survey and the discussion of the findings are presented, the project includes an extensive literature review of the ecology of both *P. argus* and of the six ant species found during this research. This ecological review is pertinent to the study as *P. argus* goes through several, distinct life stages before adulthood which have different resource requirements and because *P. argus* has a symbiotic relationship with *Lasius* ants whose distribution could be driving the butterfly's spatial occurrence. *Lasius* ants also go through several transitions before adulthood hence any consideration of their distribution, must be underpinned by an understanding of how their life stages may influence their resource requirements.

Following the Results and Discussion sections, the Conclusion looks at management initiatives designed to protect heathland areas and considers strategies which could be employed at Studland to enhance *P. argus* survival in the future.

3 Literature Review: The ecology of *P. argus* and heathland ants

The literature review in this section gives a detailed account of the life history of *P. argus* and describes the butterfly's symbiotic relationship with *Lasius* ants. The ecology of the six heathland ant species found at Studland is also explored as an understanding of each species is relevant when assessing species' habitat requirements and community dynamics in the discussion in section 6.

3.1 The morphology of *P. argus*

P. argus is a sexually dimorphic, univoltine butterfly which flies between late June and early August (Ravenscroft and Warren 1996). The male imago (see Figure 3) has a wingspan of 26-32 mm (Emmet & Heath 1990) - with an average span of 29 mm (Thomas 2007) - and can be easily distinguished from other Lycaenidae by its deep lavender blue upperwings which are bordered by a thick black margin fringed by white cilia (wing fringe). The terminal section of the wing is dark brown extending slightly inwards along the central veins (Emmet & Heath 1990). The underside hindwings are light grey/silver with a bluish tinge at the base and a wide orange band near the edge bordered outwards by black eyespots encircling a distinctive, bright blue/green pupil or stud (Thomas 2007). A series of small brown spots and irregular crescents can be seen on the inside of the orange border next to an inner sequence of white-ringed dark spots closer to the body. The forewing underside can be differentiated from other blues as no spots can be seen closer to the grey-blue body, than the centre of the wing (Thomas 2007). The antenna terminates in a brown club and is ringed in black and white (Emmet & Heath 1990). See Appendix 9.3 for morphological diagram.

Figure 3 : *P. argus* male imago on Studland Heath (Munns 2015)

The female imago (Figure 4) which has an average wingspan of 31 mm (Thomas 2007), can be identified by the iridescent brown of her upper side wings which are tinged with a blue basal flush. A row of crescent-shaped orange lunules can usually be seen on both wings and the cilia is white (Emmet & Heath 1990). The underwings mirror the orange band and studded eye spots shown in the male but on a dark brown ground colour. The female can be differentiated from other butterflies with similar colouring by two horizontal black spots at the top edge of the hindwing and by the size of her black spots which are larger than those found in female blues of other species (Thomas 2007).

Figure 4 : *P. argus* female imago

(left: N.Hulme, Iping Common June 2013; right: I.Leach, Prees Heath July 2015)

3.1.1 **The life history of *P. argus***

3.1.1.1 Imagines

The flying period for imagines is reportedly from early July to early September (Emmet & Heath 1990, Asher et al. 2001) although in this research, the first adult males were seen at Plateau Heath on June 13th 2015. Males appear first and may try to establish small territories before endeavouring to mate with newly emerged females. While the butterfly is a sedentary species (Thomas 2007) rarely flying more than ten metres (Emmet & Heath 1990) during its adult lifetime, both sexes will fly low, in warm conditions, over host food plants and vegetation with a swift, rather erratic wingbeat. Courtship takes place as soon as the female emerges and mating occurs, with closed wings, on low vegetation.

3.1.1.2 Eggs, Larvae and Pupae

Small, spherical white ova (0.3 mm tall and 0.6 mm wide) with a concave pinnacle (Emmet & Heath 1990) and tiny spikes extending from the centre (Thomas 2007) are generally laid singly close to the ground in midsummer. They are deposited on leaf litter, bare soil, twigs or the stems of food plants notably *Ericaceous* spp. and *Ulex* spp.in heathland locations (Ravenscroft and Warren 1996) where host plants fringe pockets of bare ground. The warm microclimate created by bare ground open to the sun is thought to enhance larval development (Ravenscroft and Warren 1990) giving the offspring a better chance of surviving the winter hibernation period. The small indentation (micropyle) at the centre of a strong outer shell has a thinner wall which allows gas exchange to take place (Thomas 2007) while the fluid inside the egg nourishes the growing embryo.

After overwintering in the egg stage, the larvae hatch in the spring where they feed on the buds, flowers and young shoots of host plants. The presence of strong jaws allows them to feed voraciously before reaching their full size at approximately 13 mm long (Emmet & Heath 1990). They are well-camouflaged amongst their host plants possessing black heads and green/brown onisciform bodies with a white-edged dark dorsal stripe and thick skins which protect their vital organs from insect bites (Baylis and Pierce 1993). Larvae go through four instars in total. In instar two, a dorsal gland (called the Newcomer's gland) develops on segment seven (Thomas 2007), which, along with small pore cupola organs, produce secretions which are desirous to ants – specifically of the *Lasius* genus in heathland sites (see Section 3.1.2).

Larvae go through several moults before pupation occurs in June when the body of the imago is formed within the pupa. As an obligate myrmecophilous butterfly (Fiedler 1996), the relationship between *P. argus* larvae and ants is particularly important. When larvae are fully grown, they will construct a silk cocoon at the bottom of a hole which can be up to 70 mm deep (Emmet & Heath 1990). Within this cocoon, larvae will develop into the pupal stage where the body will darken to a deeper brownish green. Larvae are dependent on the symbiotic relationship they have with ants as the later bury pupae/fully grown larvae underground where they tend them (Thomas 2007). Pupal cells at ground level may be constructed, pupae may be carried into ants' nests or a temporary nest may be established around the buried chrysalis. Pupation is thought to last for approximately three weeks before emergence of the adult imago. On emergence, Thomas 2007 states that it is common for imagines to be smothered in ants for a short period while their wings harden.

3.1.2 **The symbiotic relationship between *P. argus* larvae/pupae with *Lasius* spp.**

There is a considerable amount of research to show that *P. argus* has a symbiotic, highly evolved, relationship with *Lasius* spp. and, on heathland, with *Lasius niger* and *Lasius alienus* in particular (Thomas 2007). Several studies have shown that *P. argus* will lay her eggs only in areas where *Lasius* spp. pheromones can be detected (Thomas 1985, Asher et al. 2001, Seymour et al. 2003, Dennis & Sparks 2006) while Fiedler (2006) states that obligate myrmecophilous lycaenids are completely dependent on their association with ants for survival and that the presence of host ant nests is a key factor in butterfly distribution (Mouquet et al 2005 cited by Fiedler 2006). The relationship is mutualistic; while *P. argus* benefits from the protection it gains from ants from predation and parasitic attack (Pierce et al. 2002, cited by Fielder 2006) the ant, in return, has access to the sugar-rich liquid and amino acids secreted from the butterfly larvae's glands (Asher at al. 2001, Seymour et al. 2003) which Thomas (2007) claims is produced in large quantities hence a major food source for ant colonies increasing ant fitness (Dennis and Sparks 2006). Research by Jordano and Thomas (1992) discuss the use of pore cupola organs on the larvae's body which secrete sugary liquid while the nectary Newcomer's organ develops in the larvae's second instar secretes amino acids and sugar. Baylis and Pierce (1993) point out that amino acids are costly for larvae to produce as they transfer energy away from growth to defense strategies; secretions therefore take place only when the mutualistic ant is in attendance. Several studies have examined the tentacular organs (or tubercules) on the larvae's eighth abdominal segment which emit chemicals attractive to ants (Jordano & Thomas 1992); it has been suggested that these chemicals stimulate the host ants into frantic activity designed to lead them to larval nectary glands (Emmet and Heath 1990), and that these chemicals mimic the alarm calls of the host ant again inciting activity (Baylis and Pierce 1993).

While larvae may be tended by ants within or outside of *Lasius'* nests, larvae which are taken into ants' nests may benefit from better protection from heathland fires than those tended on ground level cells (Emmet and Heath 1990).

3.2 Ant Ecology

Ants are a common and abundant terrestrial insect which inhabit a diverse range of habitats. While there are approximately 50 different species of ant within Britain (Barnard 2011), the species found in this research, from the *Lasius*, *Myrmica* and *Formica* genera, are six of the most widespread species inhabiting heathland in the south of England. Section 3.2.1 provides a general summary of the lifecycle and behaviour of ants while section 3.3 looks at the ecology of the six species found in this research.

3.2.1 Physical Structure and Life cycle of Ants

Although there is some variation in the physical composition of ants, they have many common morphological characteristics and go through the same lifecycle stages. Like many other Hymenoptera, ant colony organisation is based upon a caste system determined by haploid or diploid egg production. Fertilised, diploid eggs produce two types of female: queen ants, who mate, are often winged, lay eggs and go on to found new colonies and worker ants who are smaller, wingless and spend time foraging outside of the nest and tending the brood (Skinner & Allen 1996). Male ants who develop from unfertilised haploid eggs, are generally winged and needed primarily for reproduction although they can also be used as a food source (Brian 1977). The reproductive ants (termed the alate ants or sexuals) will undertake a synchronised nuptial flight, cued by humidity or temperature (antARK 2017a), away from their nests to mate with sexuals from different colonies to avoid genetic interbreeding.

Once a queen has been fertilised, she will usually establish a new colony in an unestablished location (Radchenko & Elmes 2010). The sperm transferred to her from the male sexual will be stored and fertilise thousands of eggs over the course of her lifetime (Brian 1977, Skinner & Allen 1996). On alighting in a new area, the queen's wings, if she has them, will break away and she will dig a nest cell in her selected habitat where her eggs are laid. Egg development leads to an initial larval stage where legless, eyeless and initially hairless larvae develop. The larvae grow, going through several moults before developing hooked hairs by the third moult which allow the mass to be interlocked and moved more easily. The larvae feed by piercing neighbouring eggs in addition to the absorption of liquid food offered by worker ants. After several moults, the larvae will stop eating and eject the contents of the gut before developing into a pupal phase (Brian 1977). The larval skin is cast off and an elongated pupal skin forms which hardens to form the pupa. During pupation, the pale adult (imago) is formed from the adult buds inside the larvae and emergence from the cocoon is generally followed by a subsequent darkening of colour announcing the arrival of a new adult in the colony. In Formicine ants a cocoon is created as larval feeding ceases and metamorphosis takes place within the cocoon. Brian (1977) points out that initially, small female workers develop as food supplies are limited at this point in the cycle. The new workers tend the remaining brood and forage outside of the nest enabling larger, winged females to develop as food is more abundant. Males develop in the latter stages and the colony is then considered to be mature.

3.2.2 **Temperature**

Seasonal temperature is important in the ant life cycle as queens need an ambient temperature of at least 10°C (Brian 1977) to enable sperm to travel through the queen’s oviduct and fertilize the eggs which are generally laid between April and May. After winter hibernation, all colony members gather in the warm soil at the top of the nest during spring before bringing up the overwintered brood. Warm temperatures are needed to enable larval growth (North 1998) which may be suppressed if temperatures are unseasonal. Brian points out that workers and queens also need warmth to restore glandular activity and to allow sexual organs to develop. Research by Haatanen et al. (2015) in Turku, Finland on *L. niger* colonies suggests that overwintering is an expensive energy-depleting period for all ants and can be particularly costly in very low temperatures where more body fat is needed by founding queens to enable survival. Warmth is therefore needed to enable worker ants to become active outside of their nests once more.

3.2.3 **Nesting and its impact on soil components**

Ants create many different types of nest structure ranging from subterranean galleries with linking tunnels and chambers to soil and vegetation mounds. All nests are, however, constructed for the same purpose which is to provide shelter and protection from enemies; to maintain a beneficial microclimate to aid brood development (Rees 2006) and to store food (Brian 1977). Nesting has a significant impact on the soil components in the nesting zone and nearby. Ants have been described as key soil engineers (Cammeraat and Risch 2008) as nest construction changes the physical, biological and chemical structure of soil. The building of underground tunnels, shafts and chambers disturbs soil layers increasing soil porosity and potentially causing soil particles to separate (Frouz and Jilková 2008) and can consequently have an impact on hydraulic processes in the earth (Jouquet et al. 2006). Bioturbation can occur as soils from different horizons and sources are mixed together as ants excavate soil, not only from surrounding areas but from deep layers within the ground which are then brought up to surface layers. Nest-affected soil will show an increased nutrient content (especially in phosphorous and nitrogen) as food and excreta accumulate in the nest and this will have an impact on microbial activity and decomposition rates (Steila and Pond 1989). Chemical changes can also occur as the increase in nutrient levels can increase pH soil levels leading to a more neutral reading (Frouz and Jilková 2008).

3.2.4 **Foraging Behaviour**

Worker ants use a variety of environmental and chemical cues to navigate between the nest and food sources. Many ant species, especially underground foragers like *L. alienus*, leave chemical trails which can be detected through the olfactory antennae organs, to enable other workers to locate resources. Surface trackways, which are generally established in spring after hibernation, are also used by many species including *L. niger* and *F. rufa,* to provide directional cues to others especially at the beginning of spring before a worker's individual memory of food location is activated (Skinner & Allen 1996). This situation may be reversed however as the season moves on. A study by Grüter et al. (2011), looked at the foraging behaviour of *L. niger* workers comparing their use of pheromone trails laid by other workers and by their own memory of food source location. Research from eight colonies, taken from the campus grounds at the University of Sussex, found that when the colonies were kept in artificial foraging boxes, workers relied more heavily upon memory/private navigational information of food location than pheromone trails when there was a conflict between the two foraging methods. The research claimed that site fidelity, allowing repeated visits to a food source, could be remembered by the workers for many months.

Light patterning, which illuminate the shape of trees and vegetation against the sky, is also thought to provide a visual cue for surface foragers, however this is considered the least efficient foraging method as the sun's position changes constantly and is consequently of short-term benefit.

3.2.5 **Dietary Requirements and Honeydew**

Ants are an omnivorous species with a varied diet based on nectar, soft fruits, small invertebrates, seeds and aphid honeydew. Nectar, fruits and plant phloem sap in honeydew are rich in sucrose, fructose and glucose providing energy while the consumption of invertebrates provides amino acids and protein for larval growth. Honeydew is particularly important to foraging ants as it is more than just a plant exudate having passed through the body of the aphid which adds excretory products. This results in a product composed of melezitose, vitamins, amino acids and amides providing many of the components needed for growth (Brian 1977). Formicine ants particularly tend and endeavour to protect aphid colonies to harvest their excretory products. A symbiotic relationship between ants and aphids can be said to have developed; Brian's work at Hartland Moor Nature Reserve (1977) refers to the removal of the eggs of aphid predators by *L. niger* workers while *F. rufa* has been noted to repel parasitic insects which use aphids as hosts (Parmentier et al. 2015). Studies have shown the benefits obtained by *Aphis fabae* (blackbean aphid) who will actively incite the attention of *L. niger* workers by frantic leg waving as the ants' harvesting prevents cast skin adhesion to tree branches and prevents the growth of detrimental mould (Skinner & Allen 1996). *Aphis sambuci* have been found to produce more honeydew when tended by *F. rufa* and some aphids notably *Forda formicaria* are only found where ants are present (Skinner & Allen 1996).

The extra-floral nectaries from bracken fronds are another important component within the diet of *Lasius* ants who will excavate scrapes at the bottom of new fronds to ambush competitors foraging at the same plant. *Calluna vulgaris* is another key plant for many ant species as its nectar contains some protein in addition to sugars. Seed consumption, which provides starch, has also been noted by heathland ant communities especially by *Tetramorium caespitum* which stores seed piles below ground for grub consumption after hibernation. Brian (1977) refers also *to L. alienus* ingestion of the caruncle of *Ulex minor* (dwarf gorse) seeds while *L. niger* has been noted to eat the stalk and caruncle of primula seeds.

3.3 The Ecology of Individual Species

There are, however, some differences between the ant species found in this research which are pertinent to the habitat requirements of *P. argus*. Sections 3.3.1 – 3.3.5 highlight some of these key differences.

3.3.1 ***Lasius niger (Linnaeus 1758)***

The formicine black ant, *L. niger*, is widespread within Europe (Thiel and Köhler 2016) and can be found within a diverse range of habitats including urban parks, heathlands, roadside verges, gardens and grasslands (Brian 1977, BWARS 2017). The species is small (workers < 7mm in length), dark matt in colour with a single waist segment and can be identified, with the use of a microscope, by the short, soft, standing hairs found on the antenna and hind tibia and by the dense pubescence seen on the clypeus (Royal Entomological Society 1975). It can be distinguished from other dark coloured ants by the relatively short legs and segments 2-5 of the funiculus which are shorter in length than the total of the other funicular segments (see Appendix 9.4 for morphological diagram). Although *L. niger* forages both above and below ground, the eyes are not prominent and ocelli are small and undeveloped.

Open areas with patchy plant cover are typically chosen for nesting (Haatanen et al. 2015). While *L. niger* will occasionally build soil mound nests (Fowles and Hurford 1998) and have been found to inhabit disused *Formica candida* cone nests in boggy heathland terrain (Rees 2006), nesting sites are primarily located under flat stones in moist soil where a series of underground tunnels, close to the surface, are constructed in the nesting vicinity (Brian 1977). Stone crevice nests have the benefit of providing protection against trampling while simultaneously absorbing radiation and creating an equable microclimate. Tunnels are frequently multi-branched and will be covered in a soil ‘canopy’ if they break through the soil’s surface.

L. niger is generally reported to be a monogynous species (Brian 1977, Haatanen et al. 2015) although research by Sommer and Hölldobler (1995) conducted under laboratory conditions, found that after the nuptial flight, several queens may found a new colony together increasing the production of workers within a shorter period than monogyny foundation would allow. Queens have a life expectancy of 20-30 years while workers are reported to live for just one to two years (Haatanen et al. 2015). Colonies are reported to be large, compared with other UK species, typically having at least 10,000 members (Sommer & Hölldobler 1995).

L. niger has the most northern UK range and is reported to favour cooler, wetter habitats (average 15°C in June, Brian 1977) than the other five ant species found at Studland. Research on Hartland Moor National Nature Reserve, Dorset (Brian 1977) considered the factors influencing a queen's selection of a new colony site on southern heathland. Brian reported that while *L. alienus* was found to nest on high, dry heathland dominated by *Agrostis setacea*, *L. niger* colonised wetter, more densely vegetated areas where *Molinia caerulea* was most abundant in conjunction with *E. tetralix.* Brian's (1977) ant traps set up during the nuptial flying season (July- August) on the southern heath discovered that *L. niger* queens consistently chose the coolest parts of the heath to alight; an occurrence which was replicated under artificial gradient conditions. This could be a response to vegetation requirements, as *L. niger* will tend coccids found on moisture-loving *Molinia caerulea* grasses, collect nectar from Ericaceae spp. as well as forage for aphid honeydew on birch/gorse vegetation. It could also be a response to interspecific competition; *L. niger* is better adapted to wet conditions than the other five species found, as the hirsute nature of its body enables it to trap air more successfully enabling it to live in a less competitive area. Brian (1977) acknowledges however that results from the nuptial flying traps could have been influenced by predation as *L. niger* queens alighting in drier areas, colonised by *L. alienus* or *T. caespitum*, could have been predated before capture in the research.

3.3.2 ***Lasius alienus***

The formicine brown ant, *L. alienus,* is also widespread within the UK but has a more southerly range than *L. niger* and is rarely found north of the Midlands. Brian (1977) defines *L. alienus* as primarily a heathland species preferring the warmer, dry heath where nest construction takes place in shallow, subsurface soil where a series of galleries and shafts are constructed. Foraging takes place underground, especially in the presence of interspecific competition from *L. niger* who are potentially able to suppress the territory range of smaller, competing species (Sommer & Hölldobler 1995). Thiel and Köhler (2016) consider interspecific competition to be a main structuring dynamic in local communities, such as heathland sites, where strategies such as aggression and avoidance are routinely employed. *L. alienus* can be distinguished from *L. niger* by its slightly small size (<6mm) and by the absence of standing hairs on the scape of the antenna and hind tibia (Skinner and Allen 1996). While *L. alienus* is weaker and less aggressive than *L. niger*, it also uses formic acid ejection as a defence system and is reportedly more agile with better group organisation and cooperation systems than its competitor *L. niger* (Brian 1977).

3.3.3 ***Formica rufa* (Linnaeus 1761)**

The Formicinae ant, *F. rufa* (southern or red wood ant) is a woodland species found in England and Wales predominantly in the south although their range in the C21st has been recorded as far north as Cumbria (BWARS 2017). The species is characterised by large, long-legged workers (over 7mm), a shiny frontal area with commonly a dark head and abdomen with a contrasting red/orange thorax (Skinner & Allen 1996). In addition to size, the *Formica* genus can be differentiated from genus *Lasius*, by the elongated spiracle on the propodeum, the short maxillary palp and the distinctive double row of bristles on the underside of the hind tibia (Royal Entomological Society 1975).

F. rufa construct large (1m+) deep-layered, mound nests from vegetation debris in sunlit woodland glades and shape the nest dome to intersect with rays of infra-red radiation to maximise warmth. The size of the mounds provides a stable nest while the diverse range of organic and inorganic materials used in its construction, create many microhabitats which are beneficial to a range of myrmecophilic organisms including Coleoptera, Hemiptera and Aranae (Parmentier et al. 2014). *F. rufa* can regulate nest temperatures during the summer season and inner nest chambers have been found to be many degrees warmer than the ambient air temperature due to the ants' own body temperatures and possibly to the decomposition of vegetation within the nest (Sorvari et al. 2016).

F. rufa nests have been found in coniferous, mixed and deciduous woodland but can be located in scrubby heathland and open forest rides (BWARS 2016). Nests can be individual or part of a linked colony and generally have several queens and contain up to 400,000 workers. New colonies are created when colonies divide and a newly fertilised queen will found a new nest often by social parasitism of a more submissive *Formica* species (Brian 1977).

3.3.4 ***Formica fusca* (Linnaeus 1758)**

F. fusca has a wide distribution across southern Britain and the Midlands with local distributions found in northern England, Ireland, the Channel Islands, Lundy and the Isle of Man (BWARS 2017). The species can be recognised by its uniform matt black colour and relatively large size with many workers reaching up to 6-7mm. The species lack the long femora hairs found in other *Formica* species (such as *F.lemani*) and have few hairs on top of the pronotom (Skinner & Allen 1996).

F. fusca can be found in a range of habitats including heathland, moorland and open woodland where it creates vertically chambered nests under stones, tree bark or within the soil (Brian 1977). In Brian's study of Hartland Moor Nature Reserve (1977) *F. fusca* nests were found alongside those of *T. caespituum*, in intermediate areas between the dry heathland favoured by *L. alienus* and the wetter heath favoured by *L. niger*.

F. fusca is a polygene ant with many queens inhabiting a colony. Sexuals appear between June and August and disperse sporadically rather than through synchronisation while mating takes place on the ground before the fertilised queen founds a new nest. *F. fusca* nests are particularly subject to invasion from *F. rufa* queens seeking to found a new nest who may be brought in unwittingly by *F. fusca* workers (Helantera et al. 2014). The invading queen will kill the host queen and the host workers will tend parasitic, *F. rufa* brood. Research by Helantera et al. (2014) found that *F. fusca* workers, in laboratory experiments, seemed to be able to discriminate between the eggs of nest mates and non-nest mates. The research suggests that while parasitic *Formica* queens, seeking to invade the nest, lay eggs which do not have the cues used by workers to ascertain egg provenance, the parasitic eggs are still often accepted by host workers. The research suggests that this may only occur where a parasitic queen has some similar cues to the host ant or has taken on (or may be mimicking) a similar chemical profile to nest members by being in the nest environment.

3.3.5 **Myrmicinae**

Myrmicinae are easily identified by their double-waist segment consisting of the petiole and postpetiole (Skinner & Allen 1996). The species are generally small (<7mm), a uniform red/brown in colour and have 12 antennal segments with no outgrowth or flange separating the antennal scape from the funiculus. Myrmicinae mandibles have teeth while the propodeum has a set of spines and the petiole upper surface is held at a sharp angle to the hind face (Royal Entomological Society 1975). Myrmicinae are equipped with a sting for defence, rather than the formic acid used in Formicinae ants; the double-waisted segment in the body allows great flexibility enabling the ant to bring the gaster up under the thorax until the tip and jaws meet which enables the ant to sting the opponent while simultaneously holding it with its jaws.

3.3.5.1 Myrmica scabrinodis (Nylander 1846)

M. scabrinodis is a widespread and common ant in the UK which inhabits a range of habitats including bogs and moorland, grasslands, forests and open woodland (Radchenko & Elmes 2010). Nests can be built in the soil or under bark and in very boggy heathland areas, can be established in dense *Sphagnum* clusters especially where they rise above ground water levels (Boyce 2003). *M. scabrinodis* also build solaria, constructed from vegetation and chewed earth fragments to incubate their brood during the summer months (Boyce 2003). Brian (1977) suggests that *M. scabrinodis* can potentially live in hotter, more arid habitats than other *Myrmica* species as their nest construction features thick, mud-filled walls which retain moisture. Workers are kept on the surface of the soil throughout the year (although inactive when the temperature falls below 8°C) giving the species a territorial advantage during the spring (Brian 1977).

Like *Lasius* ants, *Myrmica* ants are also known to be myrmecophilous benefitting the larvae of lycaenid butterflies such as the *Maculinea* genus (Elmes and Wardlaw 1982). Eggs are laid on specific, larval host plants and after developing into the fourth instar phase, the larvae will move to the base of the plant and wait for adoption by *Myrmica* ants (MACMAN Project 2002-6 cited by Witek et al. 2016).

3.3.5.2 Myrmica ruginodis (Nylander 1846)

M. ruginodis can be distinguished from *M. scabrinodis* by the former's long propodeum spines which have the same measurement in length as the distance between the tips (Skinner & Allen 1996). *M. ruginodis* is a common and widespread species in the UK able to inhabit cooler habitats than other *Myrmica* species (Radchenko & Elmes 2010) hence it can be found in woodland clearings, forests, boggy areas and grasslands. Nests are frequently constructed under bark and rotten wood in forests but in grasslands, a shallow soil nest is built (Radchenko & Elmes 2010). In boggy areas, particularly, solarium soil structures are built over mossy nests for brood development. Brian (1977) considers these ants to be a nomadic species moving regularly to colonise new habitats. For this reason, he claims that their nests are far less robust than those created by *M. scabrinodis* being more lightly built with thinner walls. They do however build deep underground chambers for overwintering. While the species is generally monogynous, Brian and Brian (1949 cited by Radchenko & Elemes 2010) observed polygynous nests in West Scotland where the queens (termed microgynes) were smaller than in monogynous nests. The research suggests that colony fission of polygynous nests enables *M. ruginodis* to monopolise new habitats quickly while *monogynous* queens who seek new areas independently, form more short-lived, nomadic colonies.) Brian & Brian (1949, cited by Radchenko & Elemes 2010) also note that monogynous nests sometimes recruit microgynes after initial nest establishment perhaps to colonise more areas more rapidly. Like other *Myrmica* species, the nuptial flight of *M. ruginodis* takes place between August-September when mixed *Myrmica* swarms congregate for mating.

4 Research Methods

4.1 Sample Sites

To investigate the distribution of *P. argus* across the peninsula, 40 sample plots were selected using a random stratification method based on the selection of GPS easting and northing grid references. Two sample points had to be reselected due to the inaccessibility of the sample location. There are many inaccessible areas on the peninsula which do not become reachable even in the summer; these areas include the boggy woodlands around Little Sea and most of the marshes especially at the northern end of Third Ridge. Figure 5 shows the layout of the sample plot while Figure 6 shows the location of each sample point (Appendix 1 gives the GPS Easting and Northing references of all plots). The map in Figure 7 gives the compartment names for each area.

In February 2015, once sample points had been selected, a 13cm core of earth was extracted at each of the 40 sites using a bulb planter and separated into 2 samples (3cm and 10cm) for laboratory analysis. During April and May 2015, the vegetation community within a 5m² radius of the core soil sample, was recorded at each sample plot. This included species identification, percentage cover and mean height (cm) and was determined using five 1m² quadrats placed within the sample zone depicted in Figure 5. The central quadrat included the soil sample while the remaining 4 quadrats were placed at compass points. Bryophyte identification was included at species level but *Cladonia* was recorded at genus level only. General records of habitat type were also kept for each site.

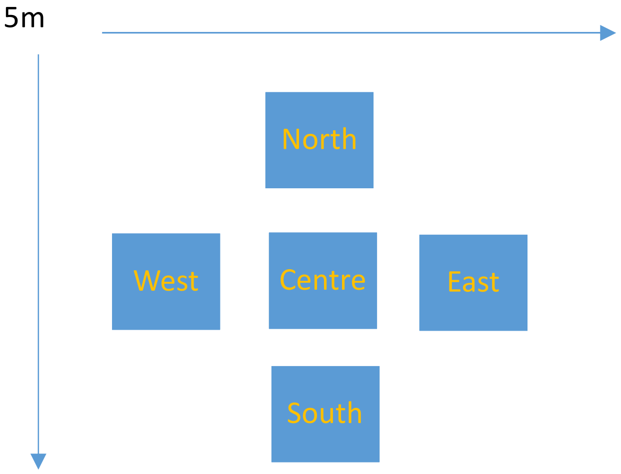

Figure 5: Vegetation grid in 5m² zone at each sample point

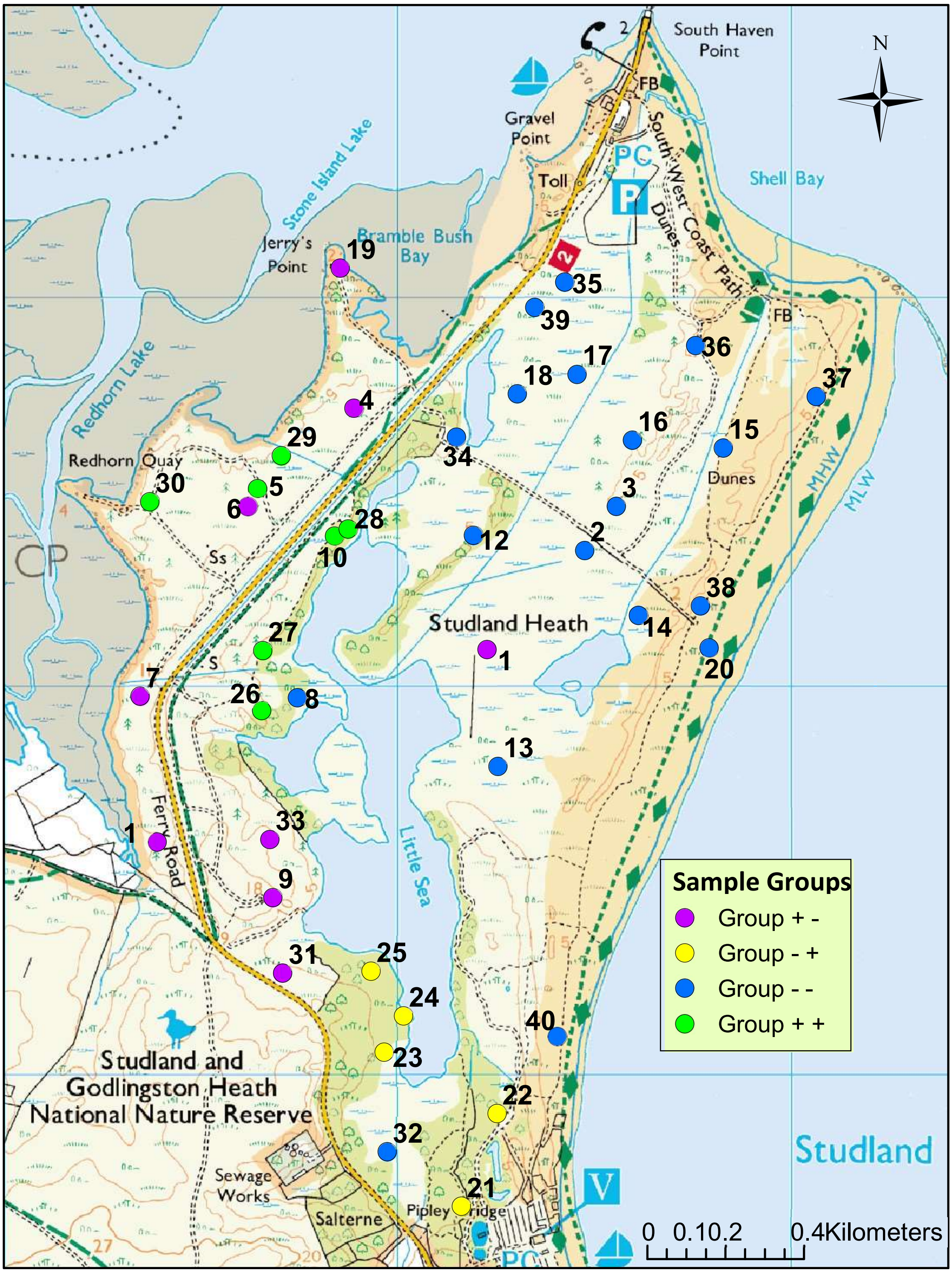

Figure 6 : The position of each of the 40 sites in the 4 sample groups across the peninsula (Amended from EDINA Digimap Ordinance Survey Service: Studland Peninsula ArcMap 10.2.2.)

(**Group + -** Sample plots where *P. argus* presence was recorded in 2015 research but not recorded in the 1930s.

Group - + Sample plots where Diver recorded *P. argus* presence in the 1930s but not found in 2015 research.

Group - - Sample plots where *P. argus* has had no recorded presence in Diver's records or in this 2015 research.

Group + + Sample plots where *P. argus* was recorded by Diver in the 1930s and seen in 2015 research).

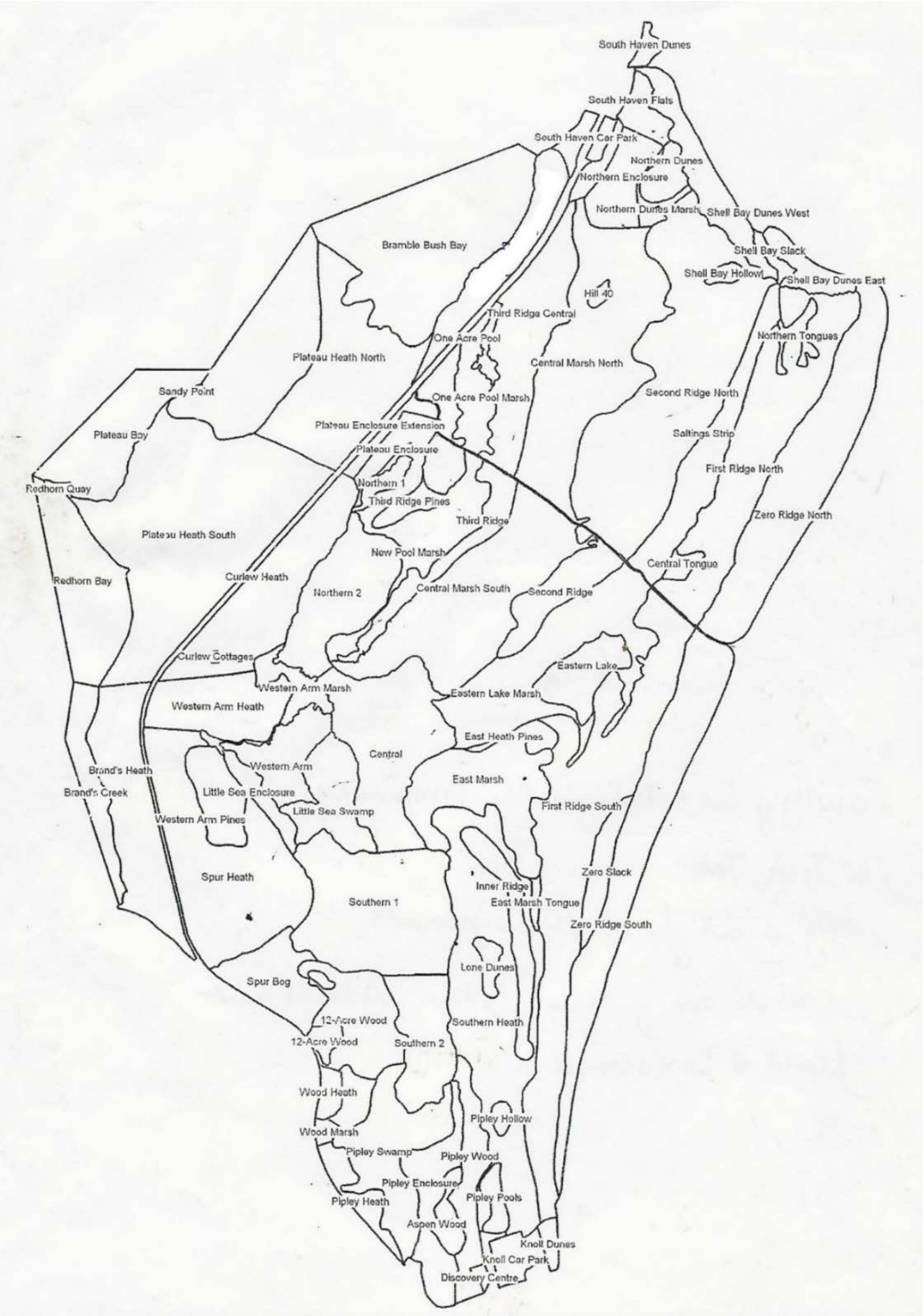

Figure 7 : Compartment names on Studland peninsula which correspond with Diver's survey names (The Cyril Diver Project, National Trust, Studland, Dorset)

Plant identification was confirmed through keys and handbooks (Fitter et al. 1984, Pratt 2008, Streeter 2009, Atherton et al. 2010) and verified by the Cyril Diver Project Botanical group led by environmental chemist and botanist Robin Walls, botanical author Edward Pratt and botanist Bryan Edwards (Dorset Environmental Records Centre).

4.2 The recording of *Plebejus argus*

The number of *P. argus* at each plot was determined by the construction of six transect routes across the peninsula which were walked once a week between 11:30 and 14:30 from June 10th until July 29th 2015. The transect routes crossed all sample points and butterflies were counted in a $2m^2$ zone around the centre of the sample point as the transect line was walked. The transect routes were loosely based on the Pollard and Yates 1993 model as the transects were fixed routes, walked weekly and butterflies were recorded within a fixed width band. The transects were not set up with sections linked to habitat types however as they followed the randomly selected sample points. The transect system was chosen as a suitable methodology due to the large numbers of *P. argus* anticipated in a small area. Walking the transect within a fixed band of $2m^2$, made it easier to count all the butterflies as they flew up and avoided double-counting of individuals. Initially, time-related counts at each sample point was considered but it was decided that it would be very difficult to avoid double-counting due to the large number of individuals anticipated in a small zone.

4.3 Group membership

When the butterfly counts were completed, each sample point was allocated to one of four groups. Group membership was based on four criteria as shown in Table 1. Table 2 shows the grouping of each of the sample points while Figure 6 shows group identity by colour-coding.

Table 1: The categorisation of the sample points into four groups

Group category	**Description of site category**
Group + -	Sample plots where *P. argus* presence was recorded in 2015 research but not recorded in the 1930s.
Group - +	Sample plots where Diver recorded *P. argus* presence in the 1930s but not found in 2015 research.
Group - -	Sample plots where *P. argus* has had no recorded presence in Diver's records or in this 2015 research.
Group + +	Sample plots where *P. argus* was recorded by Diver in the 1930s and seen in 2015 research.

As sample points were generated randomly at the beginning of the research, it was not known initially at which sample points *P. argus* would be recorded during this research. Diver's distribution map was helpful in determining where Diver had recorded the butterfly in the 1930s while all other results, which influenced group membership, were from sightings in this study. Consequently, each of the four groups has a different number of sample points. This is due to the random selection method which allocated sample points over the whole peninsula regardless of vegetation type or the likelihood of finding *P. argus* there. This method was used so that comparisons could be made between habitats and vegetation where *P. argus* had a presence or was not recorded.

Table 2 : Sample points with the 4 groups

Group category	**Description of site category**	**Site numbers**
Group + -	Sample plots where *P. argus* presence was recorded in 2015 research but not recorded in the 1930s.	1.4.6.7.9.11.19.31.33.
Group - +	Sample plots where Diver recorded *P. argus* presence in the 1930s but not found in 2015 research.	21.22.23.24.25.
Group - -	Sample plots where *P. argus* has had no recorded presence in Diver's records or in this 2015 research.	2.3.8.12.13.14.15.16.17.18.20.32.34.35.36.37.38.39.40.

Group category	**Description of site category**	**Site numbers**
Group + +	Sample plots where *P. argus* was recorded by Diver in the 1930s and seen in 2015 research.	5.10.26.27.28.29.30.

4.4 Laboratory Analysis on soil samples

The soil samples were tested in the laboratory for: the pH level; conductivity (μS/cm); percentage of total nitrogen, total carbon and organic mass; the total nitrogen: carbon ratio and for a range of other variables including mg/kg of cadmium, copper, iron, potassium, magnesium, manganese, sodium, phosphorous, lead and zinc. Soil samples were collected during a three - week period in February 2015 and were stored in airtight containers, in the fridge, until analysis. All soil samples were air dried for 72 hours before weighing on a digital balance to calculate bulk density. Each sample was then broken up, passed through a 2mm sieve to remove coarse material/roots and homogenised using an electric blender. The cylinder volumes were: 84.82cm³ for the 3 cm samples and 282.74cm³ for the 10 cm samples. Soil pH was obtained using a metre and probe in soil/water solution (HANNA Grocheck-Combo pH/TDS Metre with Smart Electrode) and conductivity was found using an EC metre and probe (HANNA EC, TDS and Temperature Tester). Organic matter was calculated by loss on ignition while carbon and nitrogen ratios were discovered using the Flash Elemental Analyster 1112 Series. Metal content was found using an Inductively Coupled Plasma: Varian Vista ProCCD ICP-OES. All laboratory work was carried out at Bournemouth University and at Brockenhurst College.

4.5 Ant Collection

Ant traps were set up at each of the 40 sites and collected from throughout May and September 2015. At each site, five sugar traps (using an overturned clay pigeon disc) and five pitfall traps (using water only and a tough, fine mesh) were set up at each sample point. Pitfall traps were emptied 24 hours after installation while disc traps were checked 72 hours after they were set up. All traps were visited five times during the collection period. Ant abundance at the disc traps was counted using perspex and a fine mesh grid which could be placed under the disc before flipping it over. Ant specimens were collected at each visit for identification using a light microscope borrowed from the National Trust. Ant samples taken during the project were checked against two identification keys, Skinner and Allen 1996 and the Royal Entomological Society Handbook for the Identification of British Insects 1975. Specimens were also checked against a comprehensive, hymenoptera collection taken from Studland Heath in 2013 which had been verified by ecologist/myrmecologist A. Abbot (Environmental Consultants Wareham, Dorset).

4.6 Statistical Tests

All statistical tests were carried out using IBM SPSS Statistics Version 23. Principal Component Analysis (PCA) was carried out on the soil sample and vegetation results to reduce the number of variables influencing each plot and to see if any patterns or associations emerged (Wheater 2000). The loadings for the components extracted were saved as factor scores and Multiple Linear Regression (MLR) was then carried out to determine if a significant regression equation would result when *P. argus* and the symbiotic ant, *L. niger*, were regressed against the extracted components. Kruskal Wallis H tests were used, where data was nonparametric, to investigate whether there were any significant differences between the component distributions across the four groups. Where significant differences between groups did exist, post hoc Mann Whitney U tests were employed to identify the location of the differences between paired groups (Wheater 2003).

In addition to PCA, Spearman's Rank Coefficient Correlation was also carried out on independent vegetation variables to ascertain whether a relationship could be found with either *P. argus* or *L. niger* and Binary Logistic Regression was carried out to predict if the presence of *P. argus* in a plot was more likely in conjunction with any of the 70 independent variables.

5 Results

Results from the study have been divided into two main sections to reflect the key research questions specified in section 2.3. Section 5.1 gives the abundance and distribution results for *P. argus* across the sample plots while Section 5.2 presents results pertinent to the question of what drives *P. argus* distribution. As there are more possibilities to consider in 5.2 than in the first research question, the section has been broken down into sub-sections. Section 5.2.1 -5.2.3 present the results relevant to vegetation (named resources) as a driver influencing *P. argus* abundance and distribution while Section 5.3 considers the importance of ants to the butterfly and presents results showing the relationship between the ant species found with vegetation components. Section 5.4 considers soil components as a possible driver of *P. argus* number and distribution and presents results pertinent to this.

5.1 What was the distribution of *P. argus* across Studland peninsula?

A total of 612 *P. argus* imagines, both male and female, were recorded at plots in Groups **+ -** (sample plots where *P. argus* presence was recorded in 2015 research but not in the 1930s) and **++** (sample plots where *P. argus* was recorded by Diver in the 1930s and seen in 2015). Of this total, 414 (68% of total sightings) were recorded in Group **+ -** while 198 (32% of sightings) were seen in Group **+ +**. Figure 8 shows the position of *P. argus* plots across the peninsula while Figure 9 and Figure 10 illustrate the number of sightings at each plot within each group. The total of 612 imagines seen slightly exceeds results from the UK Butterfly Monitoring Scheme (UKBMS) transects for 2015 at Studland which totalled 349 imagines; 63 at Studland Heath and 286 on the Ferry Road. This is likely to be due to the position of hotspot plots 4, 9 and 27 in this research which are situated in the middle of Plateau Heath where large numbers of *P. argus* feed as the area is densely vegetated with *E. tetralix*. The route of the Butterfly Monitoring Scheme Ferry Road transect skirts along the eastern edge of Plateau Heath where there is a pathway and less vegetation.

Figure 8 shows the distribution of *P. argus* to be predominantly congregated on the western side of the peninsula running along both sides of Ferry Road with Plateau Heath on the western side and Curlew Heath and Western Arm Heath on the eastern side. Plot 1 with 3 sightings, was the only plot with *P. argus* presence to the east of Little Sea.

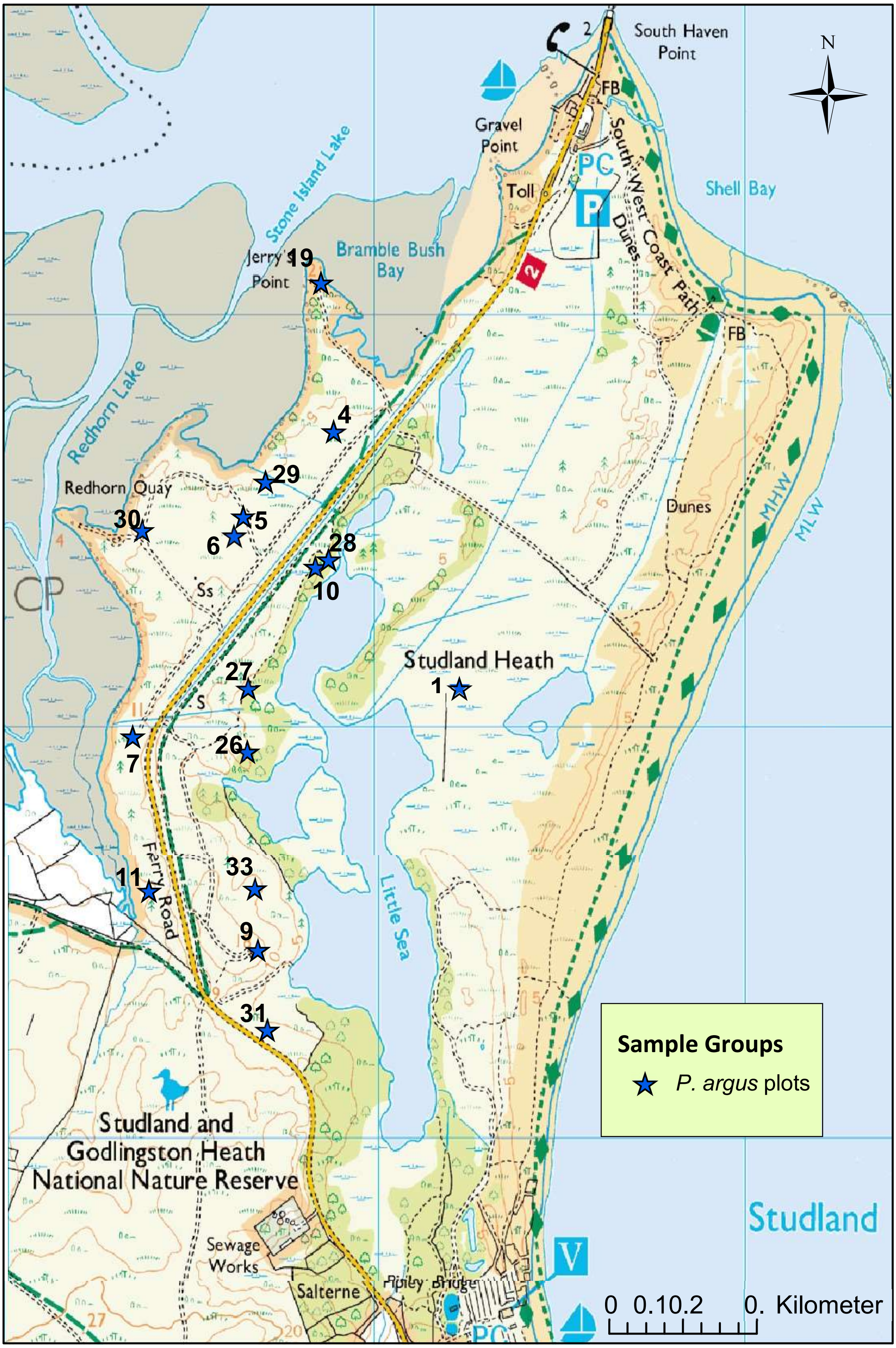

Figure 8 : The distribution of *P. argus* across Studland peninsula in 2015 (Amended from EDINA Digimap Ordinance Survey Service: Studland Peninsula using ArcMap 10.2.2.)

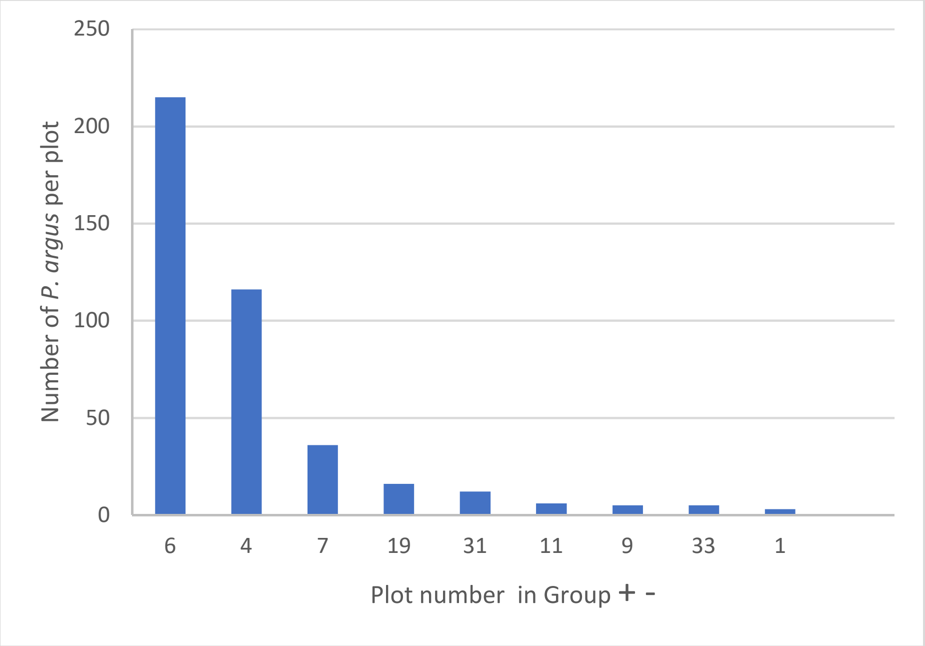

Figure 9 : Hotspot plots- The number of *P. argus* adults (male & female) at each plot in Group **+ -** (sample plots where *P. argus* presence was recorded in 2015 research but not 1930s)

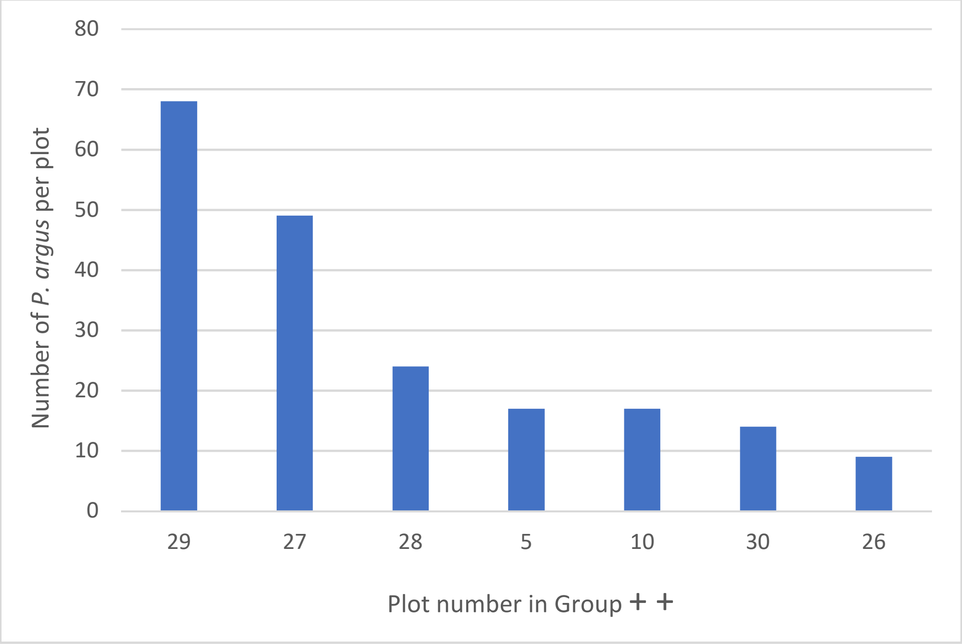

Figure 10 : Hotspot plots: The number of *P. argus* adults (male & female) at each plot in Group **+ +** (sample plots where *P. argus* was recorded by Diver in the 1930s and seen in 2015).

Results from a Mann Whitney U test showed that differences in the abundance of sightings between the two groups **+ -** and **+ +** was not significant (p= .314, Appendix 9.5).

The box plot in Figure 11 however, shows that Group **+ -** has 2 outliers in plots 4 (point 2) and 6 (point 3) on Plateau Heath reflecting the hotspots which were higher than the median value of 12. The range in Group **+ +**, which had a median of 17, was more evenly spread with no outliers.

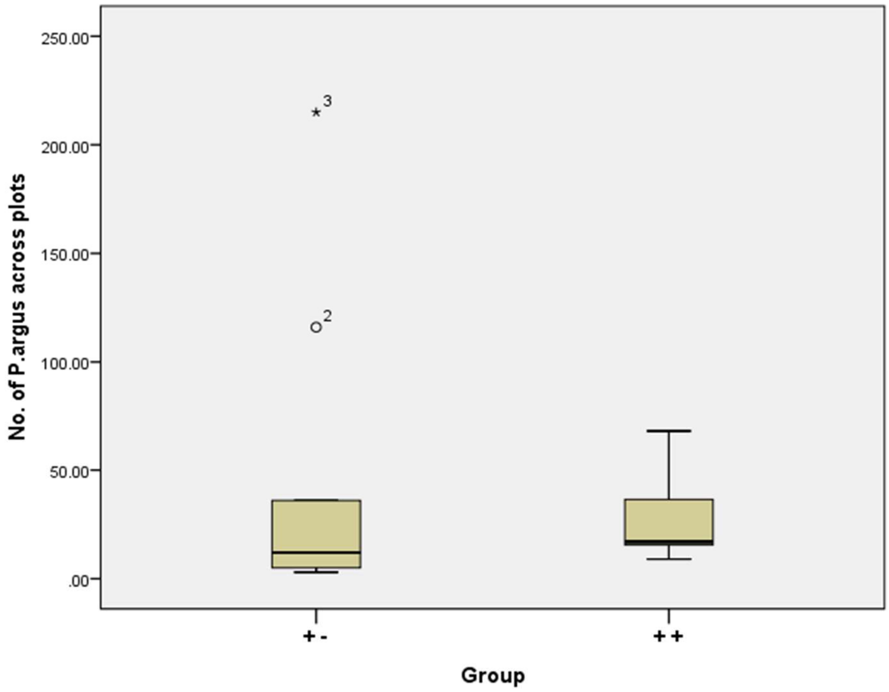

Figure 11 : Box plot showing the range of *P. argus* in Group **+ -** (sample plots where *P. argus* presence was recorded in 2015 research but not in the 1930s) and Group **+ +** (sample plots where *P. argus* was recorded by Diver in the 1930s and seen in 2015) with 2 outliers at point 2 (plot 4) and point 3 (plot 6).

5.2 What factors are driving the distribution of *P. argus* across the peninsula?

5.2.1 **How important is vegetation?**

It is probable that, like many other insects, *P. argus* responds to a combination of resource factors which renders a habitat either desirable or unsuitable. Principal Component Analysis (PCA) was therefore carried out to reduce the number of individual resource variables influencing plot diversity and to investigate any underlying associations which could show patterns in resource composition. Resource data included vegetation variables and *L. niger* abundance recorded at each plot. *L. niger* abundance was included as a resource variable due to the findings of myrmecological research which suggests a significant correlation between the presence of *P. argus* with *L. niger* abundance (see Literature Review in Section 3). Where pairings with very high correlations were present (9.0 +), one variable within the pairing was removed from the analysis; likewise, results with very low correlations were also removed. There was, for example, a high correlation in many plant species between percentage cover and height; height results were therefore removed from the analysis. Table 3 illustrates the seven extracted components or groupings which emerged from 16 resource variables in the PCA analysis where the Eigenvalue is above 1. The seven components accounted for 72.35% of the variance between plots (Appendix 9.6). Table 4 shows the percentage of variance for each component with the total variance of 72.35% highlighted. Factor Analysis Rotation using Varimax with Kaiser Nomalization was conducted to emphasise any patterns or groupings emerging in the data. The Rotated Component Matrix in Table 3 shows the loading score for each variable within the seven extracted components. The blue shading in the table depicts the highest loadings within each component.

Table 3: Rotated Component Matrix in Resource Data

	Component						
Independent Resource Variable	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Ground plant % cover	**.871**	-.075	.084	-.049	.017	-.013	-.042
Flowering plant % cover	**.860**	.022	-.065	-.037	-.037	.039	-.062
Leaf litter / deadwood % cover	**.546**	.402	.229	-.226	.226	-.082	.156
Bare ground % cover	.095	**.801**	-.117	-.057	-.116	.220	-.088
Grass 5-10 cm % cover	-.142	**.718**	.312	.148	.121	-.220	.179
C.introflexus % cover	-.096	.065	**-.845**	-.034	.171	-.192	.134
Tree % cover	-.092	.186	**.621**	-.084	.428	.035	.138
E. cinerea % cover	-.151	.018	.029	**.761**	-.079	-.095	-.112
E. tetralix % cover	-.272	-.259	.200	**-.597**	-.497	-.178	-.077
C.vulgaris % cover	-.206	-.140	-.550	**.546**	-.099	-.269	.000
Shrub % cover	-.050	-.115	.094	-.171	**.782**	.212	-.009
L. niger abundance	-.216	-.128	.121	-.191	**-.576**	.430	.315
Grass 2-5 cm % cover	-.079	-.129	.086	.090	.143	**.777**	.113
Bryophyte/Sphagnum % cover	-.117	-.251	-.172	.183	-.006	**-.683**	.047
Grass under 2 cm % cover	-.024	.096	-.053	-.097	.003	.048	**.916**
Cladonia spp. % cover	-.047	-.346	.033	.479	-.272	.129	**.485**

Table 4 : Total variance explained; the seven components extracted from Resources PCA making up the total of 72.35% of variance which is highlighted in blue (with Kaiser-Meyer-Olkin Measure of Sampling Adequacy at .557)

Component	**Initial Eigenvalues**			**Extraction Sums of Squared Loadings**			**Rotation Sums of Squared Loadings**		
	Total	**% of Variance**	**Cumulative %**	**Total**	**% of Variance**	**Cumulative %**	**Total**	**% of Variance**	**Cumulative %**
1	2.87	17.95	17.95	2.87	17.95	17.95	2.05	12.84	12.84
2	2.10	13.13	31.08	2.10	13.13	31.08	1.68	10.55	23.39
3	1.70	10.62	41.71	1.70	10.62	41.71	1.68	10.52	33.91
4	1.44	9.02	50.73	1.44	9.026	50.73	1.66	10.41	44.33
5	1.31	8.21	58.95	1.31	8.214	58.95	1.59	9.96	54.30
6	1.10	6.92	65.87	1.10	6.926	65.87	1.57	9.84	64.14
7	1.03	6.47	**72.35**	1.03	6.475	72.35	1.31	8.20	72.35

5.2.2 **Which resources are important to *P. argus*?**

Having extracted seven main components from the Resources PCA, Multiple Linear Regression (MLR) was then calculated to predict the abundance of *P. argus* based on resource factor scores resulting from the seven components identified in the PCA extraction (Appendix 9.7). A significant regression equation was found (F (7,32) = 3.606, p= .006) with R^2 of .441. Within individual components however, only Component 5 was significant (Standardized Coefficient -.50, p=.001) which explained only 8.21% of the variance (see Initial Eigenvalues in Table 4) and was a negative predictor of *P. argus* abundance; as shown in Figure 12.

Although Resource Component 5 has two of the higher loadings for *E. tetralix* % cover at -.497 and *L. niger* abundance at -.576, the highest loading figure is for shrub % cover at .782 which, along with tree cover at .428 is also influencing the component result (see Table 3). Notably, both *E. tetralix* and *L. niger* have negative results suggesting an inverse relationship between *E. tetralix* and *L. niger* with shrub and tree cover. Results do show however a grouping between *E. tetralix* with *L. niger* and between shrub and tree percentage cover.

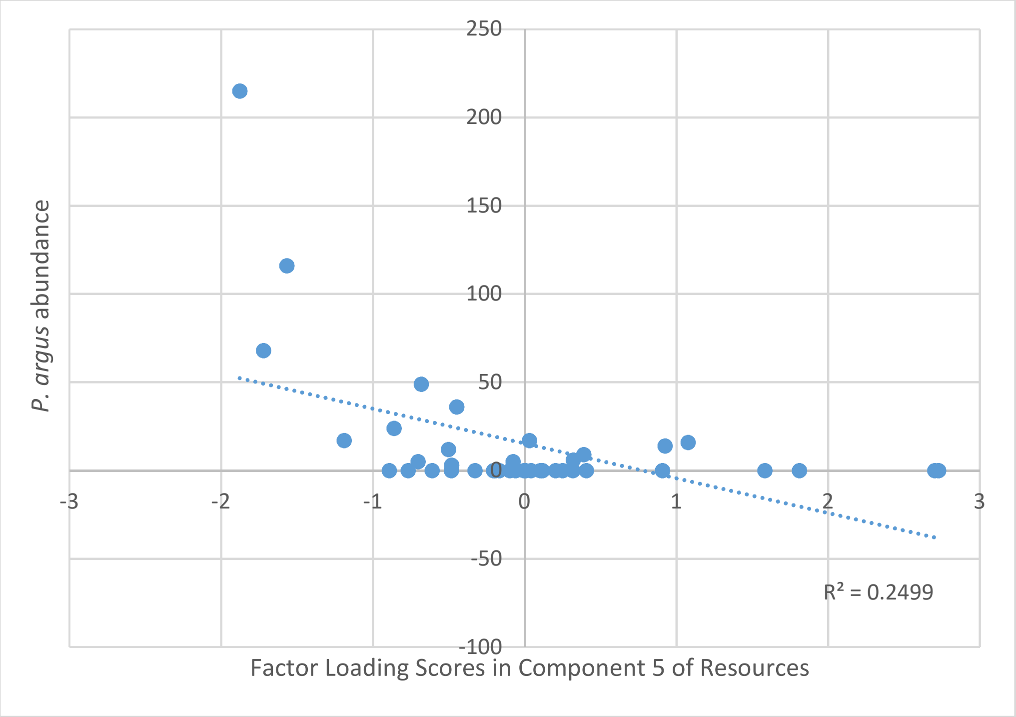

Figure 12: A significant, negative prediction between *P. argus* abundance and Component 5 of Resources (including outliers)

Although a Kruskal Wallis H test found no significant difference in the distribution of Component 5 across the four groups, the box plot in Figure 13 shows outliers at points 3, 7, 20, 24, 26 and 27 which could be could be influencing the negative relationship between *P. argus* with Component 5. When the outliers are removed however, the relationship remains negative as shown in Figure 14.

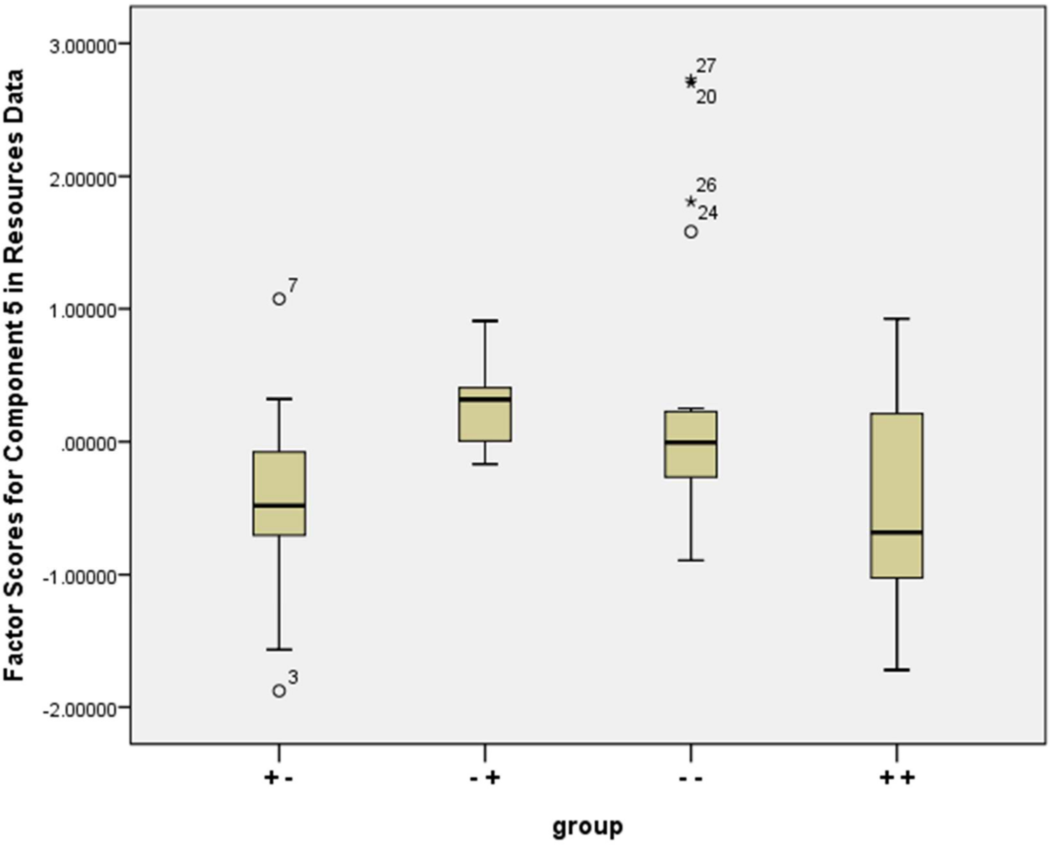

Figure 13 : Box plot showing differences in the distribution of Component 5 in Resources across all groups with outliers. The symbols o and ★ indicate outliers; ★ more extreme than o.

(**Group +** - Sample plots where *P. argus* presence was recorded in 2015 research but not recorded in the 1930s.
Group - + Sample plots where Diver recorded *P. argus* presence in the 1930s but not found in 2015 research.
Group - - Sample plots where *P. argus* has had no recorded presence in Diver's records or in this 2015 research.
Group + + Sample plots where *P. argus* was recorded by Diver in the 1930s and seen in 2015 research).

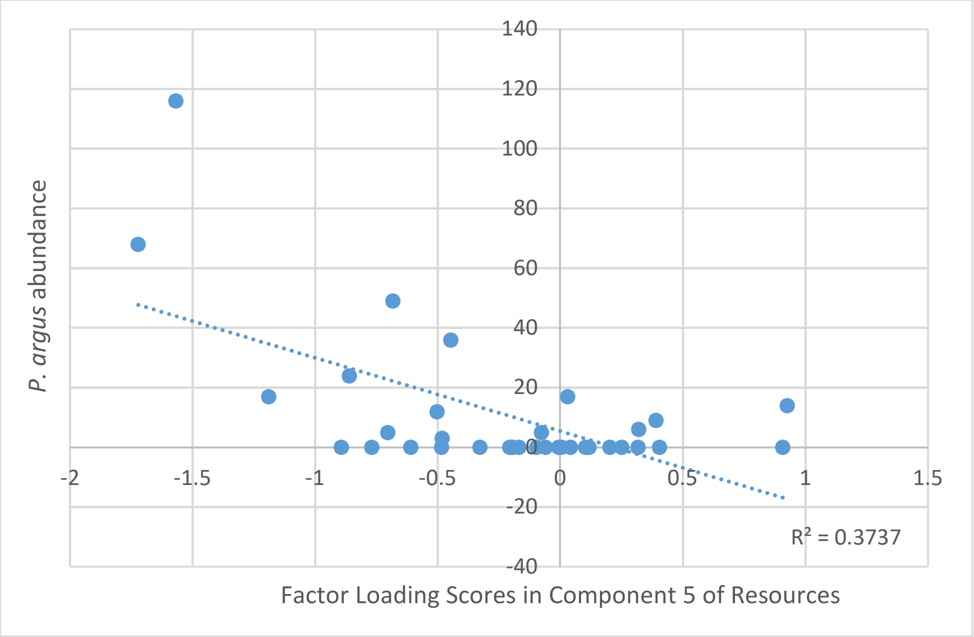

Figure 14 : A significant, negative prediction between *P. argus* abundance and Component 5 of Resources (excluding outliers at points 3, 7, 20, 24, 26 & 27).

As it is possible that the relationship between *P. argus* with *E. tetralix* and *L. niger* is being adversely affected by the high loadings for shrub and tree cover in Component 5, Spearman's Rank Correlation Coefficient was also conducted to investigate the relationship between *P. argus* with Resource variables. As this test is outside of the Resources PCA, the height variables which had been excluded from the PCA due to high correlations with plant cover, were replaced as variables for testing. Spearman's Rank Correlation Coefficient test was selected as it is a robust test which is not sensitive to outliers and as a nonparametric test, data does not have to be evenly distributed for the results to be valid. Much of the Resources data, outside of the PCA with its factor scores, is not normally distributed and many of the variables have significant outliers. Table 5 displays the Correlation Coefficient and Significance value for all Resources variables when correlated against *P. argus* abundance. Results from the Spearman's Rank Correlation Coefficient test showed a significant, positive relationship (where $p < .05$) between *P. argus* with *E. tetralix* % cover (r=.396), *E. tetralix* height (r=.346) grass 2-5 cm % cover (r=.367) and with the abundance of *L. niger* (r=.706). In all four cases, positive, linear relationships with *P. argus* were shown where values rose simultaneously. The correlation coefficient value for *L. niger* at r=.706 shows a particularly strong association with *P. argus*.

Table 5 : Spearman's Rank Correlation Coefficient results when *P. argus* is correlated with Resource variables outside of the Resources PCA (Significant values where p<.05 are highlighted in yellow).

Name of Resource variable	**Correlation coefficient (r)**	**Significance (2-tailed) p value**
E. tetralix % cover	.396	**.011**
E. tetralix height (cm)	.346	**.029**
Grass 2-5 cm % cover	.367	**.020**
L. niger abundance	.706	**.001**
Bare ground % cover	.253	.115
Bryophytes/*Sphagnum*	-.334	.055
C.vulgaris % cover	.038	.815
C.vulgaris height (cm)	.028	.865
C.introflexus % cover	-.243	.131
Cladonia % cover	.272	.089
E.cinerea % cover	-.046	.778
E.cinerea height (cm)	-.031	.847
Flowering plant % cover	-.072	.657
Flowering plant height (cm)	-.120	-.084
Grass under 2 cm % cover	.191	.237
Grass 5-10 cm % cover	.054	.742
Grass over 10 cm % cover	.044	.788
Leaf litter % cover	-.175	.279
Rush/sedge % cover	-.060	.712
Rush/sedge height (cm)	-.060	.713
Shrubs % cover	-.011	.948
Shrub height (cm)	.002	.991
Tree % cover	.057	.728
Tree height (cm)	.051	.753

Figure 15 and Figure 16 show the significant, positive relationship between *P. argus* and *E. tetralix* as numbers of the butterfly increase as plant density and height increases. The box plots in Figure 17 and Figure 18 depicting the distribution of *E. tetralix* across the peninsula show outliers in group **- -** (sample plots where *P. argus* was not recorded in 2015 or in the 1930s).

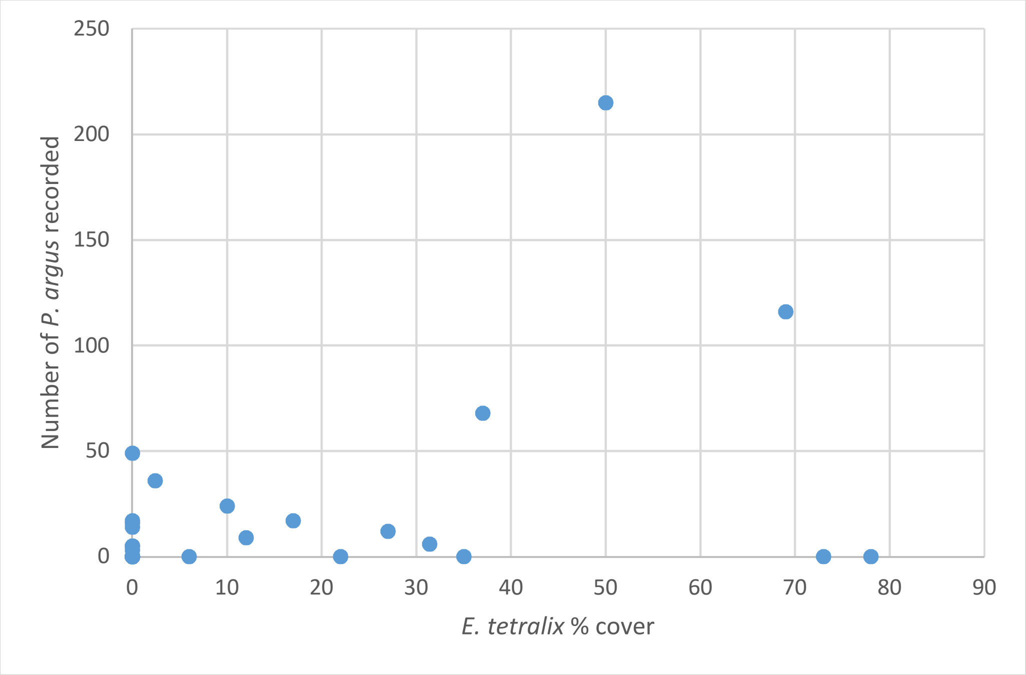

Figure 15: A significant, correlation (r= .396, p=.011) found between *P. argus* abundance with *E. tetralix* % cover when considered outside of the PCA

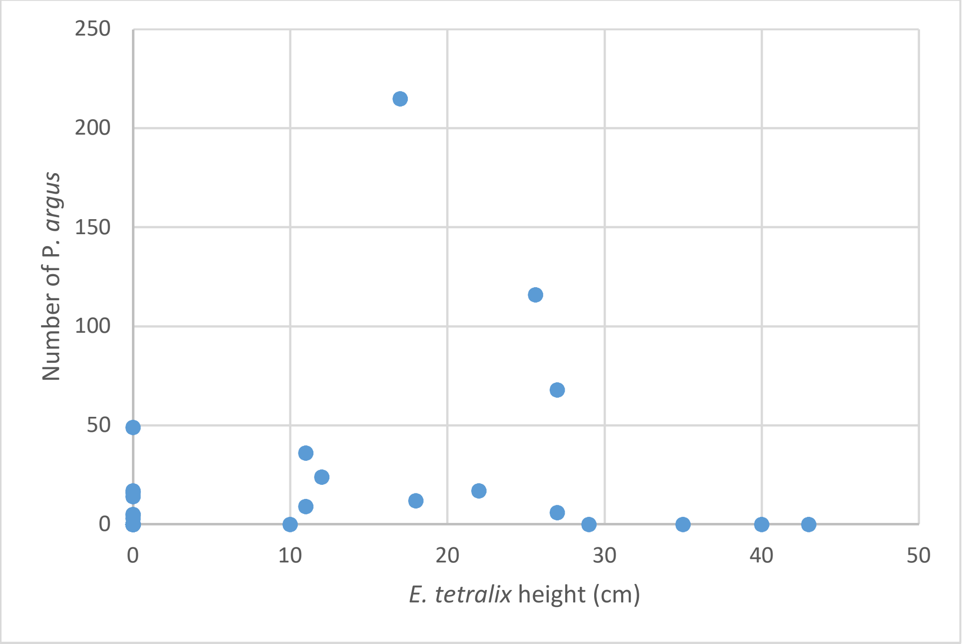

Figure 16 : A significant, correlation (r= .346, p=.029) found between *P. argus* abundance with number of *E. tetralix* height (cm) when considered outside of the PCA

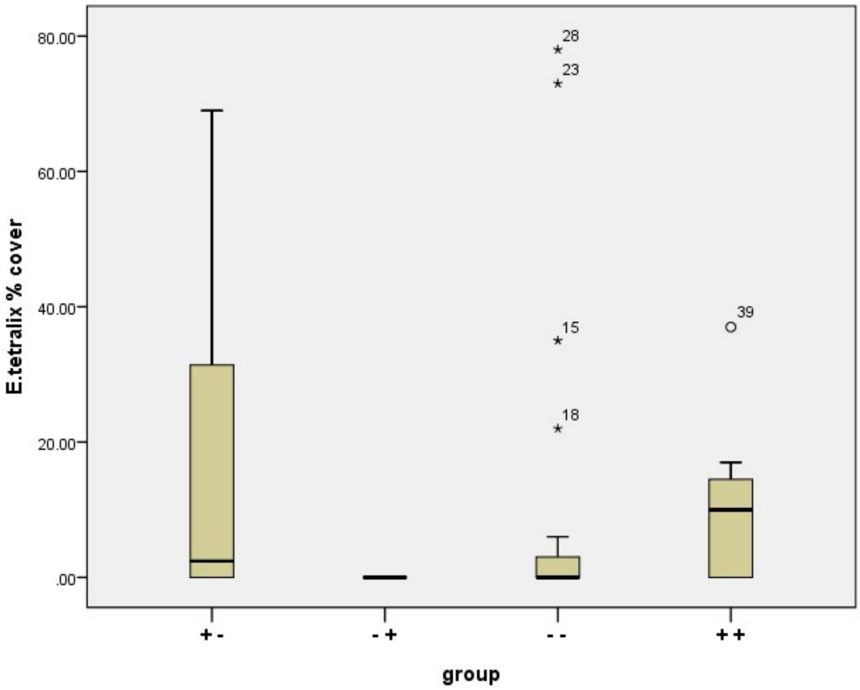

Figure 17 : Box plot showing distribution of *E. tetralix* % cover across all four groups with outliers at point 15 (plot 21), point 18 (plot 24), point 23 (plot 12), point 28 (plot 38), and point 39 (plot 32).

(**Group +** - Sample plots where *P. argus* presence was recorded in 2015 research but not recorded in the 1930s.
Group - + Sample plots where Diver recorded *P. argus* presence in the 1930s but not found in 2015 research.
Group - - Sample plots where *P. argus* has had no recorded presence in Diver's records or in this 2015 research.
Group + + Sample plots where *P. argus* was recorded by Diver in the 1930s and seen in 2015 research).

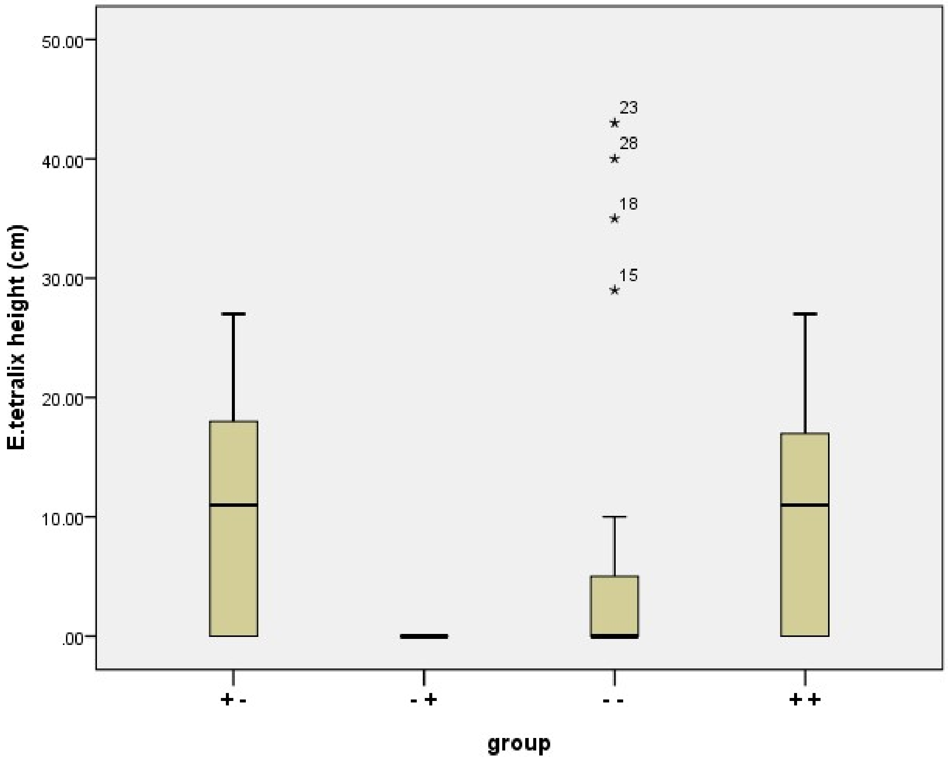

Figure 18 : Box plot showing median heights (cm) of *E. tetralix* cover across all four groups with significant outliers at point 15 (plot 21), 18 (plot 24), 23 (plot 12) and 28 (plot 38).

(**Group + -** Sample plots where *P. argus* presence was recorded in 2015 research but not recorded in the 1930s.
Group - + Sample plots where Diver recorded *P. argus* presence in the 1930s but not found in 2015 research.
Group - - Sample plots where *P. argus* has had no recorded presence in Diver's records or in this 2015 research.
Group + + Sample plots where *P. argus* was recorded by Diver in the 1930s and seen in 2015 research).

Results from the Spearman's Rank Correlation Coefficient also showed a significant, positive correlation between *P. argus* with both percentage cover of grass 2-5 cm and with *L. niger* abundance. Figure 19 and Figure 20 show these correlations while Figure 21 and Figure 22 show the distribution of grass percentage 2-5 cm and *L. niger* abundance across all groups with outliers marked.

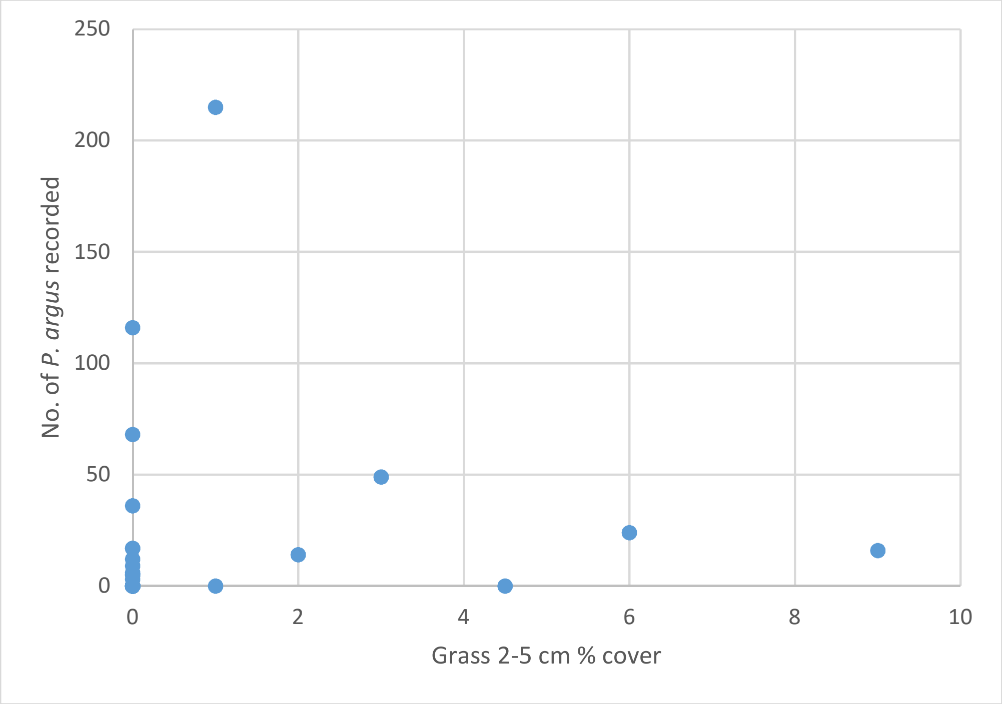

Figure 19: A significant, correlation (r= .367, p=.020) found between *P. argus* abundance with grass 2-5 cm % cover when considered outside of the PCA

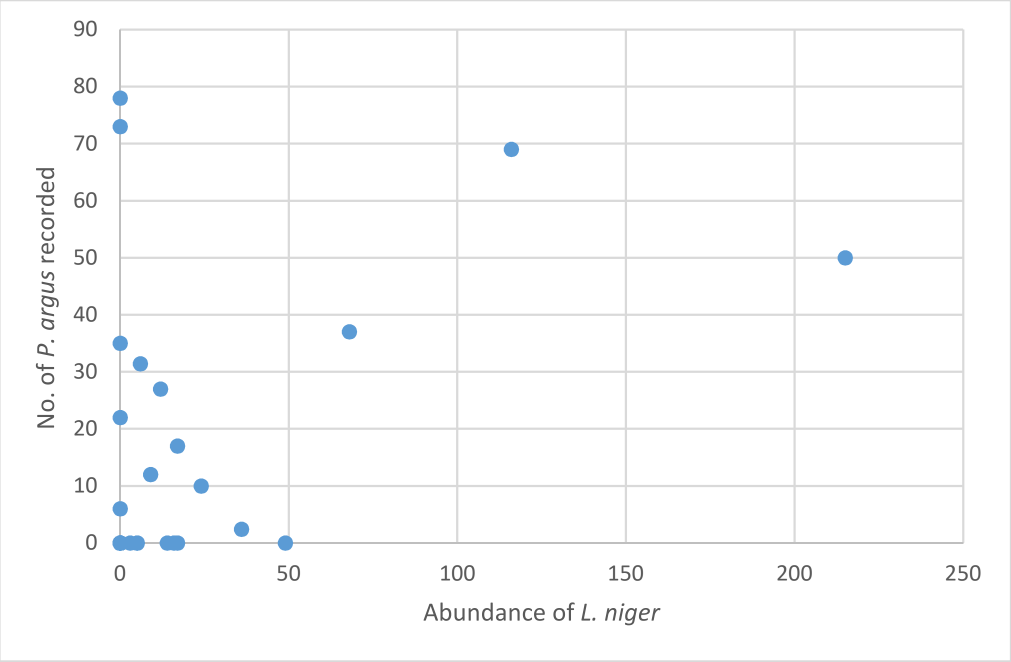

Figure 20 : A significant, correlation (r= .706, p=.001) found between *P. argus* abundance with number of *L. niger* when considered outside of the PCA

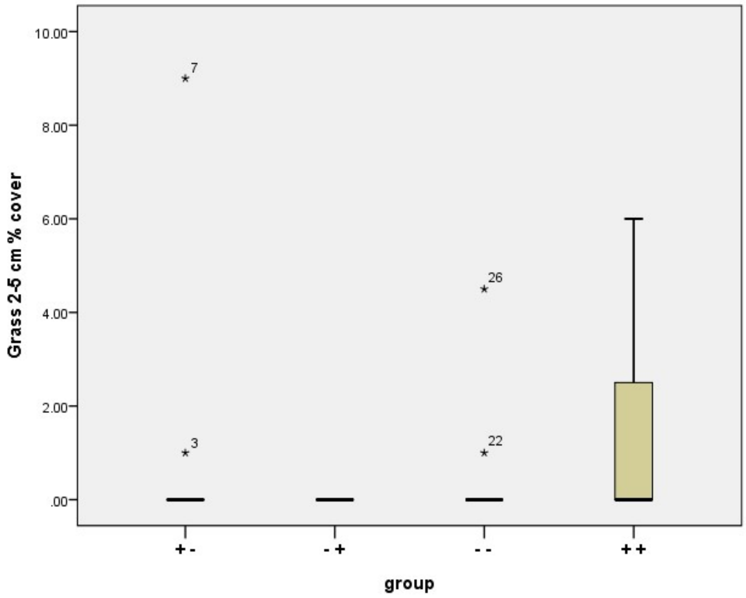

Figure 21 : Box plot showing distribution of grass 2-5 cm % cover across all groups with significant outliers at points 3 (plot 6), 7 (plot 19), 22 (plot 16) and 26 (plot 32).

(**Group +** - Sample plots where *P. argus* presence was recorded in 2015 research but not recorded in the 1930s.
Group - + Sample plots where Diver recorded *P. argus* presence in the 1930s but not found in 2015 research.
Group - - Sample plots where *P. argus* has had no recorded presence in Diver's records or in this 2015 research.
Group + + Sample plots where *P. argus* was recorded by Diver in the 1930s and seen in 2015 research).

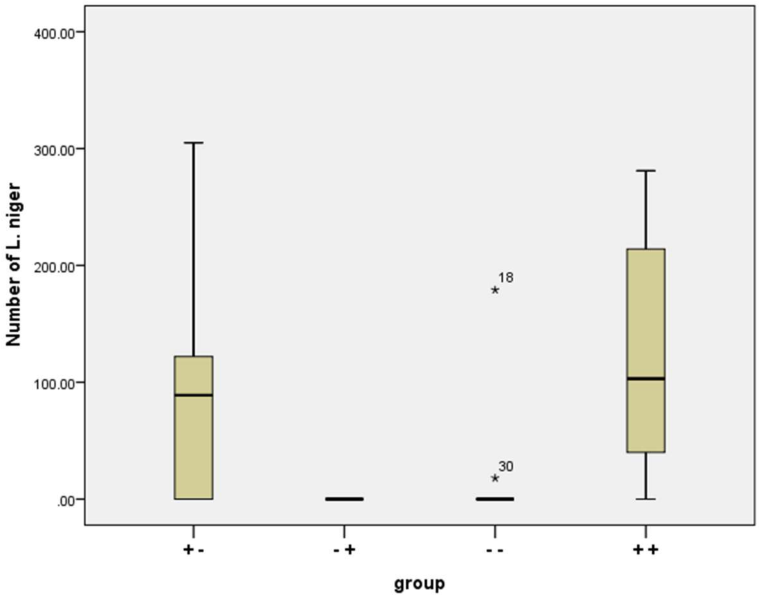

Figure 22 : Box plot showing distribution of *L. niger* across all four groups with significant outliers at point 18 (plot 12), and point 30 (plot 37)

(**Group +** - Sample plots where *P. argus* presence was recorded in 2015 research but not recorded in the 1930s.
Group - + Sample plots where Diver recorded *P. argus* presence in the 1930s but not found in 2015 research.
Group - - Sample plots where *P. argus* has had no recorded presence in Diver's records or in this 2015 research.
Group + + Sample plots where *P. argus* was recorded by Diver in the 1930s and seen in 2015 research)

The strong association between *P. argus* with *L. niger* was confirmed when Binary Logistic Regression (BLR) was carried out to predict if the presence of *P. argus* in a plot was more likely in conjunction with any of the 70 independent variables. The results confirmed that the presence of *L. niger* was a significant factor (where p=0.01) in determining *P. argus* presence with an odds ratio of 28.1 (Appendix 9.8) where the predictive capacity of the model was at 87.5%. The presence of *L. niger* led to an 18% increase in the chances of finding *P. argus* present.

5.2.3 How important are ants in driving *P. argus* abundance and distribution?

As the presence of *L. niger* has been identified as a key element within the habitat requirements of *P. argus*, a comparison of the abundance of all six ant species found in the 40 sample plots was plotted (Figure 23).

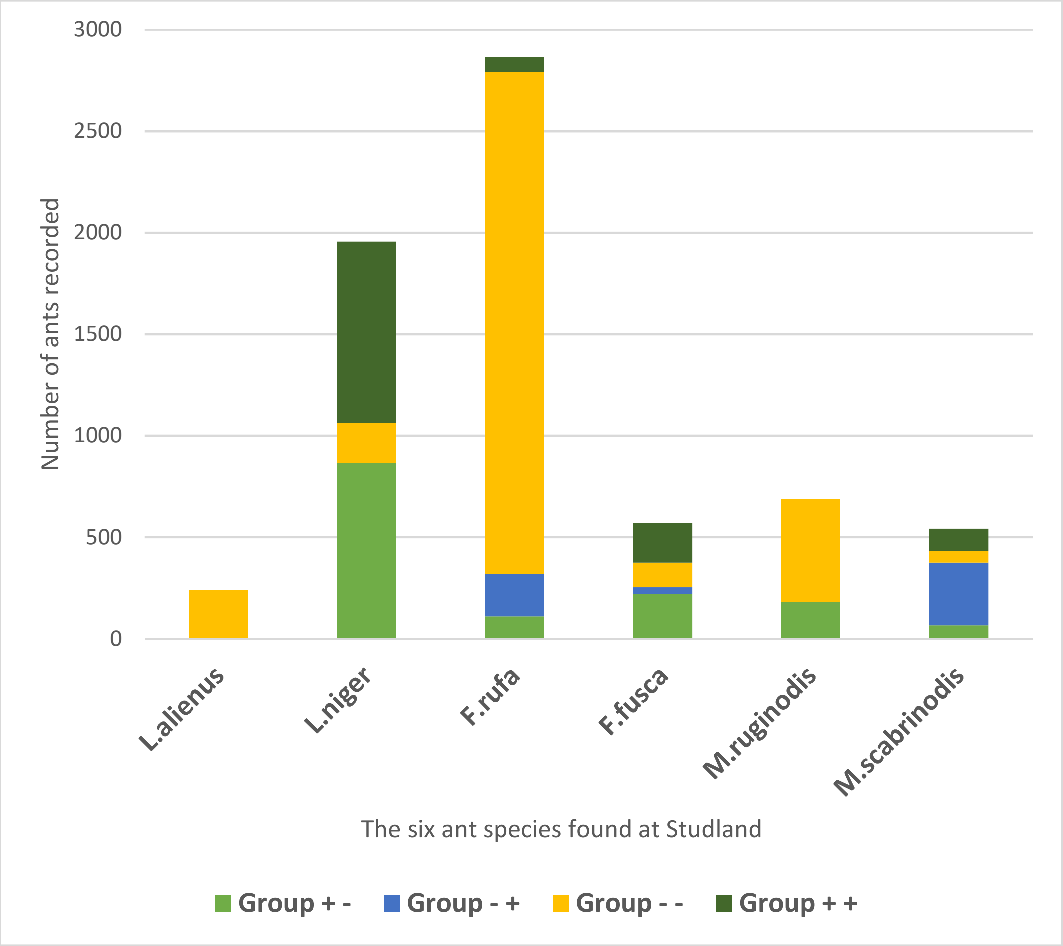

Figure 23 : The abundance of each of the six ant species found in the 4 groups

(**Group +** - Sample plots where *P. argus* presence was recorded in 2015 research but not recorded in the 1930s.

Group - + Sample plots where Diver recorded *P. argus* presence in the 1930s but not found in 2015 research.

Group - - Sample plots where *P. argus* has had no recorded presence in Diver's records or in this 2015 research.

Group + + Sample plots where *P. argus* was recorded by Diver in the 1930s and seen in 2015 research)

5.3 Do ants have specific habitat requirements?

As results from the linear regression tests found a significant prediction could be asserted between *P. argus* abundance with the presence of *L. niger* in section 5.2, further investigation was undertaken to determine any diversity in the resource requirements of the ant species found within the 40 sample plots. The literature review in Section 3 indicates that the six species found at Studland have some differences in habitat requirements which could be influencing their distribution across the peninsula.

Principal Component Analysis (PCA) was again carried out to reduce the number of individual vegetation variables between plots and to see if any patterns or connections emerged which could potentially be influencing ant distribution (Appendix 9.9). As with the Resources PCA, high and low correlations (9.0+) were removed from the analysis. Plots with no ant presence (namely plots 13, 20, 32 and 38) were also removed from the analysis. The six components accounted for 67.61 % of the variance between plots (see Table 7. The Rotated Matrix results in Table 6 illustrates that some groupings within vegetation seem to exist. Component One (which accounts for 19.78% of the variance) shows a grouping between the percentage cover of trees, grass 5-10 cm and leaf litter/dead wood which have the highest loadings in the group. Component two (which accounts for 12.26% of the total variance) has high loadings for percentage cover of ground plants and flowering plants. Component three (which accounts for 11.3% of the total variance) shows an association within the Ericaceae family with E.cinerea and E. tetralix showing the highest loadings (albeit inversely). The remaining three components (4-6) do show some vegetation associations but individually each component accounts for less than 10% of the total variance. Table 7 gives the variance percentages for each component which make up the overall 67.61%.

Table 6: Rotated Component Matrix showing six components in Vegetation PCA (with the highest loadings in each component highlighted in blue)

	Component					
	1	2	3	4	5	6
Grass 5-10 cm % cover	**.799**	-.150	.161	.013	.013	-.146
Tree % cover	**.798**	.005	-.124	.061	-.073	.353
Leaf litter/deadwood % cover	**.569**	.459	-.208	-.108	.145	.069
Ground plant % cover	-.009	**.906**	-.073	.012	-.045	.082
Flowering % cover	-.022	**.872**	.008	-.034	-.064	-.085
E. cinerea % cover	-.030	-.203	**.746**	.123	-.099	-.006
E. tetralix % cover	-.199	-.288	**-.734**	.170	-.276	-.133
Grass 2-5 cm % cover	-.205	-.100	.019	**-.788**	.228	.072
Bryophyte/Sphagnum	-.149	-.115	.099	**.716**	.138	-.011
Grass under 2 cm % cover	.222	-.180	-.277	-.057	**.627**	.121
Rush/sedge % cover	-.001	-.012	-.118	.057	**-.554**	-.020
C.introflexus	-.298	.049	.165	.389	**.566**	-.143
Shrub % cover	.148	.064	.055	-.176	.127	**.831**
Bare Ground	.497	.187	.073	-.281	.218	**-.529**
C.vulgaris% cover	-.392	-.213	.584	.402	.113	-.167

a. Rotation converged in 9 iterations.

b. Bold font has been used to highlight the variables with the highest loadings within each component

Table 7: Total variance expalined; the percentage of variance contributed by each component and the total variance at 67.61% highlighted in blue (with Kaiser-Meyer-Olkin Measure of Sampling Adequacy at .517)

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	2.96	19.78	19.78	2.96	19.78	19.78	2.26	15.10	15.10
2	1.83	12.26	32.04	1.83	12.26	32.0	2.08	13.88	28.98
3	1.69	11.30	43.35	1.69	11.30	43.35	1.66	11.08	40.07
4	1.36	9.23	52.58	1.38	9.23	52.58	1.62	10.81	50.89
5	1.13	7.55	60.13	1.13	7.55	60.13	1.28	8.57	59.46
6	1.12	7.47	67.61	1.12	7.47	67.61	1.22	8.14	67.61

Multiple Linear Regression (MLR) was then calculated to predict the abundance of each of the ant species based on vegetation factor scores resulting from the six components identified in the PCA extraction (Appendix 9.10).

5.3.1 What are the vegetation requirements of *L. niger*?

Results from the MLR, as illustrated in Table 8, showed that a significant regression equation could not be made between *L. niger* abundance with any of the six components extracted by the PCA analysis (Appendix 9.10.1). This would suggest that the species is not associated with any specific vegetation communities as highlighted in the PCA analysis.

Table 8: Regression results using the 6 PCA Vegetation components extracted with ant species

Species Name	**ANOVA F value**	**R^2**	**ANOVA df**	**ANOVA Significance all variables in MLR**	**Coefficient Component Significance If p<.05**		
					Sig. in MLR	**Individual component number**	**Sig.**
L. niger	1.739	.265	6,29	.147	.147	n/a	n/a
F. rufa	2.566	.347	6,29	**.041**	.041	**6**	**.001**
L. alienus	.329	.064	6,29	.916	.916	n/a	n/a
F. fusca	.849	.149	6,29	.543	.543	n/a	n/a
M. scabrinodis	1.325	.215	6,29	.278	.278	**1**	**.041**
M. ruginodis	2.124	.553	6,29	.081	.081	**2**	**.016**
M. ruginodis	2.124	.553	6,29	.081	.081	**4**	**.042**

The box plot in Figure 24 however, demonstrates differences between the median values for *L. niger* across the four groups suggesting an uneven distribution across Studland. Results from a Kruskal Wallis H test showed these differences to be significant (Chi-square (3) =12.043, p=.007, Appendix 9.10.2). Post Hoc Mann Whitney U tests, which were then conducted to discover where differences between group pairings lay, found significant differences in three pairings (as illustrated in Table 9) one of which (group - - with group ++) was significant after a Bonferroni adjustment was made to the Alpha level to avoid error (.05/6 = p.008). The two outliers seen in the box plot were both in group - - which, despite being the group with the largest number of plots, had *L. niger* presence in two plots only point 18 (plot 12 with 179 ants) and point 27 (plot 34 with 18 ants).

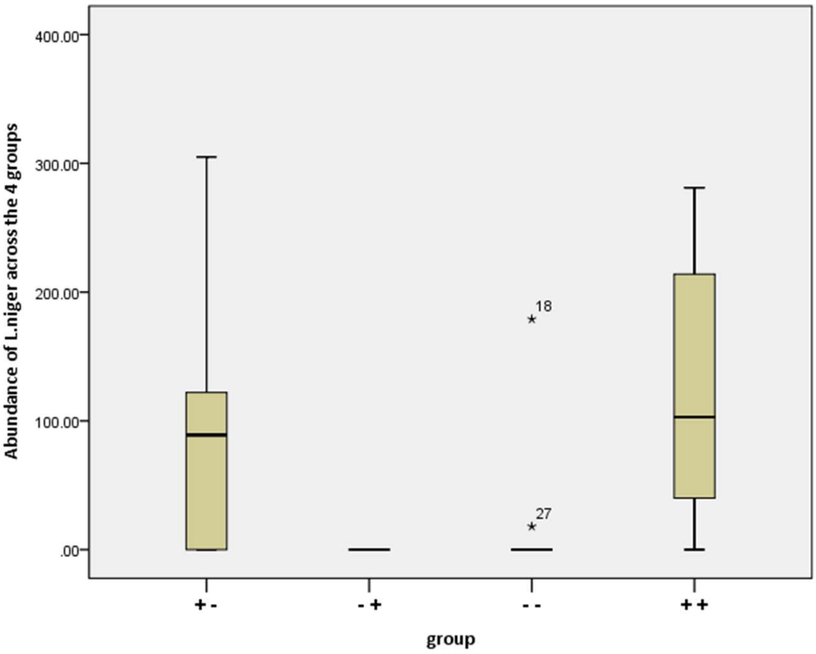

Figure 24: Boxplot showing distribution of *L. niger* across the 4 groups with two outliers at plot 12 (indicated by ★18) and plot 37 (indicated by ★27)

(**Group + -** Sample plots where *P. argus* presence was recorded in 2015 research but not recorded in the 1930s.
Group - + Sample plots where Diver recorded *P. argus* presence in the 1930s but not found in 2015 research.
Group - - Sample plots where *P. argus* has had no recorded presence in Diver's records or in this 2015 research.
Group + + Sample plots where *P. argus* was recorded by Diver in the 1930s and seen in 2015 research).

Table 9: Results from Kruskal Wallis H test showing *L. niger* distribution across groups (The * symbol and use of bold font indicates a significant difference between pairings where p has been adjusted to .008)

Results from Kruskal Wallis H test showing *L. niger* distribution across groups	***L. niger* abundance**
Group Pairings	**Asymp. Sig. (2 tailed)**
+ - and - +	.052
+ - and - -	.024
+ - and + +	.625
- + and - -	.402
- + and + +	.024
- - and + +	**.009***

Although results suggest that *L. niger* did not have specific habitat requirements in terms of vegetation associations, the distribution of plots with *L. niger* presence are primarily concentrated on the western side of the peninsula as shown in Figure 25. It is possible that the components from the PCA are masking the relationship *L. niger* has with individual vegetation variables as the six components extracted from the Vegetation PCA did not account for a high degree of variance individually (see Table 7).

Spearman's Rank Correlation Coefficient was therefore carried out using vegetation data outside of the PCA to see if any correlations existed between ants and the vegetation variables. Table 10 shows that there are significant correlations between three ant species with particular plants; *L. niger* had a significant, positive association with *E. tetralix* % cover and height, with grass under 2 cm % cover and 2-5 cm % cover and a negative association with bryophyte/sphagnum % cover. *F. rufa* had a positive, significant association with leaf litter % cover, shrub % cover and height while *M. ruginodis* showed a positive, significant association with rush and sedge % cover and height.

The positive, significant correlation between *L. niger* with each of the four variables is shown in Figure 26, Figure 27, Figure 28, Figure 29 and Figure 30. The correlation between *L. niger* and *E. tetralix* was not surprising given the distribution of the species on the wetter, western side of the peninsula where *E. tetralix* dominates. There is however, dense cover of *E. tetralix* elsewhere on the peninsula where this species was not found. Possible explanations for this and for the negative correlation with bryophyte/*Sphagnum* cover are suggested in the Discussion in section 6.

Table 10: Spearman's Rank Correlation Coefficient analysis of vegetation components ant species in ant plots (with significant p values <.05 highlighted)

	L. niger		***F. rufa***		***M. ruginodis***		***M. scabrinodis***		***L. alienus***		***F. fusca***	
Name of resource variable	**r**	**p value**	**r**	**p value**	**r**	**p value**	**r**	**p value**	**r**	**p value**	**r**	**p value**
E. tetralix % cover	**.427**	**.009**	-.114	.508	-.094	.586	-.166	.344	.074	.667	.138	.423
E. tetralix height (cm)	**.397**	**.017**	-.123	.475	-.080	.642	-.179	-.296	.101	.559	.436	.134
Grass 2-5 cm % cover	**.384**	**.021**	-.185	.280	-.157	.361	-.009	.957	-.108	.531	.317	.060
Grass <2 cm % cover	**.358**	**.032**	.044	.797	-.187	.275	.006	.971	-.108	.531	.359	.157
Bryophytes /*Sphagnum % cover*	**-.393**	**.018**	.011	.949	.197	.249	.153	.372	.136	.430	-.094	.585
Leaf litter % cover	-.319	.058	**.377**	**.023**	-.107	.535	.215	.207	-.178	.299	.004	.980
Shrub % cover	-.251	.140	**.456**	**.005**	.061	.726	-.206	.229	.011	.949	-.004	.981
Shrub height (cm)	-.201	.239	**.469**	**.004**	.157	.359	-.212	.229	-.234	.169	.024	.889
Rush & sedge % cover	-.073	.672	-.243	.153	**.451**	**.006**	.079	.648	-.086	.620	-.271	.110
Rush & sedge height (cm)	-.073	.672	-.243	.154	**.452**	**.006**	.079	.648	-.086	.620	-.271	.111
Bare ground % cover	.120	.487	-.208	.224	-.138	.421	.197	.251	-.103	.550	.294	.081
C.vulgaris % cover	-.144	.401	-.194	.258	.236	.165	.116	.501	.045	.797	.058	.737
C.vulgaris height (cm)	-.086	.617	-.192	.262	.244	.151	.017	.922	.038	.825	.148	.390
C.introflexus % cover	-.154	.370	.015	.933	.204	.234	-.228	.181	-.138	.422	.732	.059
Cladonia % cover	.193	.261	-.285	.092	.215	.208	.288	.089	-.195	.253	-.001	.994
E.cinerea % cover	-.209	.222	.117	.497	-.071	.682	.160	.351	.140	.414	-.152	.376
E.cinerea height (cm)	-.217	.203	.137	.426	-.026	.878	.180	.292	.098	.571	-.174	.310
Flowering plant % cover	-.025	.883	-.178	.298	.159	.353	.153	.372	-.040	.815	-.187	.276
Flowering plant height (cm)	-.025	.883	-.178	.298	.159	.353	.153	.372	-.040	.815	-.187	.276
Grass 5-10 cm % cover	-.170	.322	.111	.519	.040	.816	.296	.080	-.108	.531	-.220	.910

Table 10 : Spearman’s Rank Correlation Coefficient analysis of vegetation components ant species in ant plots (with significant p values <.05 highlighted)

Name of Resource variable	***L. niger***		***F. rufa***		***M. ruginodis***		***M. scabrinodis***		***L. alienus***		***F. fusca***	
	r	**P value**	**r**	**P value**	**r**	**P value**	**r**	**P value**	**r**	**P value**	**r**	**P value**
Tree % cover	.025	.883	.108	.529	.045	.796	.139	.417	-.205	.230	-.059	.731
Tree height (cm)	.025	.883	.108	.529	.045	.796	.139	.417	-.205	.230	-.059	.731
Water % (standing)	.106	.537	.046	.792	-.129	.469	-.141	.411	-.086	.620	.106	.537

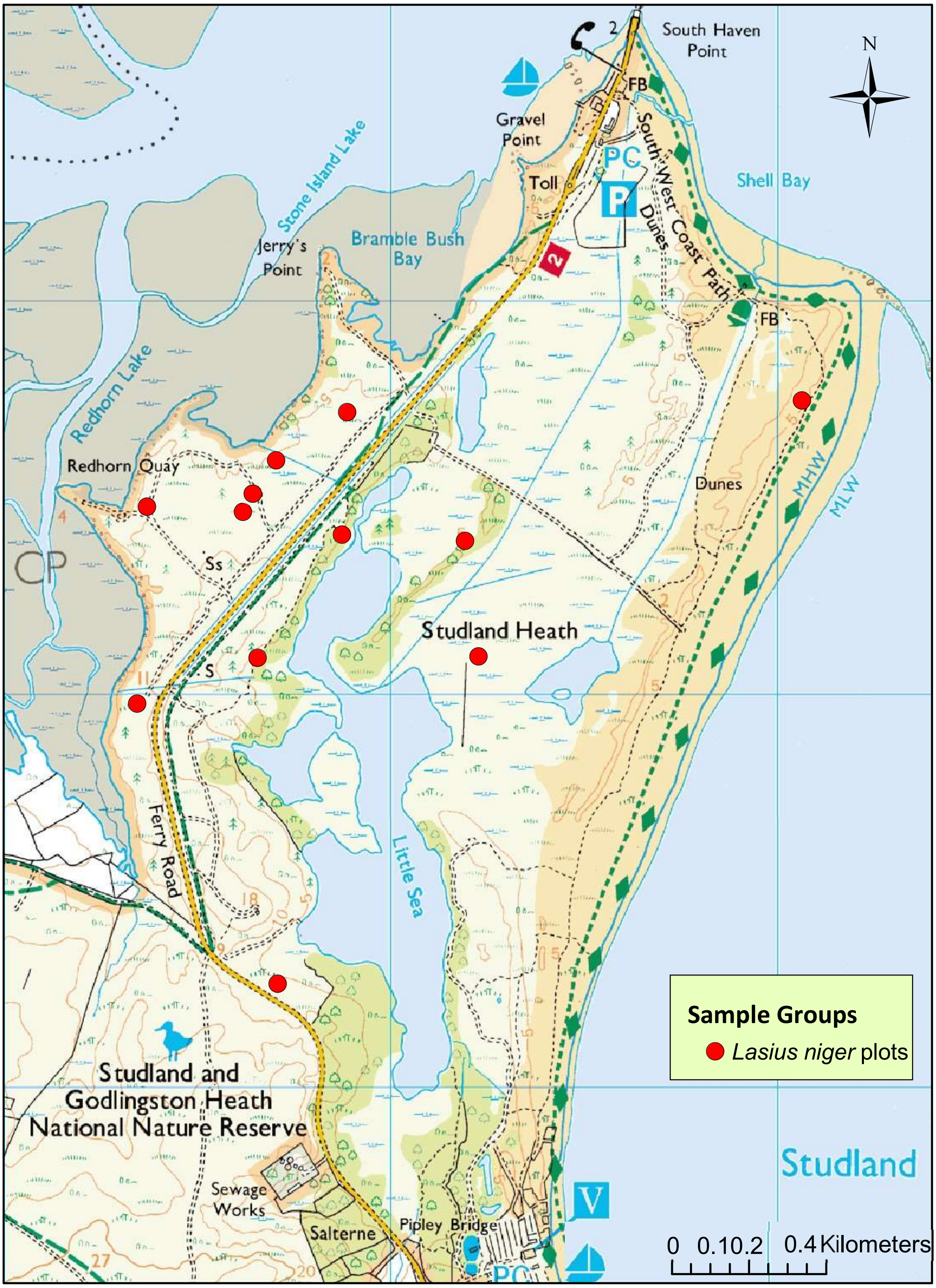

Figure 25 : The position of *L. niger* plots across the peninsula

(Amended from EDINA Digimap Ordinance Survey Service: Studland Peninsula using ArcMap 10.2.2.)

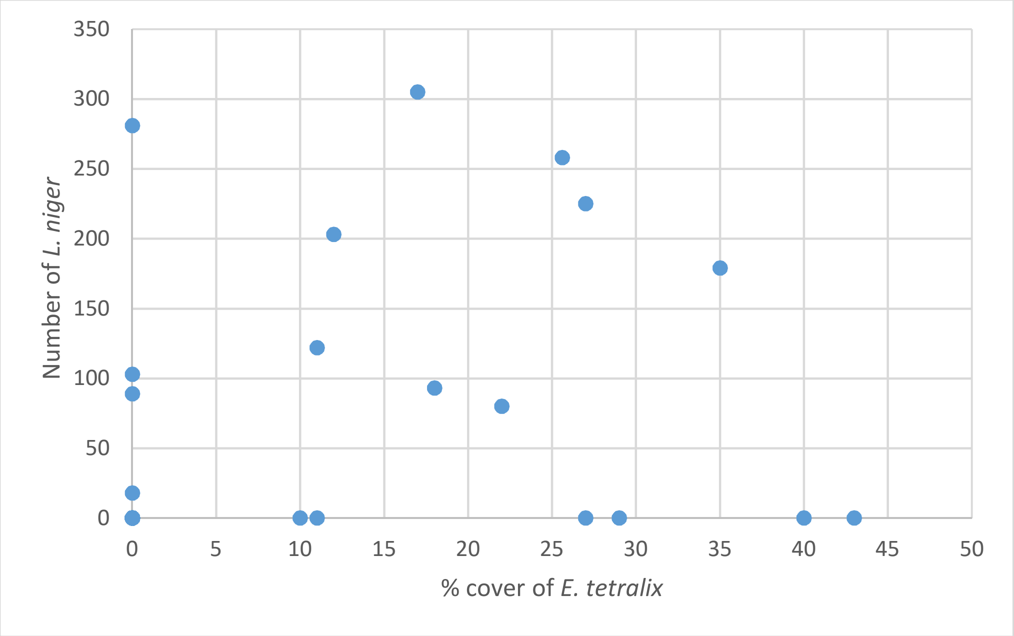

Figure 26 : The significant, positive correlation between *L. niger* and *E. tetralix* % cover (r= .427, p=.009) in ant plots using Spearman's Rank Correlation Coefficient

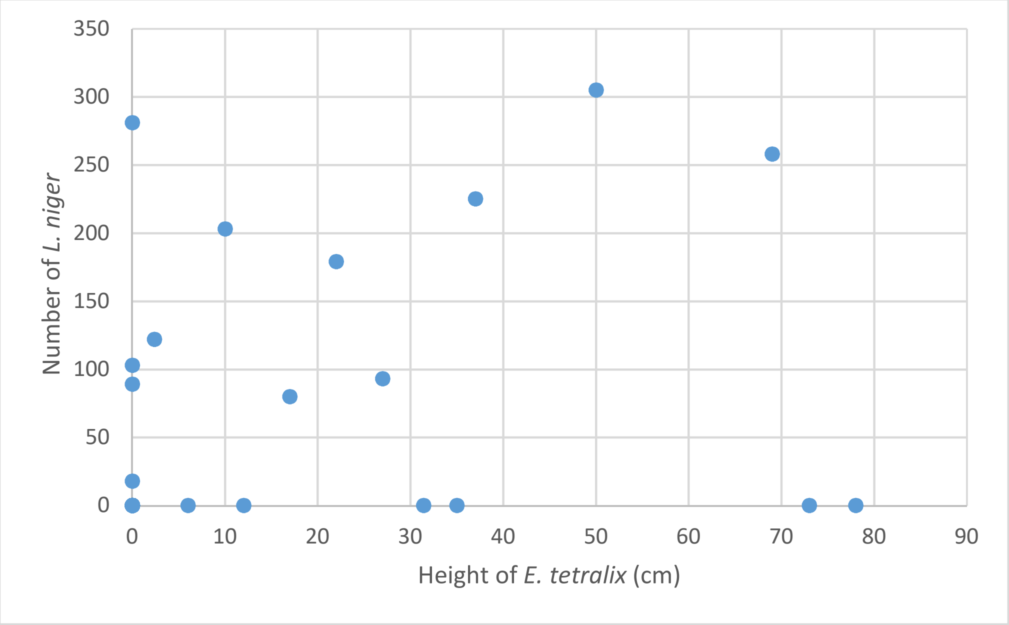

Figure 27 : The significant, positive correlation between *L. niger* and *E. tetralix* height (cm) (r= .397, p=.017) in ant plots using Spearman's Rank Correlation Coefficient

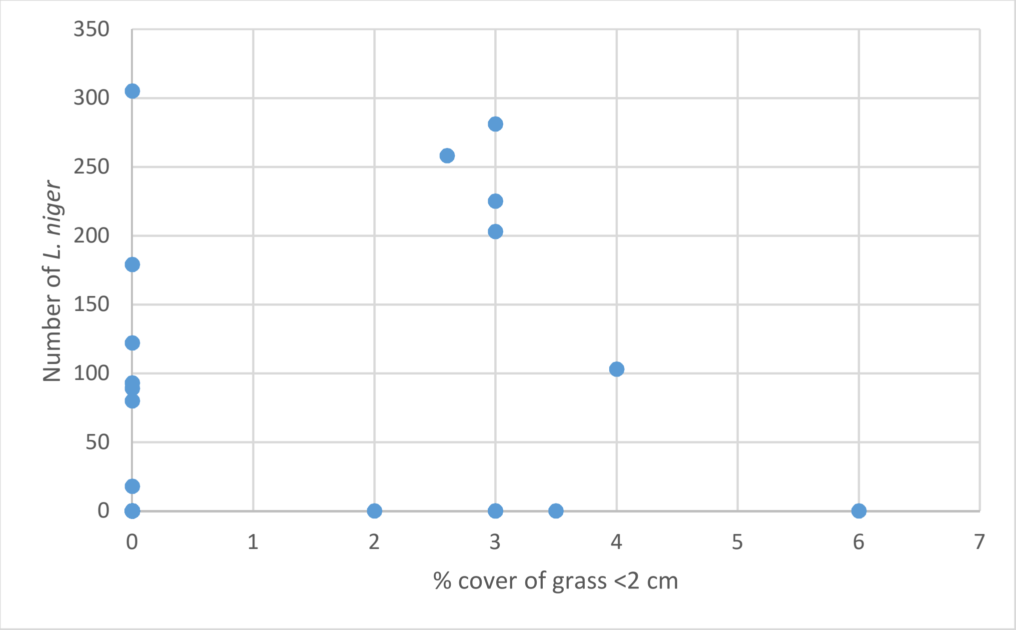

Figure 28 : The significant, positive correlation between *L. niger* and % cover of grass < 2cm (r= .358, p=.032) in ant plots using Spearman's Rank Correlation Coefficient

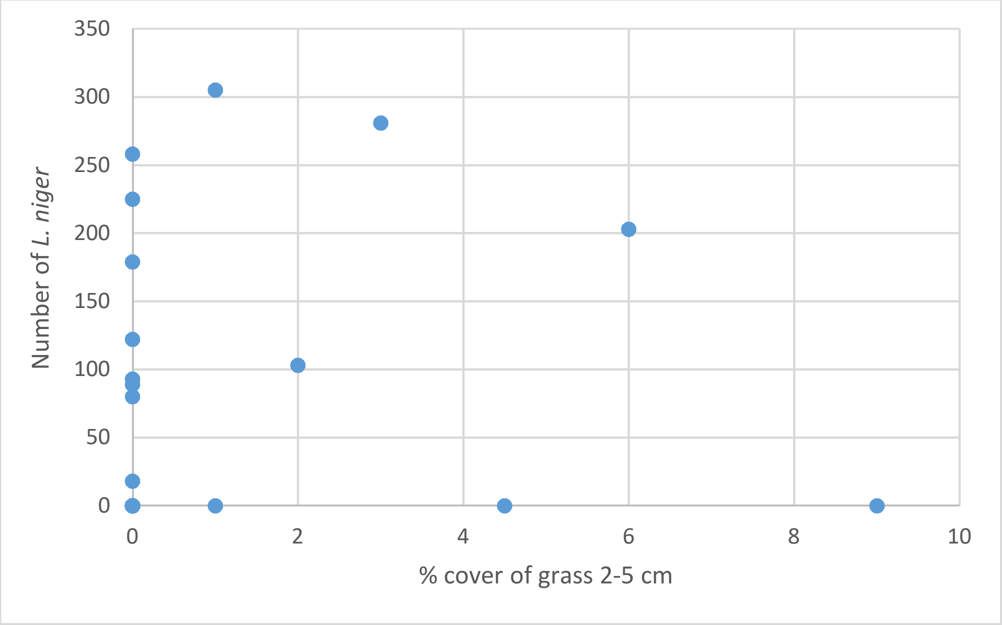

Figure 29 : The significant, positive correlation between *L. niger* and % cover of grass 2-5 cm (r= .384, p=.021) in ant plots using Spearman's Rank Correlation Coefficient

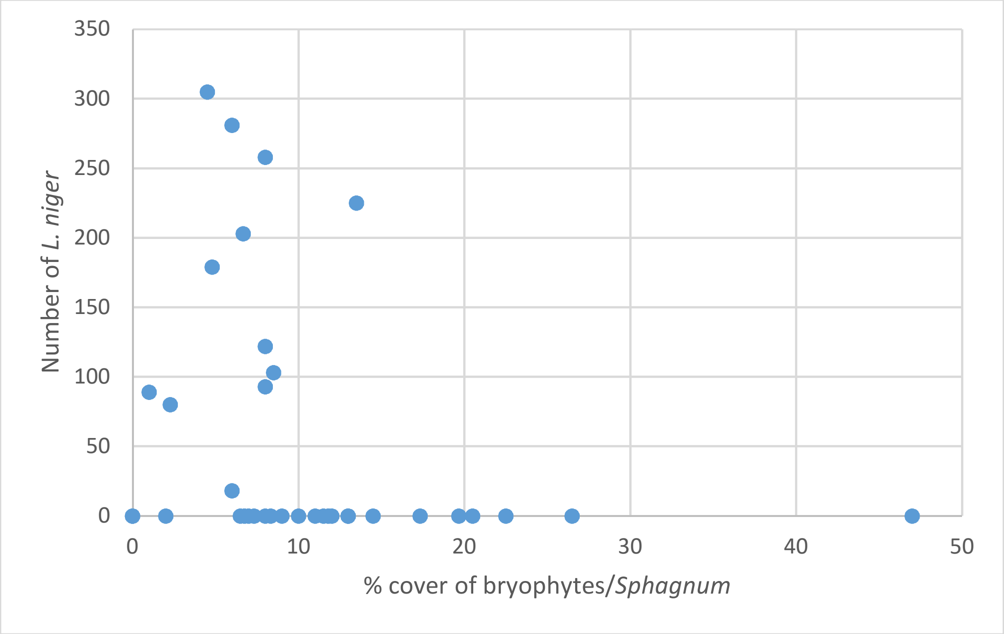

Figure 30 : The significant, negative correlation between *L. niger* and % cover of bryophytes/*Sphagnum* (r= .393, p=.018) in ant plots using Spearman's Rank Correlation Coefficient

5.3.2 ***F. rufa* and vegetation requirements**

Results from the MLR demonstrated a significant regression equation with the vegetation components extracted from the PCA. Table 8 shows that *F. rufa* had a significant relationship (p=.001, Appendix 9.10.3) with Component 6 which had the highest loadings for percentage cover of shrubs and bare ground (Table 11) and made up 7.47% of the total variance. Figure 31 shows this positive, linear relationship between *F. rufa* and Component 6.

Table 11: Components extracted from Vegetation PCA

Component Number	Highest Loadings within component group
1	% cover of grass 5-10 cm, trees and leaf litter/dead wood.
2	% cover of ground plants and flowering plants.
4	% cover of grass 2-5cm and bryophytes/Sphagnum.
6	% cover of shrubs and bare ground

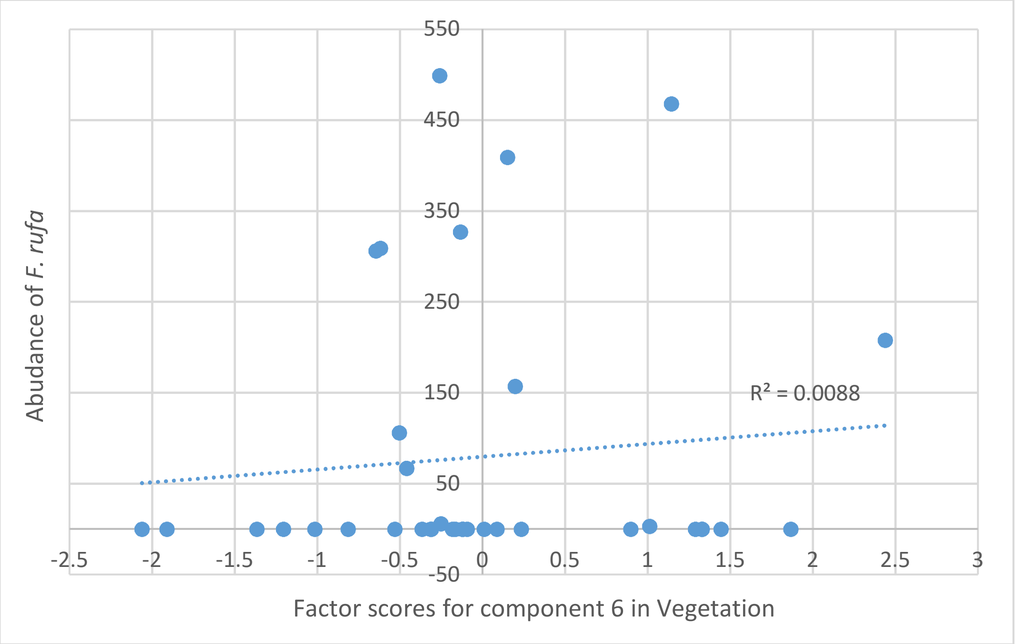

Figure 31 : The significant, positive linear regression between abundance of *F.rufa* across all sites with factor scores for Component 6 in Vegetation PCA

The box plot in Figure 32 shows the distribution of *F. rufa* across the four groups. Although differences between the groups were not found to be statistically significant when a Kruskall Wallis H test was carried out, the presence of outliers in Group + -, Group - + and Group + + in addition to the large distribution range in Group - -, indicate that *F. rufa* had an uneven distribution across the peninsula but where they were found, their numbers were generally high. In Group - - for example, the number of *F. rufa* in plots ranged from 0 (in eight plots) to 499 (plot 14) and there were at least 150 ants in each of the other six plots where presence was found. The box plot in Figure 33 illustrates the distribution of Component 6 (characterised by shrub and bare ground) from the Vegetation PCA. Although there was no statistical difference between groups when a Kruskall Wallis H test was conducted, the box plot indicates that the distribution is uneven with high outliers in Group - - mirroring *F. rufa* distribution results. The map in Figure 34 shows the distribution of *F. rufa* plots across the peninsula.

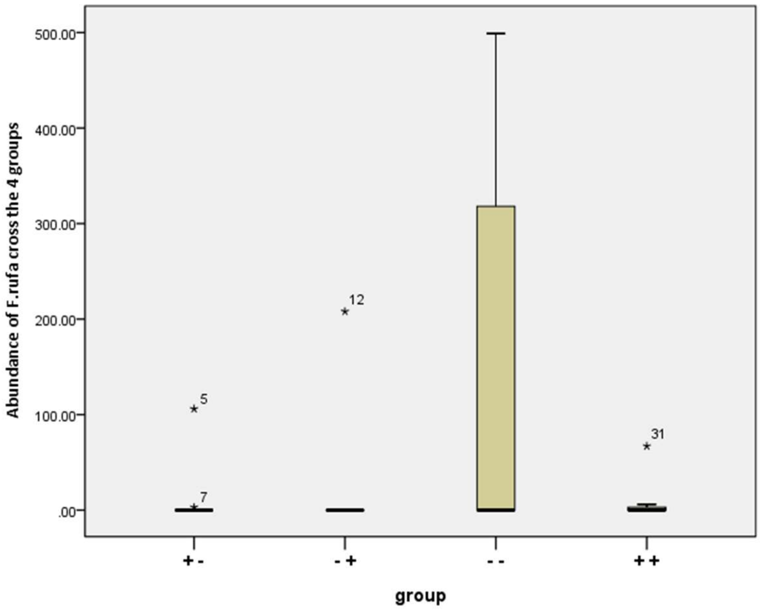

Figure 32 : Box plot showing the uneven distribution of *F. rufa* across the 4 groups

(**Group +** - Sample plots where *P. argus* presence was recorded in 2015 research but not recorded in the 1930s.
Group - + Sample plots where Diver recorded *P. argus* presence in the 1930s but not found in 2015 research.
Group - - Sample plots where *P. argus* has had no recorded presence in Diver's records or in this 2015 research.
Group + + Sample plots where *P. argus* was recorded by Diver in the 1930s and seen in 2015 research).

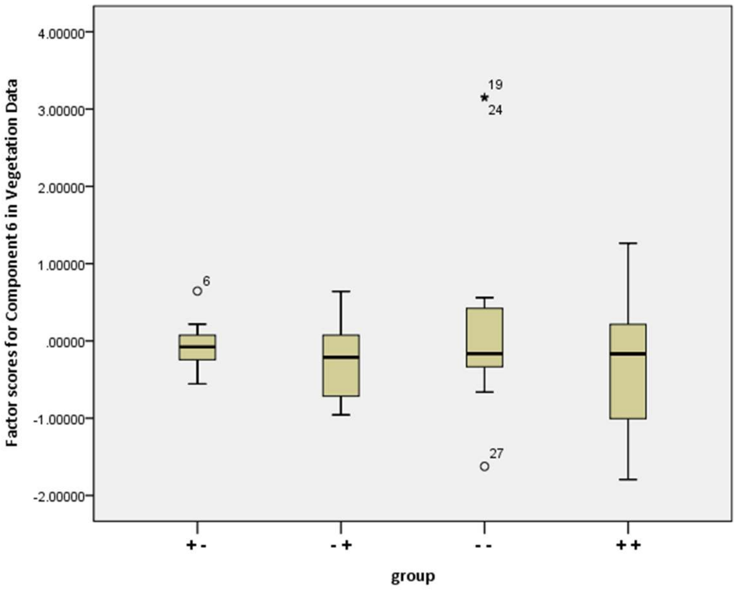

Figure 33 : Box plot showing the uneven distribution of Component 6 Vegetation across the 4 groups

(**Group +** - Sample plots where *P. argus* presence was recorded in 2015 research but not recorded in the 1930s.
Group - + Sample plots where Diver recorded *P. argus* presence in the 1930s but not found in 2015 research.
Group - - Sample plots where *P. argus* has had no recorded presence in Diver's records or in this 2015 research.
Group + + Sample plots where *P. argus* was recorded by Diver in the 1930s and seen in 2015 research).

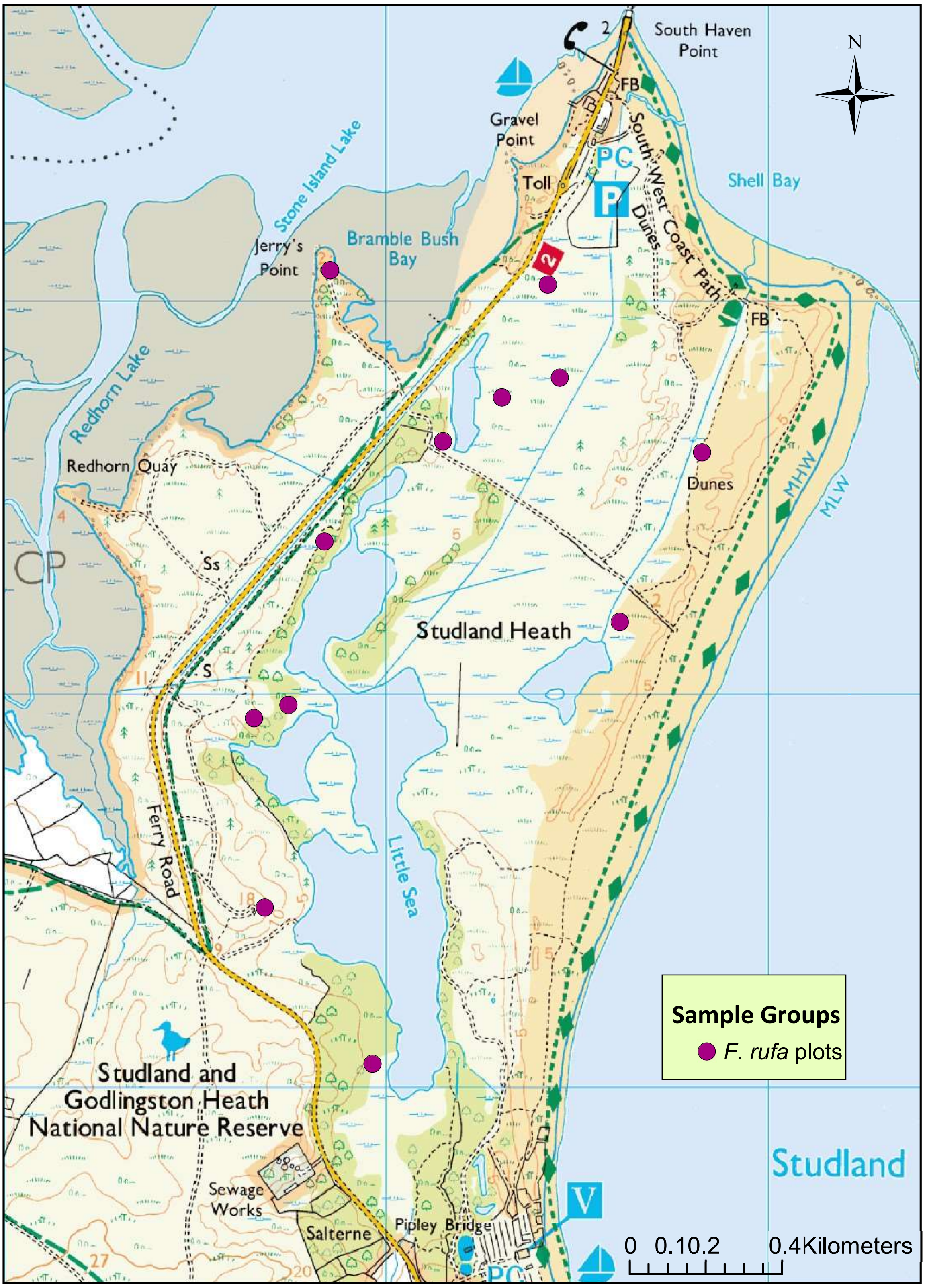

Figure 34 : The position of *F. rufa* plots across the peninsula

(Amended from EDINA Digimap Ordinance Survey Service: Studland Peninsula using ArcMap 10.2.2.)

Although *F. rufa* did show a significant regression equation with Component 6 of the PCA, this component only accounted for 7.47% of the variance however (see Table 7). Spearman's Rank Correlation Coefficient was therefore carried out to highlight any correlations between *F. rufa* with individual plant species which are being masked in the PCA extraction. Table 10 shows a significant, positive correlation between *F. rufa* with percentage cover of leaf litter (r=.377, p=.023) shrub cover (r=.456, p=.005) and shrub height (r=.469, p=.004) which is illustrated in Figure 35, Figure 36 and Figure 37.

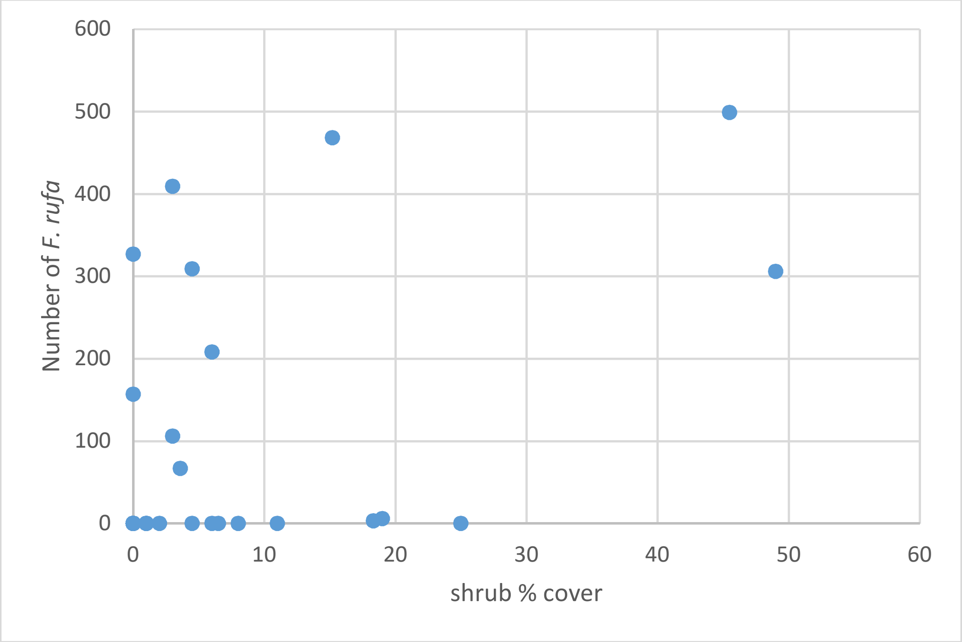

Figure 35 : The significant, positive correlation between *F. rufa* and % cover of shrubs (r=.456, p=.005) in ant plots using Spearman's Rank Correlation Coefficient

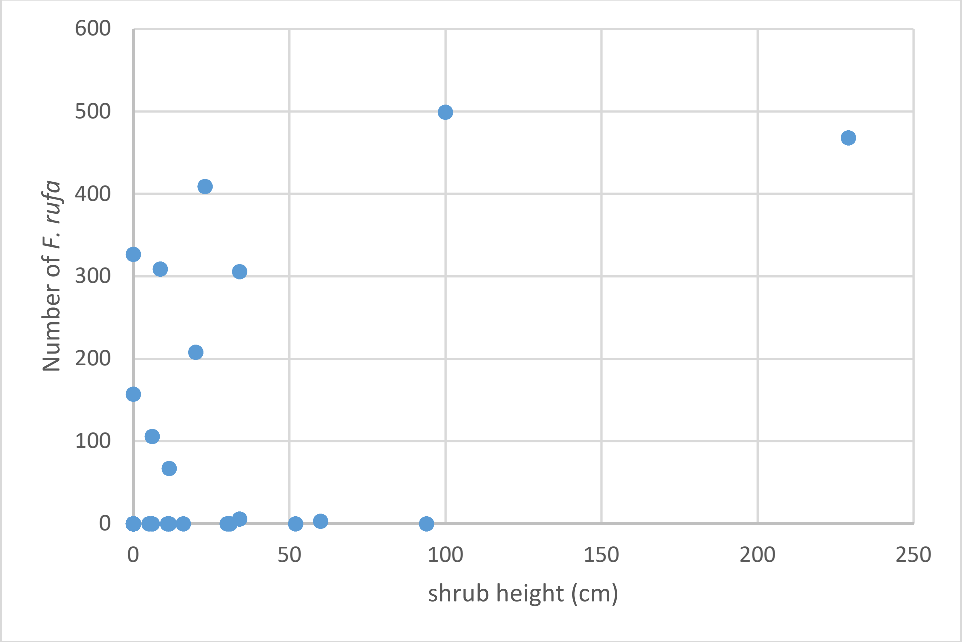

Figure 36 : The significant, positive correlation between *F. rufa* and shrub height (r=.469, p=.004) in ant plots using Spearman's Rank Correlation Coefficient

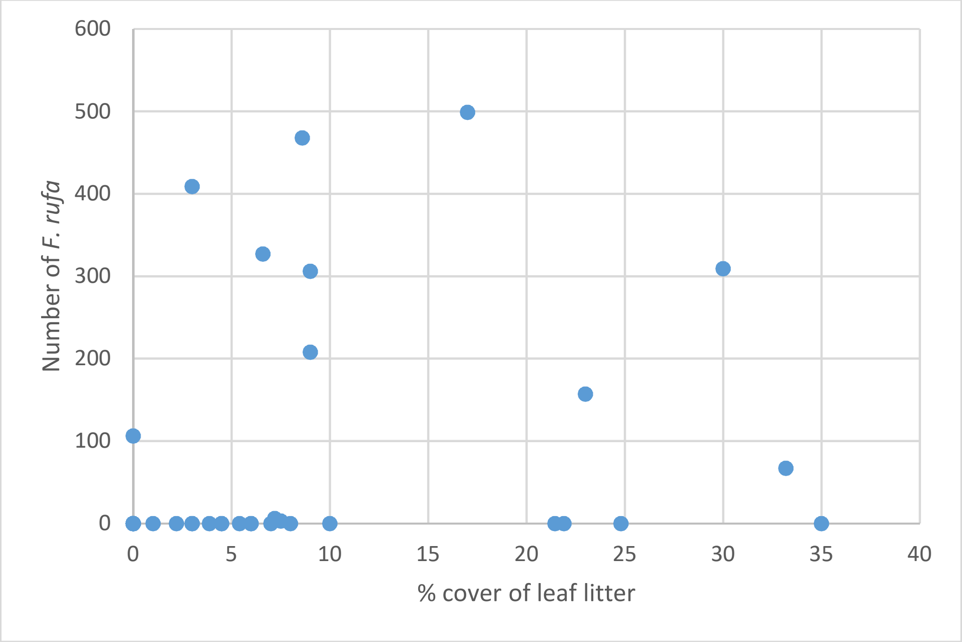

Figure 37 : The significant, positive correlation between *F. rufa* and % cover of leaf litter (r= .377, p=.023) in ant plots using Spearman's Rank Correlation Coefficient

5.3.3 *L. alienus* and *F. fusca* with Vegetation Requirements

Results from the MLR, as illustrated in Table 8, show that a significant regression equation could not be made between either *L. alienus* or *F. fusca* abundance with any of the six components extracted by the PCA analysis (Appendix 9.10.4 and 9.10.5). No specific vegetation associations were therefore identified as habitat requirements for either ant species on the peninsula. When Spearman's Rank Correlation Coefficient was carried out (Table 10) neither *L. alienus* or *F. fusca* showed a significant correlation with any of the individual vegetation variables in the ant plots. Figure 38 shows the location of the *F. fusca* plots which are predominantly on the western side of the peninsula and the two *L. alienus* plots (2 and 3) on Second Ridge.

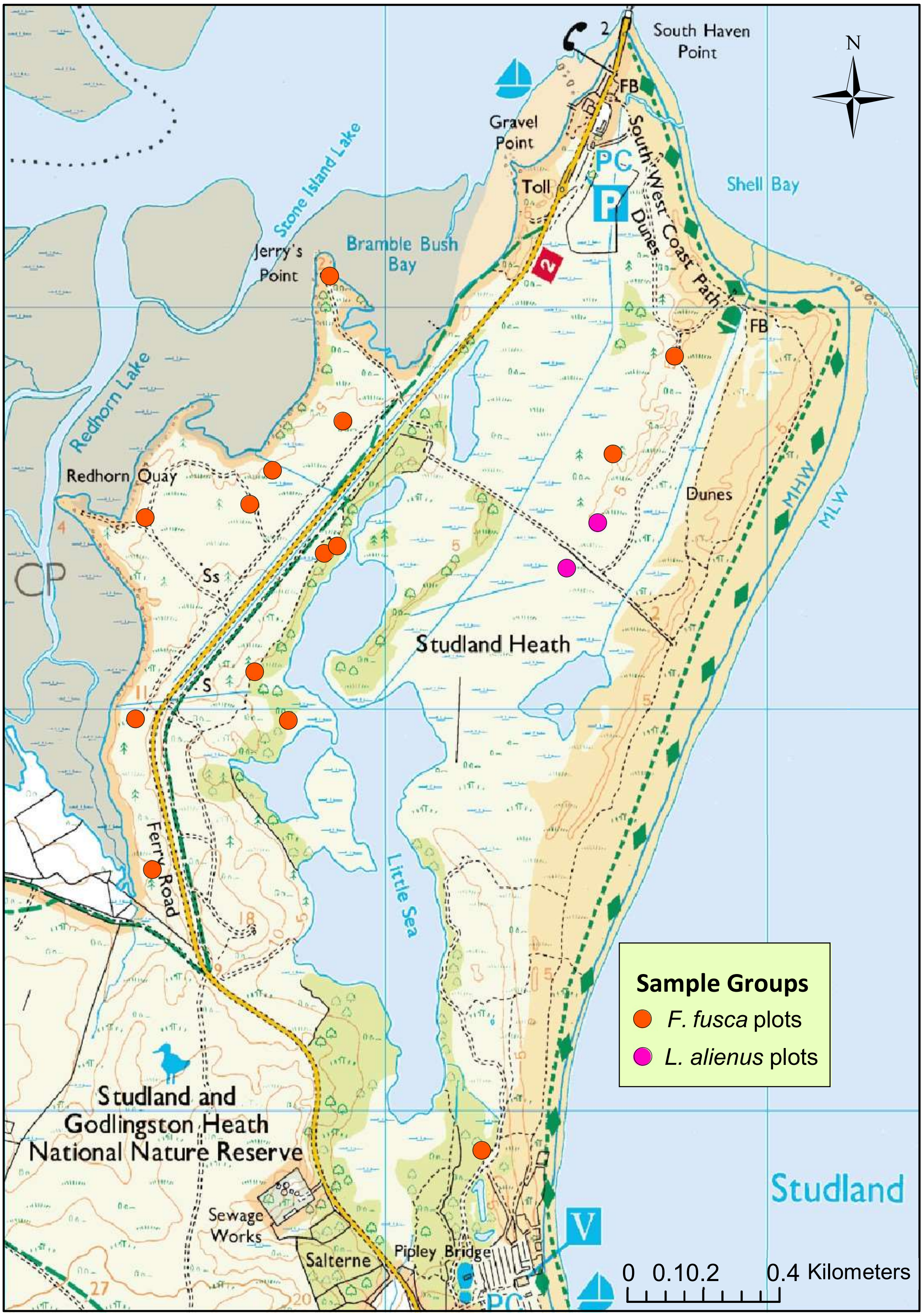

Figure 38 : The location of *F. fusca* and *L. alienus* plots across the peninsula

(Amended from EDINA Digimap Ordinance Survey Service: Studland Peninsula using ArcMap 10.2.2.)

5.3.4 *Myrmica* species and Vegetation Requirements

Results from the MLR, as illustrated in Table 8, show that both *Myrmica* species found on the peninsula, have a significant regression equation with individual vegetation components (extracted by the PCA) which may suggest specific habitat preferences. When Spearman's Rank Correlation Coefficient was carried out (Table 10), *M. scabrinodis* was not found to have a significant correlation with any of the vegetation variables in the ant plots while *M. ruginodis* did show a positive significant correlation with rush and sedge % cover and height (cm).

5.3.4.1 M. scabrinodis

While the overall MLR regression equation did not indicate a significant association between *M. scabrinodis* and vegetation, when all variables were considered together, a significant regression equation was found with Component 1 where p=.014 (Appendix 9.10.6). Component 1, as illustrated in Table 12, which made up 19.78% of the total variance, is primarily associated with percentage cover of grass 5-10 cm and leaf litter/dead wood. Figure 39 shows the positive, linear relationship between *M. scabrinodis* with Component 1 across all groups. Myrmecological research (described in the Literature Review in Section 3) reports that this ant species can be found in a wide range of habitats including open forest, woodland, bogs and marshes which is supported by the vegetation associations in Component 1. *M. scabrinodis* had a small distribution however and was found in only 5 plots (numbers 16, 24, 25, 26 and 33).

Table 12: The highest variable loadings for each component in Vegetation PCA extraction

Component Number	Highest Loadings within component group
1	% cover of grass 5-10 cm, trees and leaf litter/dead wood.
2	% cover of ground plants and flowering plants.
4	% cover of grass 2-5cm and bryophytes/*Sphagnum.*
6	% cover of shrubs and bare ground

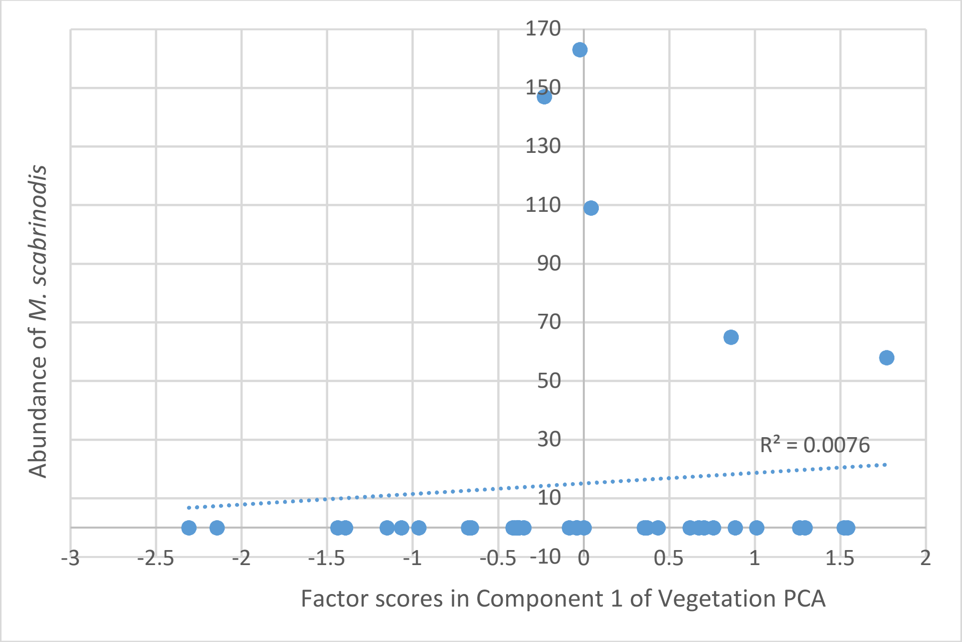

Figure 39 : The significant, positive linear regression between abundance of *M. scabrinodis* across all sites with factor scores for Component 1 in Vegetation PCA

The box plot in Figure 40 shows the distribution of *M. scabrinodis* across the four groups. Interestingly the groups were not found to be statistically significant when a Kruskall Wallis H test was carried out. This would appear to be because *M.scabrinodis* has a presence in all four groups although it is only found in 5 out of the total 40 plots. Conversely, Component 1 which *M. scabrinodis* are associated with in the MLR, was found to be evenly distributed without outliers (Figure 41) and with no significant differences between groups (Kruskall Wallis H test).

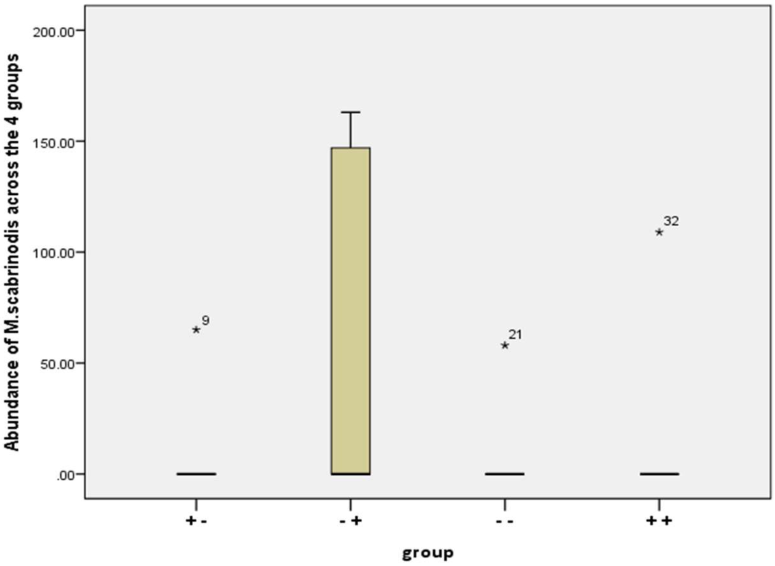

Figure 40 : Box plot showing the distribution of *M. scabrinodis* across the 4 groups

(**Group +** - Sample plots where *P. argus* presence was recorded in 2015 research but not recorded in the 1930s.
Group - + Sample plots where Diver recorded *P. argus* presence in the 1930s but not found in 2015 research.
Group - - Sample plots where *P. argus* has had no recorded presence in Diver's records or in this 2015 research.
Group + + Sample plots where *P. argus* was recorded by Diver in the 1930s and seen in 2015 research).

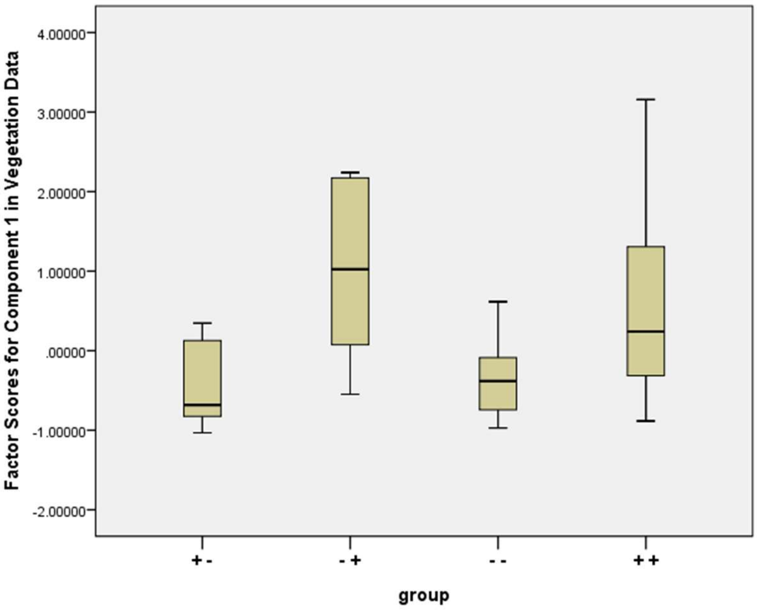

Figure 41 : Box plot showing the even distribution of Component 1 Vegetation across the 4 groups

(**Group +** - Sample plots where *P. argus* presence was recorded in 2015 research but not recorded in the 1930s.
Group - + Sample plots where Diver recorded *P. argus* presence in the 1930s but not found in 2015 research.
Group - - Sample plots where *P. argus* has had no recorded presence in Diver's records or in this 2015 research.
Group + + Sample plots where *P. argus* was recorded by Diver in the 1930s and seen in 2015 research).

5.3.4.2 M. ruginodis

While the overall MLR regression equation did not indicate a significant association between *M. ruginodis* and vegetation when all variables were considered together; a significant regression equation was found specifically with Components 2 (p=.016) and 4 (p=.042) (seeTable 8 and Appendix 9.10.7). Component 2, which made up 12.26% of the total variance, is primarily associated with percentage cover of ground and flowering plants while Component 4, which made up 9.23% of the total variance, is associated with percentage cover of bryophytes/*Sphagnum* and grass 2-5 cm in height. This ant species, which had a small distribution as it was found in only 4 out of a total of 40 plots, has been recorded in a wide range of habitats including woodland clearings, forests, boggy areas and grasslands where nests are frequently constructed under bark and rotten wood in forests and in the soil in grasslands (Radchenko and Elmes 2010). Figure 42 and Figure 43 show that in both cases, *M. ruginodis* had a negative relationship with Component 2 and Component 4.

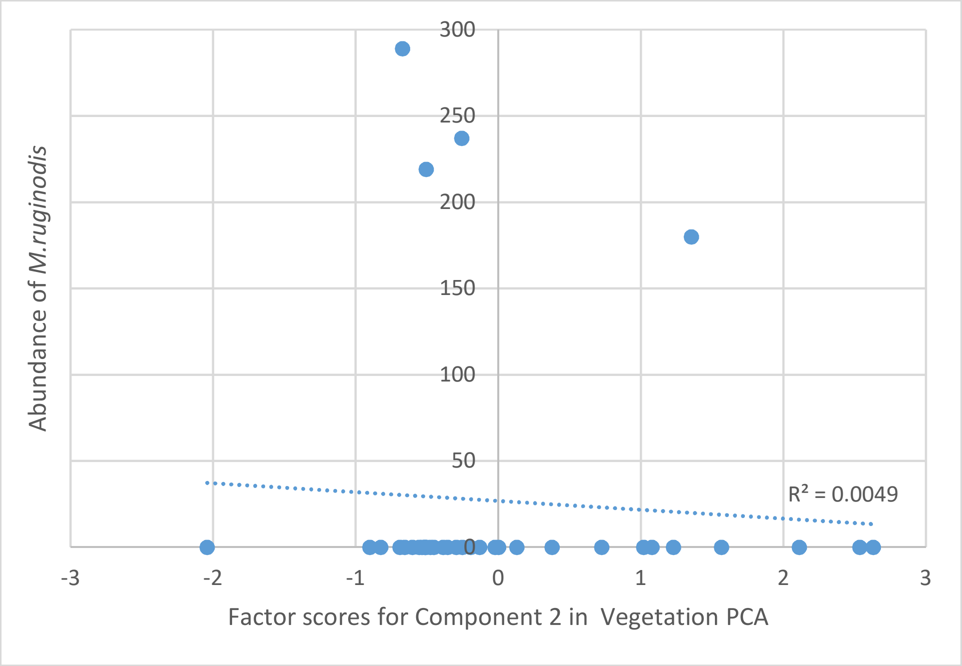

Figure 42 : The significant, negative linear regression between abundance of *M. ruginodis* across all sites with factor scores for Component 2 in Vegetation PCA

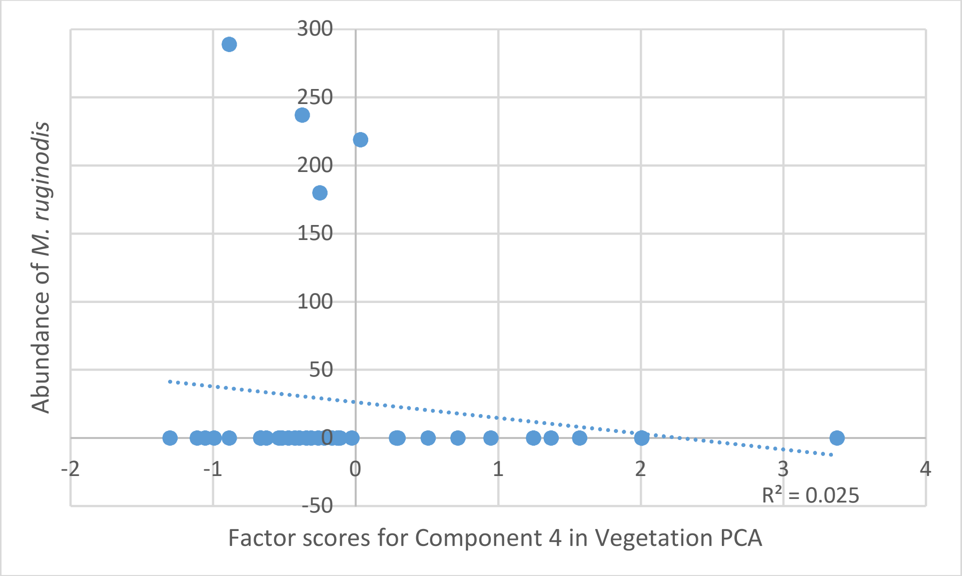

Figure 43 : The significant, negative linear regression between abundance of *M. ruginodis* across all sites with factor scores for Component 4 in Vegetation PCA

As Component 2 and Component 4 made up just 12.26% and 9.23% respectively of the total variance and due to the small number of plots found to contain *M. ruginodis*, Spearman's Rank Correlation Coefficient was carried out on independent vegetation variables to accommodate uneven distributions and extreme outliers. The results showed two positive, significant correlations (Table 10); between *M. ruginodis* with rush and sedge % cover and rush and sedge height (cm) which are illustrated in Figure 44 and Figure 45.

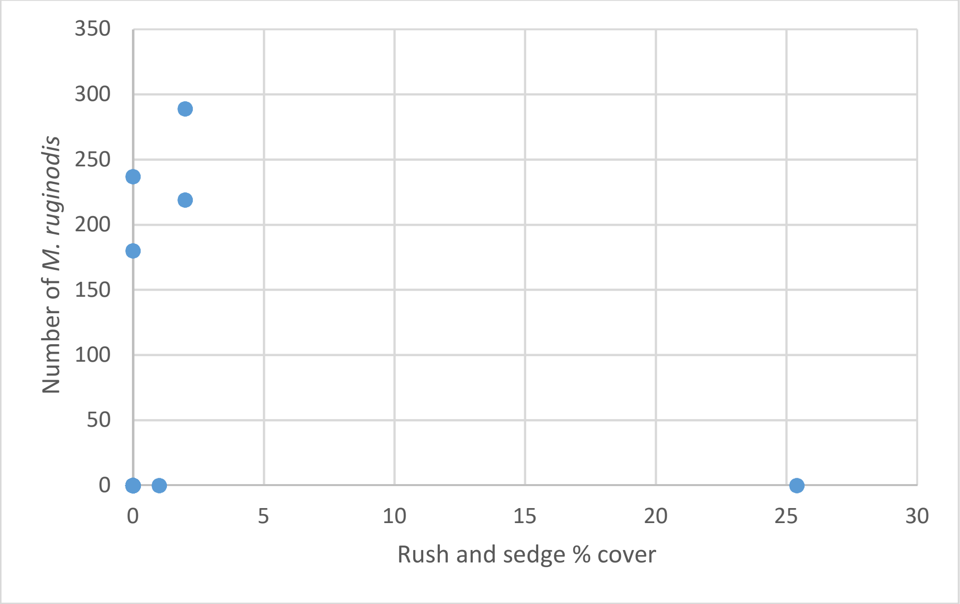

Figure 44: The significant, positive correlation between *M. ruginodis* and % cover of rushes and sedge (correlation coefficient .451, p=.006) in ant plots using Spearman's Rank Correlation Coefficient

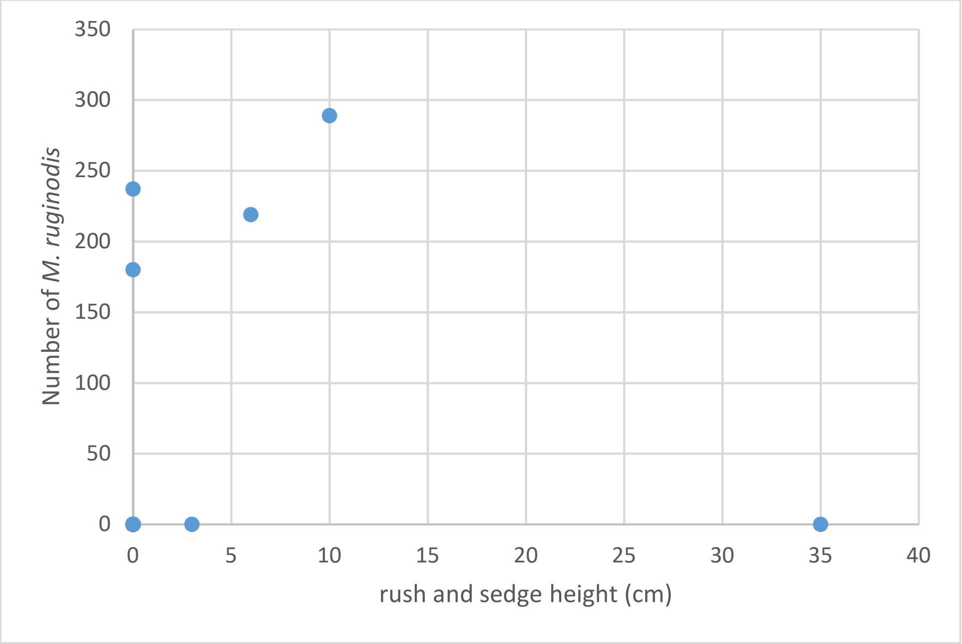

Figure 45 : The significant, positive correlation between *M. ruginodis* and height of rushes and sedge (cm) (correlation coefficient .452, p=.006) in ant plots using Spearman's Rank Correlation Coefficient

Figure 46 shows the relative locations of the *Myrmica* ant plots. The small number of plots makes analysis of preference difficult to determine (see Discussion in section 6).

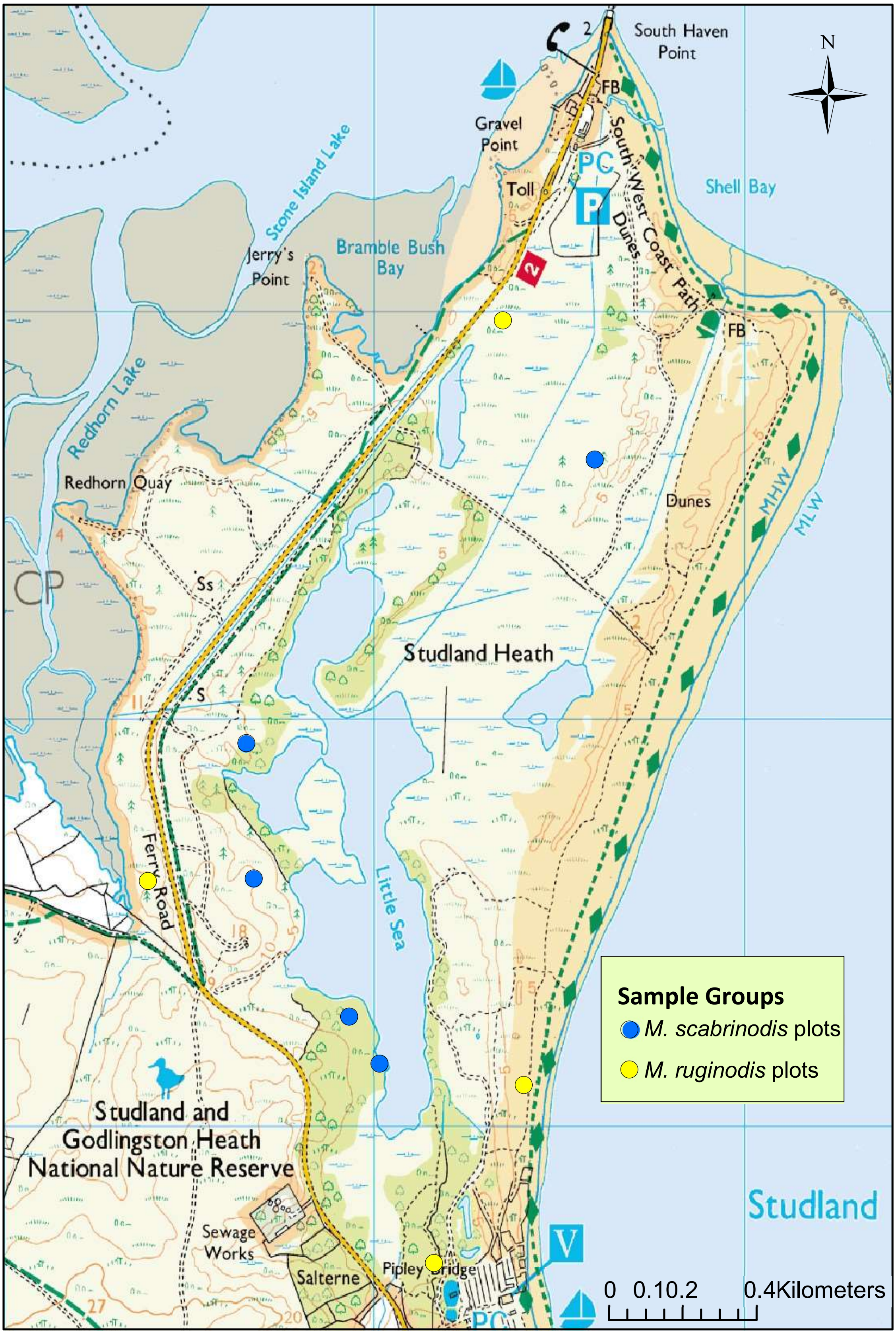

Figure 46 Location of *M. scabrinodis* and *M. ruginodis* plots across the peninsula

(Amended from EDINA Digimap Ordinance Survey Service: Studland Peninsula using ArcMap 10.2.2.)

5.3.5 How important are soil components in driving *P. argus* abundance and distribution?

To assess whether there was any association between *P. argus* abundance and distribution with soil components, PCA combined with MLR was carried out to ascertain if soil could be seen as a driver of the butterfly's distribution. Given the strong association found between *P. argus* and *L. niger* abundance in this research, the PCA factor scores in the three extracted components were also regressed against *L. niger* abundance.

PCA was employed to reduce the 32 individual soil variables influencing plot diversity and to highlight any underlying patterns within the soil chemistry. 3cm and 10cm samples were analysed separately hence PCA was performed on the 16 variables influencing each sample depth. In the 3cm soil PCA, total carbon and organic carbon results were removed from the analysis due to high correlations with organic matter while sodium was removed due to its high correlation with conductivity. Table 13 shows the three extracted components or groupings which emerged from the remaining 13 variables at the 3cm depth, accounting for 77.67% of the variance between plots in the data (Appendix 9.11). Factor Analysis Rotation using Varimax with Kaiser Nomalization was conducted to emphasise any patterns or groupings emerging in the data which might explain variance between the plots. The Rotated Component Matrix in Table 14 illustrates the loading score of each variable within the three extracted components. In each component, pairings emerge; in Component One magnesium (.905) and organic matter (.839) score most highly; Component Two shows a pairing between copper and lead (both over .9) while Component Three shows highest loadings for pH at over .9 and conductivity at .744.

Table 13: Rotated Component Matrix for 3 cm soil samples

(the blue shading indicates the highest loadings)

Soil Variable	Key Components Extracted		
	1	2	3
Magnesium mg/kg	**.905**	.151	.228
Organic Matter %	**.839**	.253	-.015
Lead mg/kg	.245	**.914**	.013
Copper mg/kg	-.009	**.906**	.076
Conductivity µS/cm	.387	.229	**.744**
pH (H20)	-.034	-.021	**.935**
Cadmium mg/kg	.783	.087	.427
Zinc mg/kg	.752	.012	-.132
Total Nitrogen %	.745	.446	.085
Manganese mg/kg	.738	.099	.250
Potassium mg/kg	.726	.296	.382
Phosphorous mg/kg	.610	.568	.106
Iron mg/kg	.580	.611	.375

a. Rotation converged in 4 iterations

b. Bold font indicates results with highest loading scores within each component

Table 14 : Total Variance Explained; the % of variance contributed by each component making up the total 77.67% which is highlighted in blue (with Kaiser-Meyer-Olkin Measure of Sampling Adequacy at.713)

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	6.98	53.73	53.73	6.98	53.73	53.73	5.24	40.37	40.37
2	1.65	12.75	66.48	1.65	12.75	66.48	2.79	21.51	61.89
3	1.45	11.18	77.67	1.45	11.18	77.67	2.05	15.78	**77.67**

Having established the presence of key soil components at the 3cm depth, it was important to consider whether these soil associations were drivers of the presence of *P. argus* or *L. niger*. Multiple Linear Regression (MLR) was calculated to predict the abundance of *P. argus* based on 3cm soil factor scores resulting from the 3 components identified in the PCA extraction (Appendix 9.12). A significant regression equation was not found (F (3,36) = .842 p= .480) with R^2 of .066. None of the 3 extracted soil components were found to be significant predictors of *P. argus* abundance.

MLR was then carried out to predict the abundance of *L. niger* based on the 3 extracted components resulting from the PCA. The combined group was not found to be a significant predictor of *L. niger* abundance (F (3,36) = 2.03 p=.127) with R^2 of .074 (Appendix 9.13). When *L. niger* was regressed against Component one only however, a significant positive regression equation was found (p=.016, Appendix 9.13.1). A significant linear relationship can therefore be seen between Component 1 (which made up 53.73% of the total variance) with the highest loadings for magnesium/organic matter and *L. niger* abundance which increase simultaneously (Figure 47).

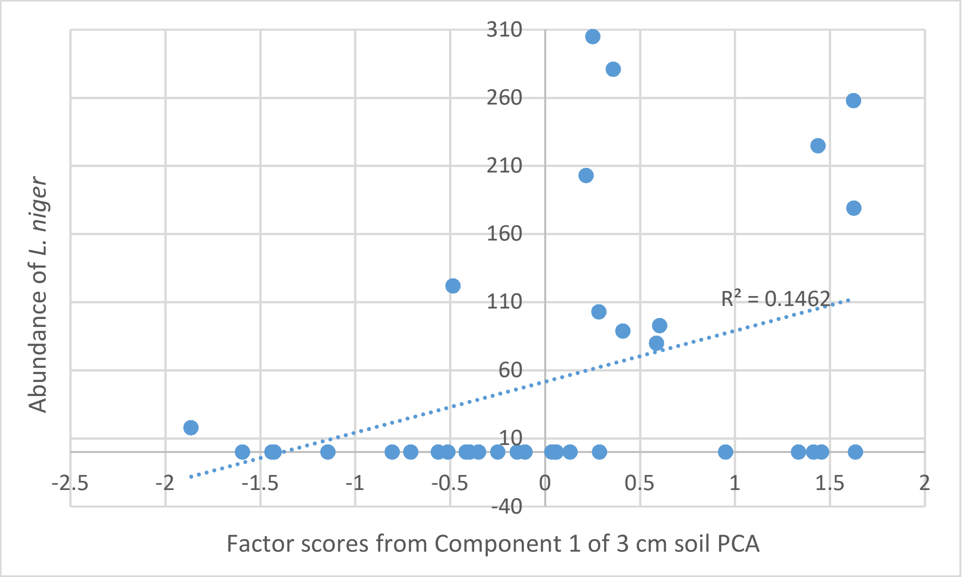

Figure 47 : Significant positive regression equation predicting abundance of *L. niger* with Component 1 in 3cm soil samples

5.4 Does soil depth at 10 cm influence *P. argus* distribution?

Having established an association between soil Component 1 at the 3cm depth with *L. niger*, it was likewise important to ascertain whether there was a relationship between either *P. argus* or *L. niger* with soil at the 10cm depth hence a new PCA was carried out. In the 10cm soil PCA, cadmium and phosphorous were removed from the analysis as both showed high correlations with magnesium (.9+). Table 15 illustrates the three extracted components which emerged from the remaining 12 variables at the 10cm depth, accounting for 72.31% of the variance between plots in the data (Appendix 9.14). Factor Analysis Rotation (using Varimax with Kaiser Nomalization) was conducted to emphasise any patterns emerging in the data which might explain variance between the plots. The Rotated Component Matrix in Table 15 illustrates the loading score of each variable within the three extracted components. Component One shows an association between magnesium and sodium which both score + .9 with total nitrogen, organic matter and copper score contributing to variance at + .8. Component Two shows pH level to be the greatest contributor to variance at .880 while Component Three shows the highest score for zinc at .770 within a generally low scoring group. Table 16 shows the percentage each component contributed to the overall variance of 72.31%.

Table 15: Rotated Component Matrix from 10 cm soil samples

(the blue shading indicates the highest loadings)

	Component		
Soil Variables	1	2	3
Magnesium mg/kg	**.944**	.149	-.104
Sodium mg/kg	**.923**	.262	.079
Organic Matter %	**.870**	-.088	.122
Total nitrogen %	**.859**	-.003	.060
Copper mg/kg	**.847**	.269	-.134
pH H20	-.071	**.880**	-.091
Potassium mg/kg	.595	**.618**	.177
Zinc mg/kg	.197	.121	**-.770**
Iron mg/kg	.130	.144	**.510**
Lead mg/kg	.773	.230	.190
Manganese mg/kg	.719	.116	-.304
Conductivity µS/cm	.491	.598	.303

Table 16: Total Variance Explained; the % of variance contributed by each component making up the total 72.31% which is highlighted in blue (with Kaiser-Meyer-Olkin Measure of Sampling Adequacy at .805)

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	6.19	51.64	51.64	6.19	51.64	51.64	5.72	47.70	47.70
2	1.37	11.47	63.11	1.37	11.47	63.11	1.78	14.88	62.59
3	1.10	9.19	72.31	1.10	9.19	72.31	1.16	9.72	72.31

Multiple Linear Regression (MLR) was calculated to predict the abundance of *P. argus* based on 10 cm soil factor scores resulting from the 3 components identified in the PCA extraction (Appendix 10.13). A significant regression equation was not found (F (3,36) = .116 p=.950) with R^2 of .098 (Appendix 9.15). None of the 3 extracted 10 cm soil components were found to be significant predictors of *P. argus* abundance. MLR was then carried out to predict the abundance of *L. niger* based on the 3 key components resulting from the PCA. Neither the combined group (F (3,36) = .982 p=.412) with R^2 of .076 (Appendix 9.16) nor individual components were found to be significant predictors of *L. niger* abundance.

Overall, soil components do not seem to be factors which influence the distribution and abundance of *P. argus* across the peninsula although there is an association between *L. niger* with Component 1 in the 3 cm soil samples. The potential reasons for this outcome are considered in the Discussion section which follows.

6 Discussion of the results

The Discussion has been divided into three separate sections which follow the three sub-questions emerging from the main research question. The first section (6.1) considers the current distribution of *P. argus* on the peninsula and discusses the resources deemed, in the Results section, to be important for the butterfly's survival. Positive drivers of distribution include the presence of the host plant *E. tetralix*, abundant cover of grass 2-5 cm and the presence of the symbiotic ant *L. niger.* Shrub and tree cover were found to be negative drivers of *P. argus* distribution.

The second section (6.2) considers the resources needed by the ant species found on the peninsula. As the presence of *L. niger* is of crucial importance to *P. argus* larvae, it is necessary to consider how resources may be affecting the distribution of *L. niger* across the peninsula. Results indicate that *L. niger* has a positive association with *E. tetralix* and with short grass (under 2 cm and between 2-5 cm) while bryophyte/*Sphagnum* cover has a negative impact on distribution. The resource requirements of the other five heathland ant species have also been discussed as interspecific competition can potentially be a driver of current *L. niger* distribution.

The third section (6.3) considers whether the distribution of *P. argus* has changed since Cyril Diver's map in the 1930s. Although there are no records to indicate the presence of *P. argus* on the peninsula after the 1930s until 1976 when Butterfly Conservation began transect recording, it is possible to compare the distribution results found in this study with Diver's distribution map as individual snapshots and to look at some of the ways in which the botanical landscape has changed over the last 80 years.

6.1 What was the distribution of *P. argus* across the peninsula?

P. argus presence was found in 16 of the 40 sample plots and the distribution was found to be on the western side of the peninsula, either side of Ferry Road (Figure 48). The only exception to this was sample plot 1 on the tip of Second Ridge where three male imagines were located in the last two weeks of transect recording. This area is a considerable distance (over 400 metres) from the western plots and is on the eastern side of Little Sea. There were four main hotspots found at plots 4, 6 and 29 on Plateau Heath and plot 27 on Curlew Heath.

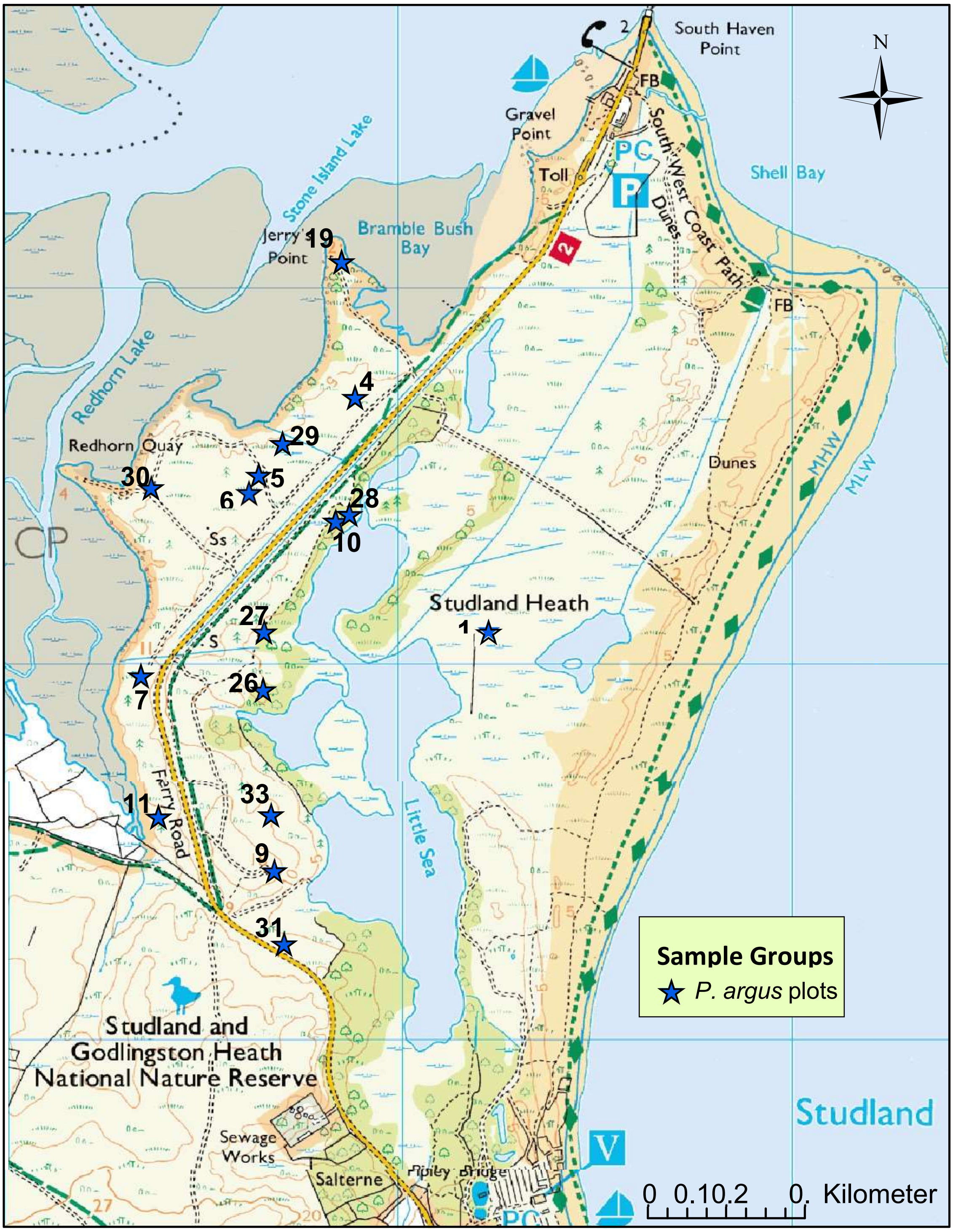

Figure 48 : The distribution of *P. argus* at sample points in Studland peninsula 2015

(Amended from EDINA Digimap Ordinance Survey Service : Studland Peninsula using ArcMap 10.2.2.)

6.2 What is driving the distribution of *P. argus*?

6.2.1 Which resources are important?

When PCA analysis was carried out to look for associations in Resource variables (Section 5.2) a significant negative regression was shown between *P. argus* with Component 5 which had the highest loadings for shrubs (.782), *L. niger* number (-.576), *E. tetralix* cover (-.497) and tree cover (.428). However, when Spearman's Rank Correlation Coefficient was carried out between *P. argus* abundance with individual variables, the butterfly was found to have a significant positive correlation with the host plant *E. tetralix*, with short grass (2-5 cm) and with the symbiotic ant, *L. niger*. It is probable that the results for *L. niger* and *E. tetralix*, which were both negative loadings within the PCA component, were shown as negatively associated with *P. argus* numbers in the MLR, due to the high loadings for shrub and tree cover.

6.2.1.1 The relationship between P. argus and E. tetralix

The four *P. argus* subspecies have developed different host plant preferences depending on habitat type and hence plant availability. Thomas (1998) states that larvae will feed on the tender buds of a wide range of plants within the Leguminosae, Cistaceae and Ericaceae but in southern heathland locations, like Studland, where the subspecies *P. a. argus* is found in wet, acidic heath, the dwarf shrub *E. tetralix* is the most important host plant for adult nectar feeding, egg laying and larval nutrition (Ravenscroft & Warren 1996, Thomas 1998). *P. argus* uses all stages of the shrub as a resource; the tender buds of young plants are important for larval feeding while the flowers of more established plants provide nectar for imagines and the straggly stems are used for egg laying. As *E. tetralix* shrubs become older and more degenerate, the erect branches begin to droop revealing gaps of bare soil which allow sunlight to warm the soil thus creating a warm microclimate for butterfly larval development.

Results from Spearman's Rank Correlation Coefficient found a moderate effect size in the positive correlation between *P. argus* with percentage cover of *E. tetralix* (r=.396, p=.011). This is supported by Figure 49 which shows that of the 14 plots where *E. tetralix* was found, nine plots were in conjunction with *P. argus*. Interestingly, there were seven plots with *P. argus* presence and no *E. tetralix* cover and five plots with *E. tetralix* cover but no *P. argus* presence. Consequently, the butterfly and *E. tetralix* occurred together in 64% of plots (see Figure 49). It is notable that in this study, *P. argus* was not recorded on the five plots on Second and Third Ridge (plots 2, 12, 13, 17 and 35) where *E. tetralix* is growing in abundance. Plots 17 and 35 have the highest percentage cover of *E. tetralix* of all plots at 73% and 78% -the mean % of all *E. tetralix* cover is 35.67% (± SD 23.95) although their respective heights of 43 cm (plot 17) and 40 cm (plot 35) are in excess of the 23.4 cm mean height (± SD 10.5 cm) for the plant. Host plant height was not found to be a barrier to the butterfly however; results from Spearman's Rank Correlation Coefficient found a positive correlation (r=.346, p=.029) between *P. argus* number with *E. tetralix* height which could be due to the increased flower abundance of many of the taller, more established plants. It is possible therefore, that the western *E. tetralix* sites are either missing an element desired by the butterfly or contain an element which the butterfly does not respond well to. Of the seven plots with *P. argus* presence but no *E. tetralix,* three had combined *C. vulgaris* and *E. cinerea* cover of at least 55% while the other four plots were in predominantly grassy locations. Although *P. argus* is a sedentary species, the imagines do fly a short distance to find host plants. There are many areas on the peninsula where *E. tetralix* is growing which were not in the randomly selected sample points and it is most likely that *P. argus*, found in sample sites without *E. tetralix*, were using areas where the plant is growing nearby.

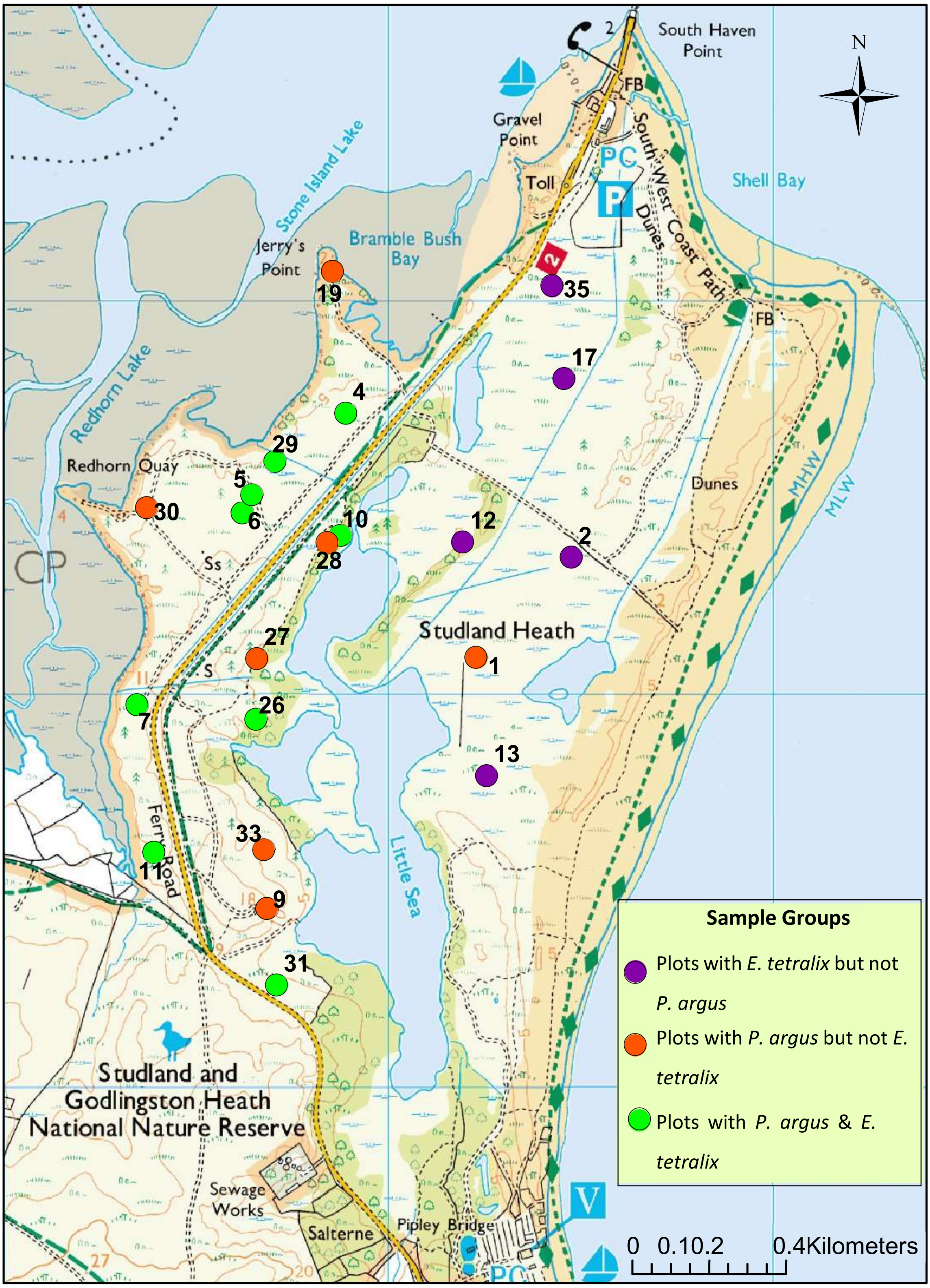

Figure 49 : The distribution of plots where *P. argus* and *E. tetralix* were recorded either together or in separate plots (Amended from EDINA Digimap Ordinance Survey Service: Studland Peninsula using ArcMap 10.2.2.)

6.2.1.2 The relationship between P. argus and L. niger

The ecology review of *P. argus* in Section 3 has ascertained the vital importance of the symbiotic *Lasius* spp. to the breeding success of this butterfly. In wet heathlands this mutualistic relationship takes place with *L. niger* rather than *L. alienus*, as the latter inhabits warmer, drier areas of the heath (Brian 1977). Results from Spearman's Rank Correlation Coefficient showed a strong relationship between the two species (r= .706, p=.001) and this was confirmed by Binary Regression Logistics which identified *L. niger* as significant to the presence of *P. argus* with an odds ratio of 28.1, p=0.01 (Appendix 9.8) where the predictive capacity of the model was at 87.5%. The presence of *L. niger* led to an 18% increase in the chances of finding *P. argus* present at a sample site.

This confirms what might be expected from a myrmecophilous, Lycaenid species, suggesting that *L. niger* presence is a key requirement for *P. argus* imagines. Analysis of the data found that *P. argus* and *L. niger* were found together in 10/16 plots (62.5%) and the number of imagines found in the six plots without *L. niger* was very small (a mean of 9.6, SD ±5) compared with plots where they were both found (a mean of 31.3, SD ± 19.3). There were two plots where *L. niger* were found without *P. argus* (179 ants in plot 12, 18 ants in plot 12); this however is to be expected as there is no evidence to suggest that *L. niger* depends on *P. argus* for survival as the ant is very wide-spread throughout the UK living in a variety of habitats which *P. argus* does not inhabit (see Section 3).

6.2.1.3 Past research on the association between P. argus and L. niger

As suggested in Section 3, there is a significant body of research which suggests that *P. argus* searches for vegetation with high densities of *L. niger* in which to mate and deposit eggs (Thomas 1989, Thomas 1993, Gutierrez et al. 2005). Ravenscroft and Warren (1996) noted in Suffolk heathland that *P. argus* does not appear in heathland locations where *Lasius* nest density is lower than 30 nests per hectare despite having a recorded presence in nearby areas where *Lasius* nests are more abundant. A study (Seymour et al. 2003) in the dune heathland of Doňana National Park, southern Spain, at the southern limit of *P. argus* distribution, found that *Lasius* spp. presence was more significant than the host plant *H. halimifolium*, for butterflies marked and released more than 100 metres away from a targeted colony of *L. niger.* Six releases of 200 butterflies per release (at a sex ratio of 1:1) took place in 2001 over 50 one-hectare plots which had similar vegetation communities and structures. The study revealed that recaptures of both male and female *P. argus*, within a 500 x 500 metre area were strongly associated with the target patch suggesting that the butterflies' search behaviour was not random. Male and female imagines moved out of release squares, which contained a higher percentage of nectar source, to move to target *Lasius* patches and butterflies which were released in target patches, showed a marked tendency to stay within the patch they were allocated. Research carried out by Seymour et al. (2003) demonstrated that recaptures from both local and continuous populations displayed the same behaviour over a variety of time periods and that males had a slightly stronger tendency to remain within a target patch even when there was a greater number of females in an alternative patch nearby. Seymour et al. (2003) conclude that this could be due to the male's patrolling behaviour fuelled by the desire to mate with emerging females directly they leave *Lasius*' chambers. Conversely, females had less compunction to remain in target patches once egg laying had taken place.

The research by Seymour et al. (2003) in Doňana National Park, has been supported by predictive habitat distribution models (Gutierrez et al. 2005), which also found that *L. niger* nest density was the major predictor of *P. argus* abundance and presence or absence. The relationship between *P. argus* with *L. niger* was discovered to be more consistent across the research and spatial range than the correlation between *P. argus* and host/larval plants. Predictive models found that egg placement was biased towards *H. halimifolium* (the main larval food plant) with *L. niger* nests at the plant base and that a stronger relationship was shown with larvae and pupae which were only found in plants with *Lasius* nests at the base. Conversely, *L. niger*, was associated with other variables including presence of vegetation, low topography and water table depth.

While benefits received by ants from Lycaenid offspring are primarily nutritional, the advantages gained by butterfly larvae from myrmecophily are thought to derive from the protection ants offer. Research by Pierce and Easteal (1986) on the myrmecophilous, Lycaenid species *Glaucopsyche lygdamus* (silvery blue) in the Gold Basin, explored some of the reasons influencing the significant correlation found between butterfly abundance with its mutualistic ant associate, *Formica altipetens*. Pierce and Easteal (1986) proposed that the protection received by Lycaenid larvae from ant associations gave it a selective advantage over unattended larvae; especially in the final instar stages. The research found that Lycaenid larvae, whose attendant ants had been excluded from research plots, had an 18% chance of survival compared with a 36% survival rate for tended larvae. In addition, the research claimed that ants that feed on honeydew and nectar secretions from other organisms, were able to protect butterfly larvae against, not only predation but from parasitoids, even those emerging from guarded pupae. While a significant correlation between oviposition and ant density did not emerge in the research, ant attendance was found to have a significant impact on larval success and potentially, local population persistence. In addition, field research on the Australian Lycaenid *Jalmenus evagoras* (Pierce et al. 1987) using ant exclusion zones, found that larvae without the protection of *Iridomyrmex* spp. (the attendant ant) are unlikely to survive predation and/or parasitism. Larvae brought into the laboratory which was tended by ants, developed over four days faster than larvae which was not tended by ants although tended pupae were smaller, less heavy and reached eclusion later than untended pupae.

6.2.1.4 The relationship between P. argus, E. tetralix and L. niger

The strong positive regression equation between *P. argus* and *L. niger* suggests that ant presence is of equal importance to the butterfly as the abundance of host plants. Results show that seven of the 16 *P. argus* plots also had a combination of *E. tetralix* and *L. niger* presence (Figure 50). These seven plots accounted for nearly 80% of the total 612 *P. argus* records. The data also shows that *P. argus* was found with *L. niger* but without *E. tetralix* in three plots, with *E. tetralix* but without *L. niger* in two plots and without either *L. niger* or *E. tetralix* in four plots, two at Spur Heath, one at Curlew Heath and one at Jerry's Point, at the tip of Plateau Heath North (Figure 50).

The high number of *P. argus* imagines at plots where *L. niger* and *E. tetralix* were both found is not unexpected. Butterflies need a combination of resource requirements which may make some areas more attractive than others. It is probable that the combination of *E. tetralix* and *L. niger* make a site more attractive than those with *L. niger* alone. Both *E. tetralix* and *L. niger* prefer the damp, wetter parts of the heath which have cooler temperatures, the former grows in acid bogs, wet heaths and moorland (Streeter 2009) while research carried out on Hartland Moor, Dorset (Brian 1977) indicated that winged *L. niger* queens chose to alight in cooler, wetter heathland slacks possibly in response to the higher density of *Molinia* spp. which may be used to provide cover in a new territory.

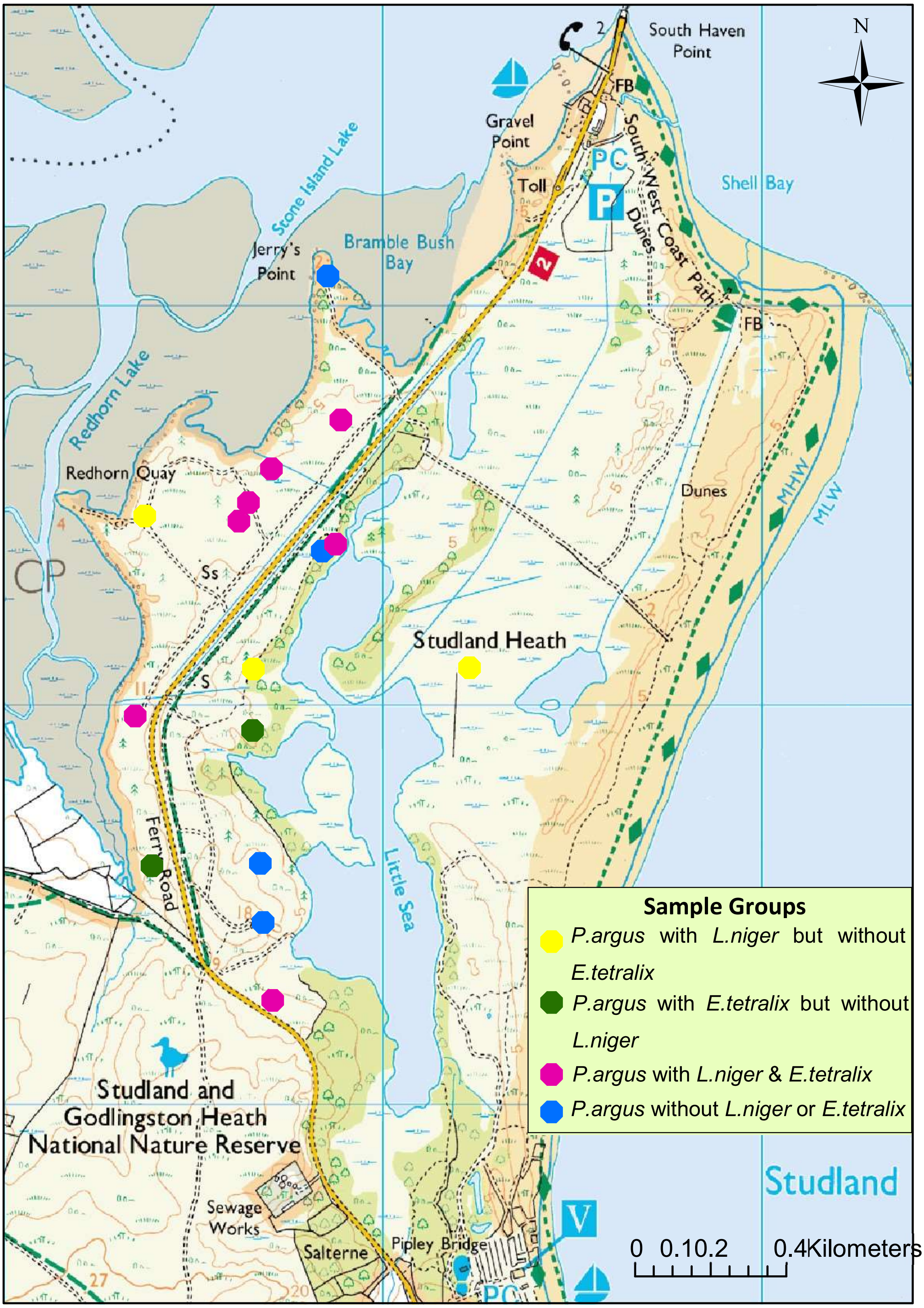

Figure 50: Distribution of *P. argus* in conjunction with *L. niger* abundance and *E. tetralix* cover

(Amended from EDINA Digimap Ordinance Survey Service: Studland Peninsula using ArcMap 10.2.2.)

6.2.1.5 Shrub, tree and grass cover

The negative regression result between *P. argus* with shrub and tree cover found in the MLR and the positive correlation found with short grass 2-5 cm (Section 5.2.3) highlighted by Spearman's Rank Correlation Coefficient, is in keeping with past research which has recorded that the butterfly needs early successional vegetation to thrive as eggs are laid close to the ground where a warm microclimate has been created through insolation (Asher et al 2001, Thomas 2007). It is likely therefore that the succession of coastal heathland to later successional processes with dominance by shrubs, pine and birch will be detrimental to *P. argus* due to the loss of a suitable microclimate for larval development. Areas of bare ground and short grass are encroached upon by shrubs and trees and if uncontrolled, relatively low-growing Ericaceae species and short grass are shaded out while nutrients build up in the soil aiding the growth of dominant large grass species (Price 2003). This is supported by research by Schirmel & Fartmann (2014) on the Baltic island of Hiddensee in Northern Germany which sampled the abundance of butterflies and vegetation along a coastal heathland gradient ranging from coastal dunes to a birch forest. The research found that *P. argus* was negatively affected by successional processes as abundance decreased significantly with increasing grass cover and vegetation density.

An alternative perspective on the impact of shrub and tree cover can be seen in research carried out by Dennis & Sparks (2006) at Great Orme's Head in North Wales. The study found that tall, vegetation structures, surrounding or close to host plants, were essential requirements for *P. argus caernensis* facilitating activities such as roosting, mating, courtship and shelter. An additional study by Dennis (2003) found that in coastal locations, *P. a.caernensis* was more likely to be abundant on taller shrubs than on host plants unless the percentage of sunshine was consistently high and wind speed low. He pointed out that as *P. a.caernensis* use different vegetation for different purposes throughout the day, it is difficult to establish the habitat preferences of the butterfly with any accuracy as it will depend on the time of day and weather conditions the survey is undertaken in.

Many of the 16 *P. argus* plots in the Studland 2015 research bordered tree and shrub-lined margins, especially on Plateau Heath, where a fringe of *Betula* spp. and *Ulex* spp.

separates the heathland from the edge of Poole Harbour. Observations during this research (but without numerical data) noted the arrival of large numbers of *P. argus* imagines from the tree-lined margins of Plateau Heath each morning as temperatures began to rise. If conditions changed, reducing the percentage of sunshine or significantly increasing wind strength, adults disappeared either to the base stems of host plants or into the shrub/trees to the west. It was therefore difficult to assess the numbers using tall vegetation at any one time but this could be addressed by future studies. The surveys in this study were undertaken once a week between 11:30 and 14:30 regardless of weather conditions; this research could be developed in the future by follow up surveys on the same transects at different times throughout the day.

The positive, correlation found between *P. argus* abundance with percentage cover of short grass 2-5 cm reflects past research which has shown short vegetation to be a key requirement for *P. argus* in both heathland and calcareous areas (Thomas et al. 1999, Davis et al. 2011). Research by Jordano et al. (1992) at Chobham Common found a negative correlation between *P. argus* and grass height possibly due to the cooler, microclimate created by tall grass coverage and the consequent loss of host plants as they become shaded out. The study points out that while microclimates created by short grass and bare ground are beneficial to *P. argus*, especially at range peripheries, warm conditions are also important for most ant species which need warmth for nesting and foraging (Skinner and Allen 1996). The temperatures required by ants are clearly an important additional factor given the dependence of *P. argus* larvae on *L. niger* populations. The next section considers the habitat requirements of the six ant species found at Studland as they are clearly pertinent to the distribution of *P. argus.*

6.3 Ant Abundance and Habitat Requirements

6.3.1 Does *L. niger* have habitat requirements which could be driving the distribution of *P. argus*?

Although the MLR results from the Vegetation PCA did not indicate any specific patterns of vegetation needed by *L. niger*, Spearman's Rank Correlation Coefficient did find positive, significant correlations with *E.tetralix*, short grass cover (under 2 cm and between 2-5 cm) and a negative association with bryophytes/*Sphagnum* when the independent variables were correlated singly outside of the PCA. The positive correlation between *L. niger* with percentage cover of *E.tetralix* (r= .427, p=.009) and *E. tetralix* height (r=.397, p=.017) mirrors the positive association between *P. argus* and *E.tetralix* discussed in the former section. Given the importance of the symbiotic ant for Lycaenid larvae, it would seem likely that *P. argus* is attracted to habitats where *L. niger* can be found rather than the reverse. *L. niger* is able to live in a wide range of environments where the conditions are suitable including gardens, parks, roadside verges and grasslands (Skinner & Allen 1996); they are not dependent on the butterfly for their existence and most of their colonies throughout the UK do not benefit from *P. argus* larval secretions.

6.3.1.1 The habitat requirements of L. niger

L. niger are an omnivorous species with a varied diet consisting of honeydew, seeds, nectar, soft fruits and other invertebrates (Skinner & Allen 1996). The positive, significant relationship between *L. niger* with *E. tetralix* is not unexpected as both species share a preference for the more humid, cooler areas of the heath. In addition, *L. niger* can obtain nectar, high in protein and flavonoids, from the plant (Panda 2005) while the straggly structure of the branches provides shelter, enables foraging and creates patches of sheltered, bare ground suitable for nest construction entrances. Brian's research at Hartland Moor, Dorset (1977) includes observations of *L. niger* appearing to use *E. tetralix* flowers as hunting grounds for invertebrate prey and nests were found to be regularly constructed in areas where the shrub met areas of bare ground.

Similarly, the positive, significant correlation found with percentage cover of grass < 2cm (r= .358, p=.032) and with grass 2-5 cm (r= .384, p=.021) is in keeping with ecological research on the position of ants' nesting sites which are predominantly in open areas where grass is short and the earth can be warmed by insolation. Ants, as ectotherms, need to create a nesting structure which maintains a warmer microclimate than the ambient air and ground temperature as this allows brood to develop as well as providing warmth for overwintering (North 1998). Nests placed under stones can reduce loss of heat to the soil through conduction while soil mound nests are predominantly constructed in sunlit areas where the mound's surface area increases the amount of heat passing into the soil. Conversely, layered nests – either above or below ground –

can provide warmer upper layers in the early spring when ants come out of hibernation in preparation for spring foraging. Humidity can also be adjusted through nest ventilation which enables ants to avoid desiccation in the summer (Sorvari et al. 2016). Holec et al. (2006) found, in research on *L. niger* mounds in former coal-mining areas in Sokolov, Czechia, that small nests were most often located on the edges of bare ground or short grass and that they were particularly vulnerable to shading from tall, dense grasses which hindered high brood production and slowed population growth. Larger nests were found to be less vulnerable to shading as the larger openings, which were found to be between 0.5-1 metre in diameter, allowed enough exposure to the sun even within densely vegetated areas. In addition, short grass patches can be used as accessible foraging grounds in the search for small invertebrate prey. The significant, negative correlation found with bryophyte/*Sphagnum* percentage cover (r= -.393, p=.018) could be due to several factors; where *Sphagnum* cover is high, the ground was possibly too wet for *L. niger* nest construction while high density of bryophyte cover may have made the ground unsuitable for nesting due to restricted insolation.

6.3.1.2 Changes in the distribution of L. niger

Given the habitat requirements of *L. niger*, suggested by the results, it is surprising that only three sample sites east of Little Sea (plots 1, 12 and 37) were found to contain *L. niger* (see Figure 52). A noticeable reduction in the distribution range of *L. niger* can be seen if Diver's map from the 1930s is compared with the distribution map from this research (Figure 51 and Figure 52). Diver's map shows *L. niger* presence to be widespread across the peninsula with records as far north as Northern Dunes, across all three ridges, in all areas around Little Sea, in addition to woodland and heathland areas. This is interesting as the distribution map from this research shows that *L. niger* distribution has contracted to sites on the western side of the peninsula with 8/12 *L. niger* plots located around Ferry Road, and just four plots on Zero Ridge North, Spur Bog, Third Ridge and at the tip of Second Ridge. This was supported by results from the Kruskall Wallis H test which found significant differences (p<.05, Table 9) between the abundance of *L. niger* across the four groups. Although group **- -** (the group where *P. argus* has never been recorded) had the highest number of sample plots (19/40), only two of them were found to have *L. niger* presence. The concentration of *L. niger* plots around Plateau Heath/Curlew Heath could be explained by *L. niger's* preference for humid, damp heathland areas where it will construct long chambers quite close to the soil surface. Winged queens have been recorded as seeming to choose *Molinia caeulea* patches which are commonly found on moorland, damp heathland and, bogs (Streeter 2009) where invertebrates may be found, in preference to areas of shorter vegetation (Brian 1977) and have additionally been shown to prefer open areas with patchy plant cover (Czechowski et al. 2009) which is provided by the *E. tetralix*-dominated heathland community found on Plateau/Curlew Heath.

There are however many similar damp habitats on the eastern side of the peninsula, particularly on the ridge leeway's which appear to have been inhabited by *L. niger* in the past. It is possible however, that choice of nesting sites in damp areas, may have been compromised by the hydrological changes noted by The Cyril Diver Project (2013-2015). As former nesting areas become too wet for use, *L. niger* may be forced to seek new terrain; much of which may have been colonised by other species. Conversely, the seeming shift in *L. niger* distribution could be a result of successional processes as the peninsula succeeds to woodland and scrub cover. *L. niger* is not a woodland species and as results from this study have found, nests tend to be constructed under stones in open grass/heathland areas where sunlight can warm the earth aiding larval development (Brian et al. 1976). There are, however, other possible explanations for distribution difference other than those caused by changes in vegetation community and structure; interspecific competition is discussed as a potential factor in the section 6.4.2.

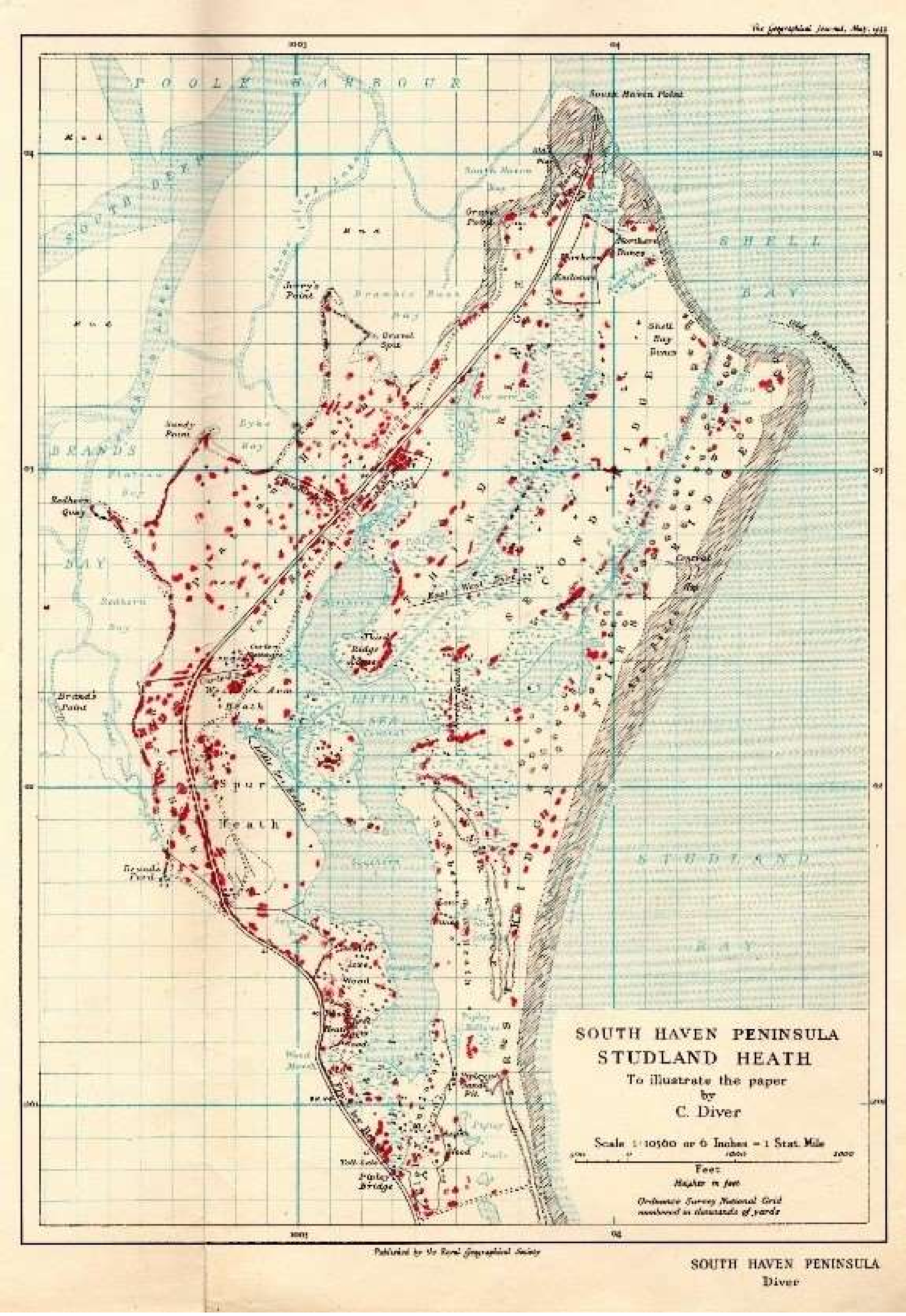

Figure 51 : The distribution of *L. niger* in the 1930s at Studland (Diver 1930s)

Dorset History Centre, Dorchester

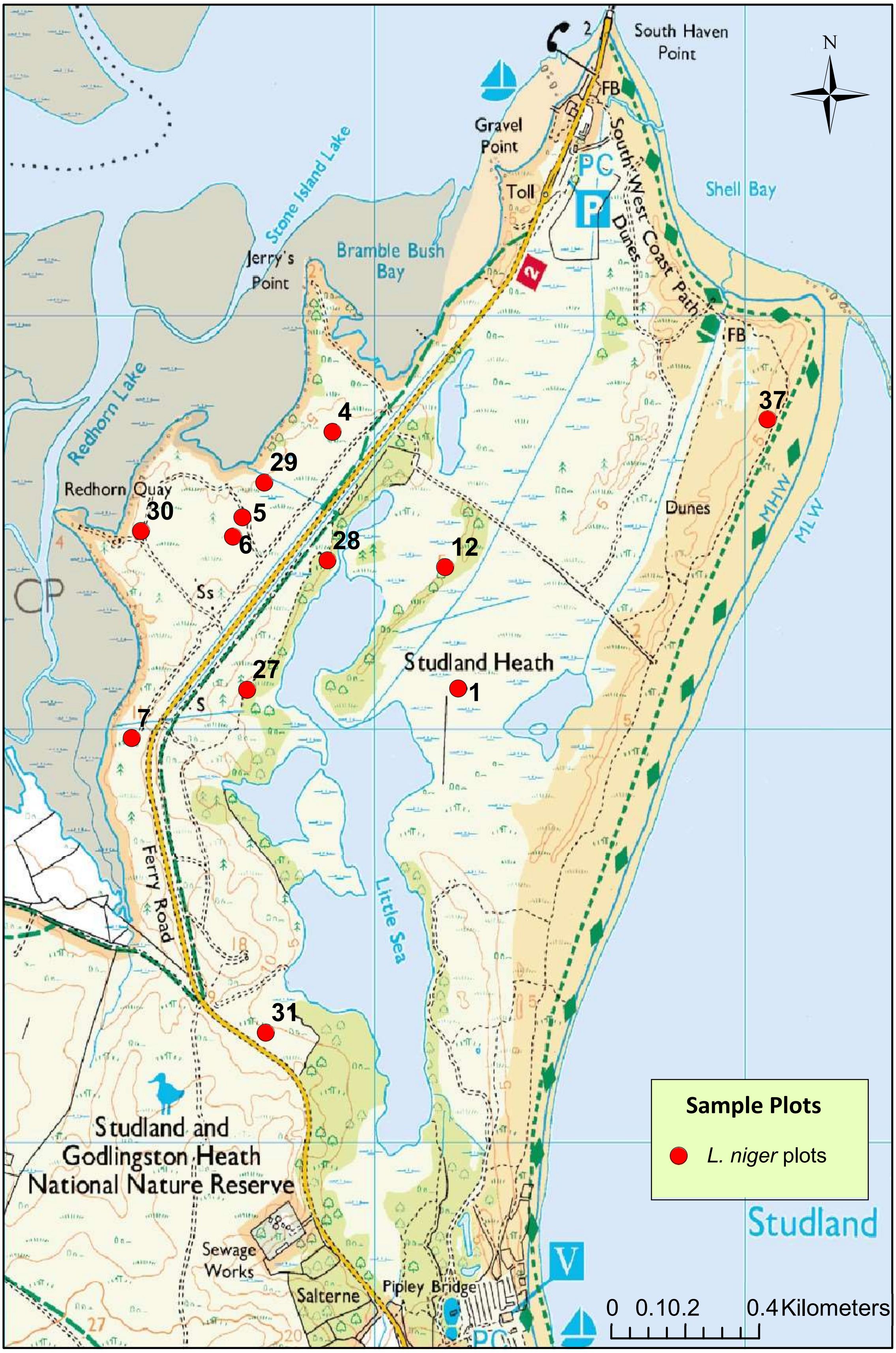

Figure 52 : The distribution of *L. niger* across the 40 sample plots

(Amended from EDINA Digimap Ordinance Survey Service: Studland Peninsula using ArcMap 10.2.2.)

6.4 Interspecific competition

6.4.1 ***F. rufa*** **habitat requirements**

Results of the PCA and MLR on Vegetation variables found a significant, positive regression equation between *F. rufa* with Component 6 of the PCA which had the highest loadings for shrub cover (.831) and percentage of bare ground (-.529). This is in keeping with myrmecological research which describes *F. rufa* as a woodland species inhabiting coniferous, mixed and deciduous woodland, scrubby heathland and open forest rides (BWARS 2016). *F. rufa* have been reported to move their nests if shadowed by overhanging vegetation and will construct their nests in sunlit clearings close to woodland edges and, often, bodies of open water (Czechowski & Vepsalainen 2009) which is illustrated in Figure 53. Brian (1977) also states that *F. rufa* will vary the shape of its nest dome in relation to infra-red ray direction as the creation of a warm microclimate is essential for larval growth.

The results from the Spearman's Rank Correlation Coefficient found positive, significant correlations with leaf litter (r= .377, p=.023), shrub percentage cover (r=.456, p=.005) and shrub height (rs.469, p=.004). This is consistent with the ecology of *F. rufa* which uses a range of organic materials, particularly pine needles, to create nesting mounds (Czechowski & Vepsalainen 2009) and which use shrubs for aphid foraging and for setting up pheromone trails. The long-legged morphology of *F. rufa* enables the larger workers to access tall trees and shrubs with ease hence shrub height is not a barrier to foraging success.

6.4.1.1 Changes in the distribution of F. rufa

Although results of the Kruskall Wallis H test did not find a significant difference between the four groups in terms of abundance, box plots did reveal an uneven distribution of *F. rufa* across the peninsula with hotspots in plots 9, 23 and 10 and the highest abundance in group **- -** (the group where *P. argus* has never been recorded) which had 86% of all *F. rufa* sightings across just 7 plots. The maps in Figure 53 and Figure 54 show the expansion of the range of *F. rufa* from a cluster of points at Curlew Cottages, Curlew Heath, South Haven Flats and North Dunes in the 1930s to a total of 12 plots including Jerrys Point, Third Ridge North, First Ridge, Spur Heath and the edge of 12 Acre Wood, where they had not been recorded on Diver's map. It is possible that the succession of vegetation noted by the Cyril Diver Project has facilitated the spread of *F. rufa* which has foraged further afield as trees and scrub have grown up. As a top invertebrate predator within the forest ecosystem (Finér et al. 2013) and with the large numbers of workers attached to each nest (Brian 1977), it is probable that *F. rufa* has exploited later successional processes very successfully.

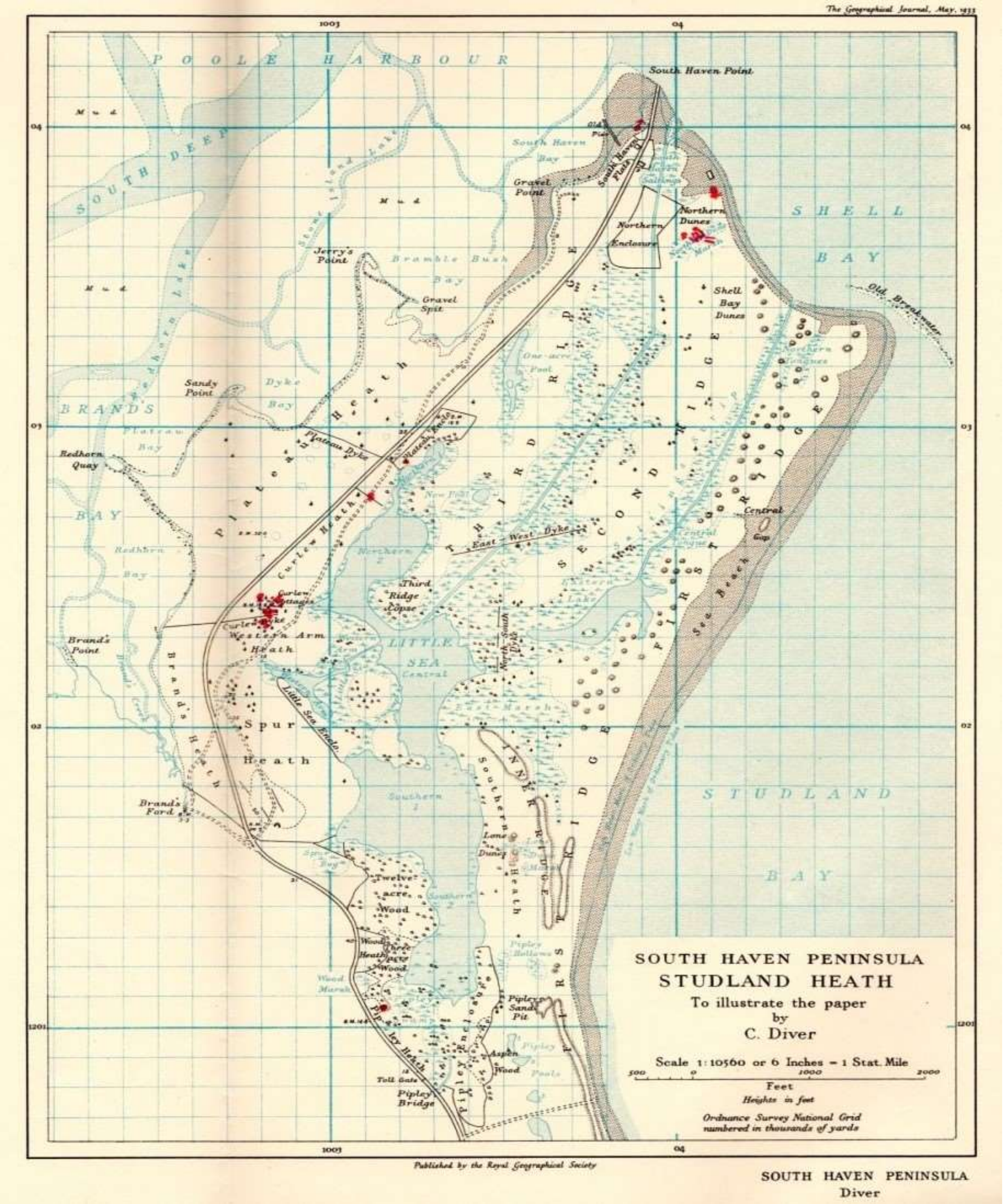

Figure 53: The distribution of *F. rufa* across the peninsula in the 1930s (Diver 1933)

Dorset History Centre, Dorchester

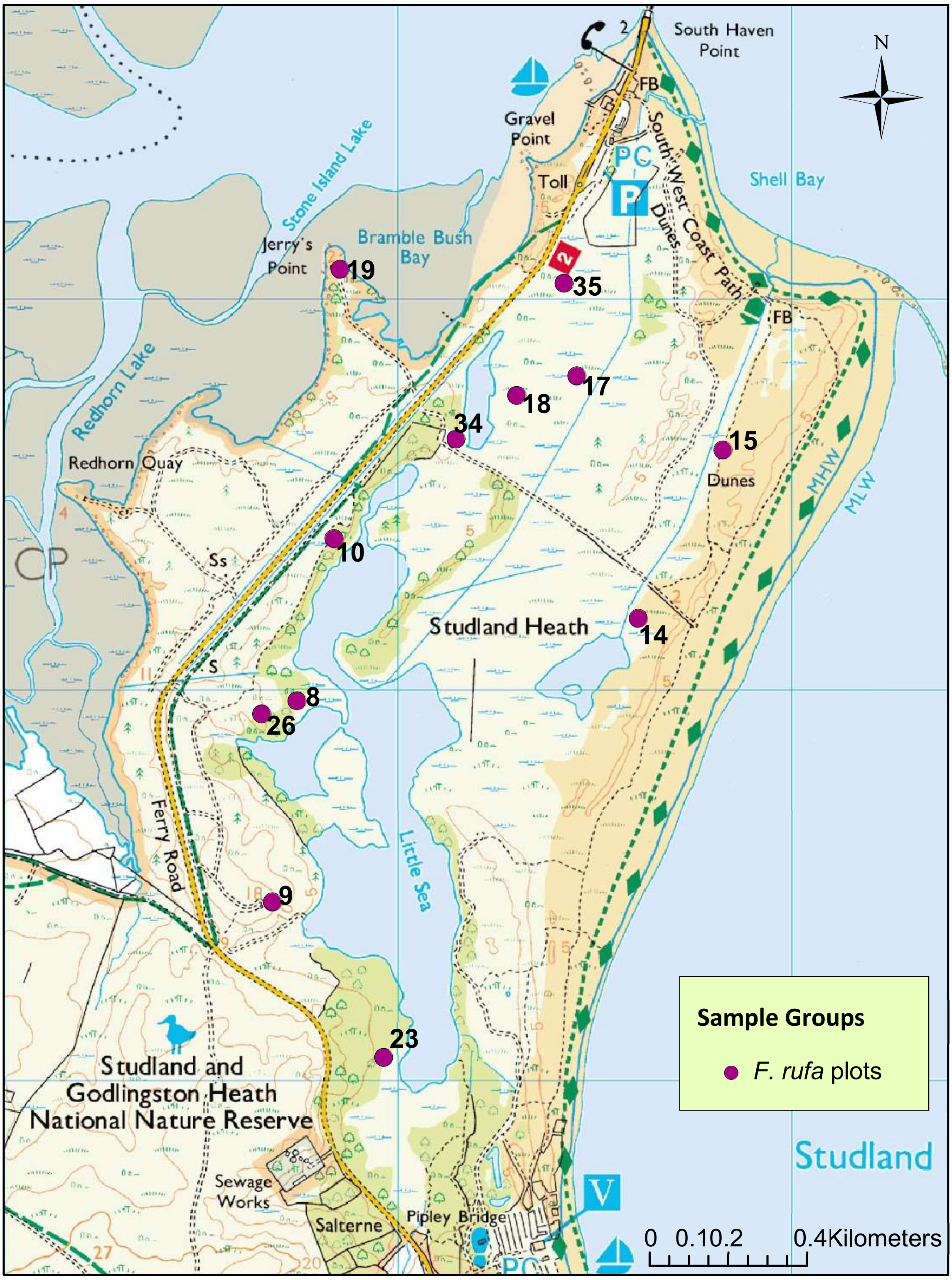

Figure 54 The distribution of *F. rufa* at Studland in sample plots taken 2015

(Amended from EDINA Digimap Ordinance Survey Service: Studland Peninsula using ArcMap 10.2.2.)

6.4.2 **Interspecific completion between *F. rufa* and *L. niger***

Research data from this study shows that *L. niger* and *F. rufa* were never present at the same plots despite having some habitat requirements and feeding preferences in common. Figure 55 compares the distribution of the two species; while *L. niger* seems confined to the western side of the peninsula with just four sites to the east, *F. rufa's* distribution is located on the eastern side of the peninsula (except plot 19) spreading as far east as First Ridge. It is not possible to determine when the ranges of either species changed without distribution records for the interval in between Diver's records and this research. It is possible that *L. niger's* distribution shift is a consequence of competitive exclusion if the ant species has been driven out of the eastern peninsula by competition from *F. rufa*. This is a possibility as the vegetation requirements of *L. niger* are still available on the eastern side of the peninsula but *L. niger* does not appear to be using them.

Fourcassie et al. (2012) state that interspecific and intraspecific competition are key determinants influencing abundance and distribution of ant communities. Interference competition is shown to be widespread with colonies regularly losing foraging sites due to attack by other species or other colonies. It is possible that *F. rufa* and *L. niger* may have temporarily inhabited the same sites before *L. niger* became excluded by the larger *Formica* species. Mackay and Mackay's research (1982) demonstrated that *F. rufa* showed especially aggressive behaviour compared with other ant species and cites Marikovsky's work (1962) which observed the movement of *L. niger* and *M. ruginodis* to underground sites when their territory was colonised by *F. rufa*. Savolainen and Vepsᾳ̈lᾳ̈inen (1988) report that, in their work on the Island of Joskᾳ̈r, Finland, territorial species (*Formica* spp.) and encounterers (*L. niger*) were never found in the same area and that both types behaved aggressively to ant members from other colonies.

It is potentially the competitive nature of *L. niger* which makes it unable to coexist with *F. rufa*. Research by Fourcassie et al. (2012) found in laboratory research, that *L. niger*, did not avoid encounters with competitors after an altercation had taken place and that they showed the same exploration and foraging behaviour as those employed before an attack. Similarly, research by Žmihorski and Slipinski (2016) carried out by sampling ant populations in clear-cuts within a deciduous and pine forest in western Poland, found that, in each sample, *F. rufa* was negatively associated with *L. niger*. The research states that *L. niger*, as an aggressive, encounterer species which defends not only its nest but its foraging territory and food source, would not be able to survive in the same location as another equally aggressive, but larger, competitive species. The study considers *F. rufa*, to be a highly competitive, efficient, dominant, and aggressive ant which is responsible for determining the distribution of other ant species within the forest. The study points out that *F. rufa* will attack other territorial species, has a detrimental impact on the fitness of subordinate ant species and predate all invertebrates within their trophic field. In addition, the research claims that *F. rufa* will have a negative impact on the foraging territory of other species by influencing the space available for use. Peral et al. (2016) refer to the known ability of dominant territorial species, like *Formica* spp. to restrict the foraging range and nest distribution of subordinate ants. Parmentier et al. (2014) also refer to *F. rufa* as top predators able to repress any outbreaks of forest pest populations while Skinner (1980) discusses the polyphagous nature of the species which relies heavily on honeydew for energy and insect prey for larval development. The next section looks more closely at the feeding requirements of both *L. niger* and *F. rufa*.

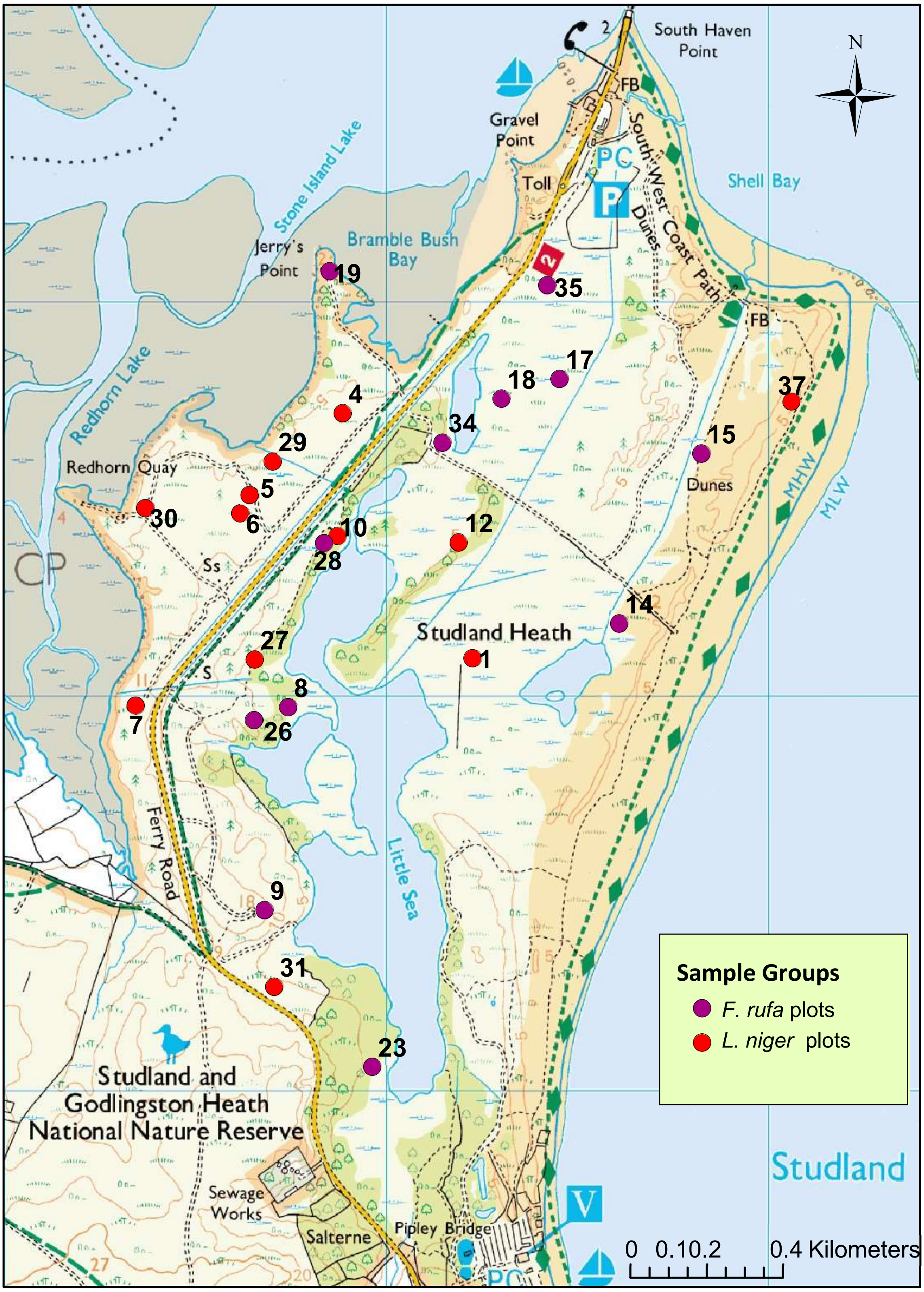

Figure 55 : A Comparison of the distribution of *F. rufa* and *L. niger* across all groups at Studland

(Amended from EDINA Digimap Ordinance Survey Service: Studland Peninsula using ArcMap 10.2.2.)

6.4.3 **What is influencing the distribution of *L. niger*?**

Predation could also be a relevant factor in ant distribution; Skinner and Allen (1996) point out that *F. rufa* nests have been found to contain the remains of many invertebrate species including *F. rufa* from other colonies, winged and unwinged *Lasiu*s species and *Myrmica* spp. Skinner (1980), who carried out research on *F. rufa's* feeding habits in limestone woodland in the UK, found that although the species predation patterns changed throughout the year, the greatest abundance of prey items were Lepidoptera larvae, aphids and honeydew in spring and early summer. Research has shown that *L. niger*, like many other ant species, will also actively harvest aphid honeydew and will forage on the ground, tree branches and bushes (often *Betula* spp.*)* in search of carbohydrate nutrition (see Literature Review). Both *F. rufa* and *L. niger* will actively protect aphids from predators or other foragers and both will defend their foraging territories and nests when threatened by another colony or species (Brian 1977, Skinner & Allen 1996, Phillips & Willis 2005). While both species are equipped with formic acid which, when ejected, penetrates animal membrane, the higher numerical strength, more efficient organisational skill (Czechowski & Vepsalainen 2009) and large workers may have given *F. rufa* an advantage over their competitor who may have responded by moving to new nesting and foraging grounds.

Several studies comment on the significant impact *F. rufa* colonies can have on the heath and forest ecosystem as they feed from a range of trophic levels; they are key predators of invertebrate prey collected from the tree canopy and the forest floor (Finér at al. 2013) and are able, through their large size and through recruitment, to carry large invertebrates back to their nests. Skinner & Allen (1996) comment on the advantages their size, numbers and developed eyesight (which can detect movements from prey up to 10 cm away) gives them over other ants within the community. Research carried out on differential eye scaling within insects (Perl & Niven 2016) found that the compound eyes of *F. rufa* can change in area, facet number and diameter as the insect changes in size. Perl & Niven's work (2016) found that facet lens diameter increases gave better photon capture, improved sensitivity and improved eyesight over smaller colony members thus giving *F. rufa* a considerable advantage when foraging. Studies of *F. rufa* nest contents have found the body parts of a diverse range of invertebrates including Odonata in addition to *Lasius* and *Myrmica* sexuals (Skinner 1980). The location of *F.rufa* nests close to water can give the species additional opportunities perhaps not available to other ant species in the community; during the vegetation surveys in May 2015 in this research, predation of the early emerging *Brachytron pratense* (hairy dragonfly) close to the water's edge was observed three times in a period of ten days.

F. rufa also feeds on honeydew from some aphid species such as *Periphyllus testudinaceus* (Skinner & Allen 1996) but while aphid excretions are primarily used for energy, invertebrate prey aids brood development (Finér at al. 2013). Myrmecological research has shown that ants will not only defend aphids they are harvesting from predation but will attack other ant species who attend aphids before other competitors. Studies of *Formica aserva* in the forests of Deep Lake, Saskatchewan in 2003 (Phillips and Willis 2005) found that the species always attacked aphid-predators over non-aphid eating insects when both types were introduced into aphid leaf clusters. When hymenoptera aphid predators were introduced at the same time as Coccinellidae members, *F.aserva* attacked the alien ant species first in 90% of test cases often leaving the Coccinellidae to predate the aphids without restraint. Phillip and Willis (2005) conclude that *Formica* spp. appear to be able to distinguish between the level of threat to their aphid resources and will attack rival ants immediately to reduce the likelihood of an organised ant raid from another colony in the future. The energy costs associated with this behaviour are deemed to be worthwhile in terms of the benefits gained from long-term protection of invaluable aphid resources.

In conclusion, therefore, it would seem logical to argue that the expansion in distribution of *F. rufa,* due to the growth of woodland and scrub across the peninsula, may have led to a constriction in *L. niger* distribution to the western side of Ferry Road. This, in turn, has potentially made the sample plots on Plateau Heath and to the west of Ferry Road more attractive to *P. argus* imagines who have shown a preference for egg laying in habitats where *L. niger* has a presence in conjunction with *E. tetralix*, a key larval food plant for the butterfly.

6.4.4 *The impact of F. fusca on L. niger* **distribution**

Although coexistence in foraging territory is claimed to be rare in hymenoptera (Czechowski and Vepsalainen 2009), *F. fusca* was found to coexist in 7/12 *L. niger* plots, in 3/12 *F. rufa* plots and in one plot with *M. ruginodis*. Conversely, *L. alienus* and *M. scabrinodis* were not found to coexist with any other species. Figure 56 compares the distribution of combined plots (predominantly on the western side of the peninsula) where *L. niger* and *F. fusca* shared foraging grounds with plots where the two species were found to forage alone. This tendency of *F. fusca* to forage with other species has been supported by Brian (1977) who decribes *F. fusca* as a lone forager who frequently hunts in the territories of other ant species escaping attack, from competitive ants like *L. niger,* through their agility and larger size. Brian's research at Hartland Moor, Dorset found that *F. fusca* was unable to forage underground or construct covers over its food supplies or foraging tracks hence it was found mostly in sheltered areas. The species only defended nest sites rather than foraging territory and preferred to nest in drier areas than those used by *L. niger.*

It does not appear that *F. fusca* is having a detrimental impact on *L. niger* distribution as competitive exclusion does not seem to have taken place. Diver's map of *F. fusca* distribution from the 1930s (Figure 57) suggests that, like *L. niger*, the species has undergone a contraction of sites having lost territory in the middle of the peninsula, on Second and Third Ridge and at the southern end at Pipley Enclosure. This could potentially be due to the expansion of *F. rufa* which may have had an impact on *F. fusca* distribution over the last 80 years since Diver's surveys in the 1930s. Savolainen and Vepsäläinen (1988), in their work on boreal ants in the Tvärminne archipelago, discuss the competitive ant hierarchy which they state is based on colony organisation and foraging density with aggression correlating positively with foraging density. They describe *F. fusca* as a submissive at the bottom of the hierarchy, defending only their nests, while encounterers (like *L. niger*) will also defend their food supply and territorials (*like F. rufa*) at the pinnacle of the hierarchy, will additionally defend their foraging grounds. The study found, through using baited traps and mapping nest location, that submissive ants showed, 'complementary abundances' (Savolainen and Vepsäläinen 1988) sharing the foraging grounds of territorials and nesting on periphery areas of dominant ant territories. When baited traps used both carbohydrates and proteins, the submissive species shifted to carbohydrate in the presence of the more powerful species while encounterers and territorials had equal numbers on each type of bait. The research, which modelled the long-term impact of the bait experiments, found that although submissives can coexist with more powerful species, ultimately, foraging numbers and nest densities were reduced. The field work showed that food sources for *F. fusca* were often small and irregular with a high renewal rate leading ultimately to a decrease in fitness for the species when nesting on the peripheries of territorials. The study further illustrated that expansion in the territories of the dominant ants drove weaker territorials and encounterers out of the foraging area leading to emigration or the death of the weaker colony.

It is possible therefore that the expansion of *F. rufa* across the peninsula is having an impact on the distribution of *L. niger* and *F. fusca* who are moving towards the western side of the peninsula. This will have implications for the future management of the peninsula, discussed in Section 7.

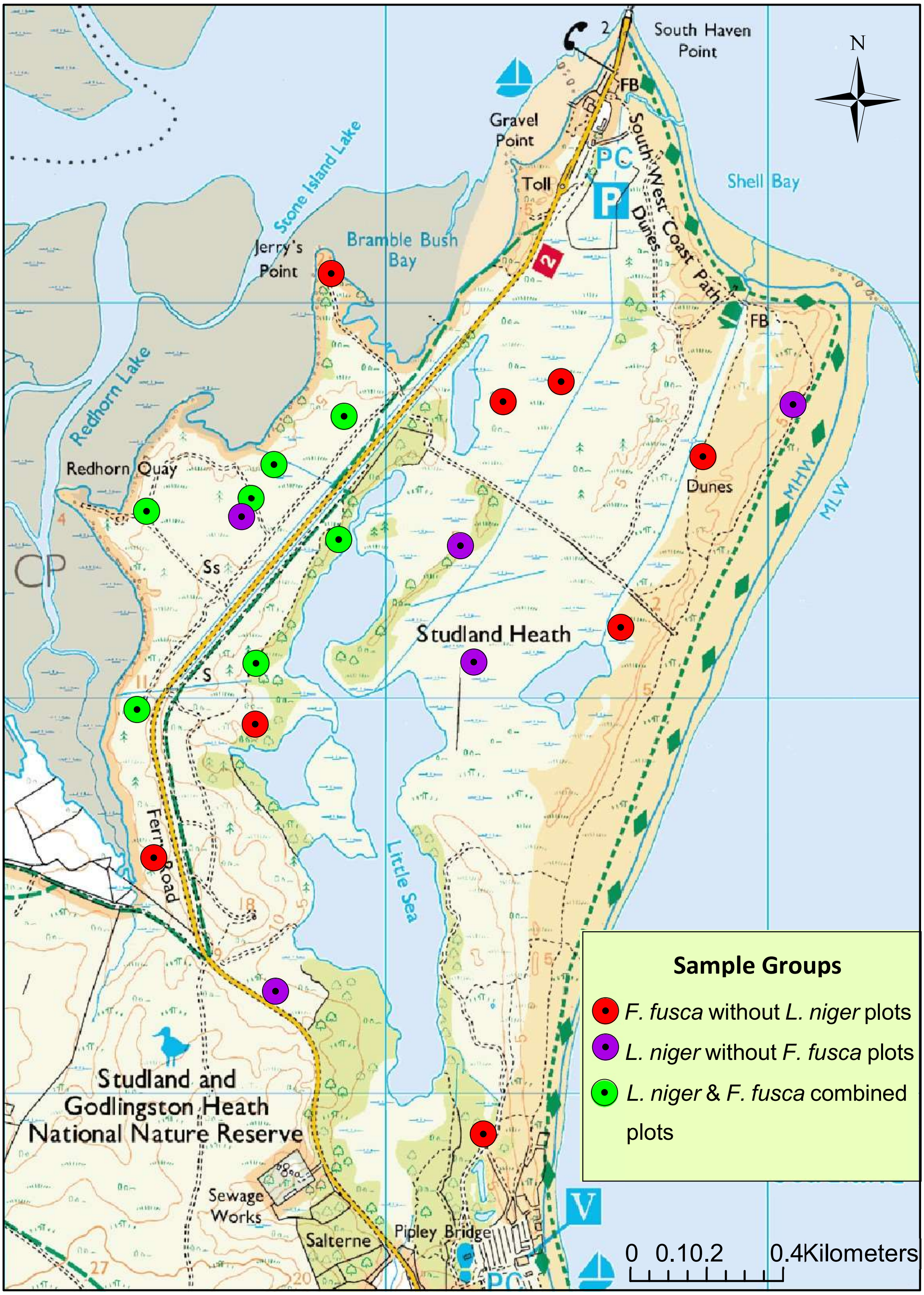

Figure 56 : The distribution of *L. niger* and *F. fusca* plots across the peninsula

(Amended from EDINA Digimap Ordinance Survey Service : Studland Peninsula using ArcMap 10.2.2.)

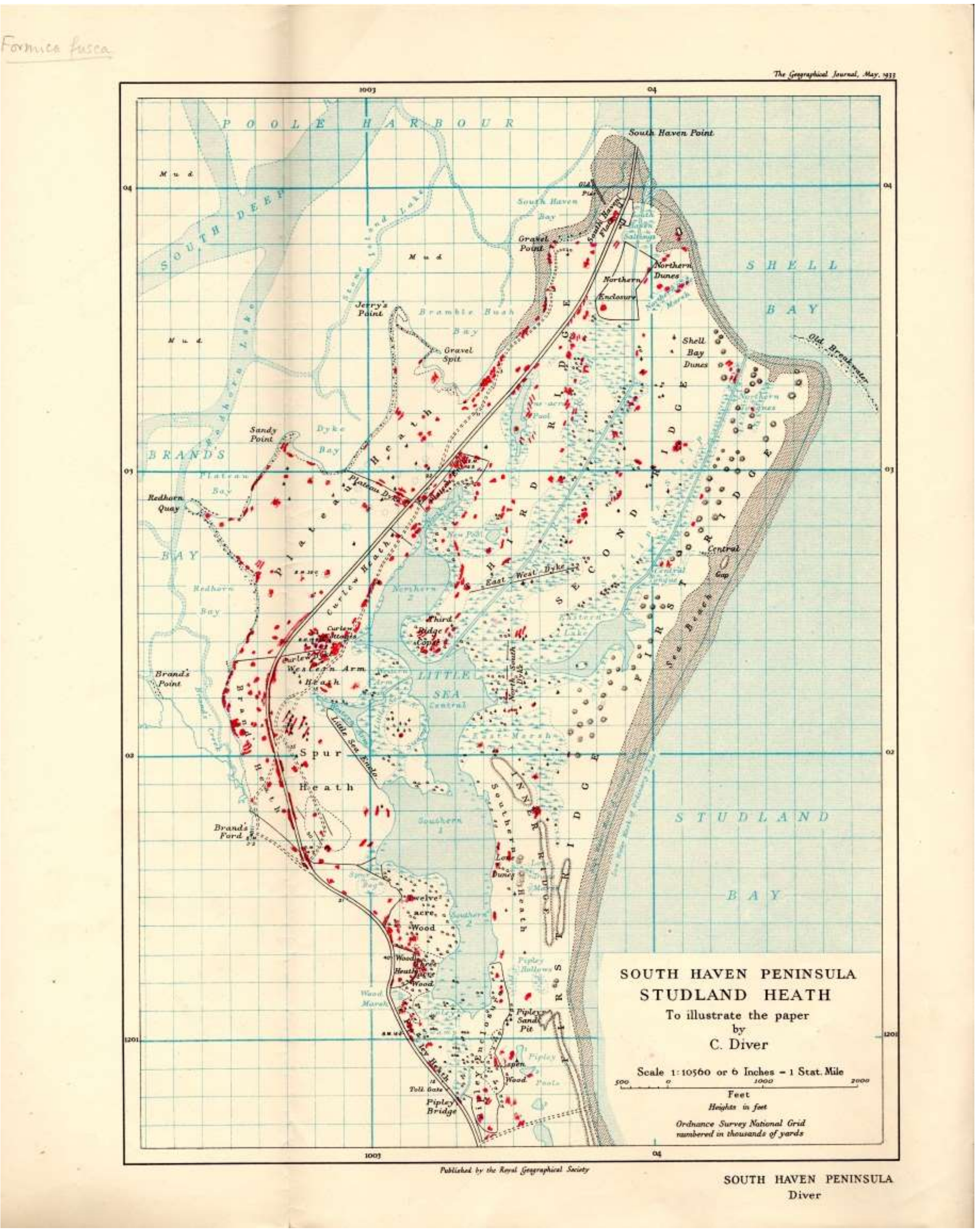

Figure 57 : The distribution of *F. fusca* in the 1930s (Diver 1933)

Dorset History Centre, Dorchester

6.4.4.1 *The impact of L. alienus, M. scabrinodis and M. ruginodis on the distribution of L. niger*

The three other ant species found on the heathland are not thought to be influencing the distribution of *L. niger*. *L. alienus* was found in two sites only (plots 2 and 3) which are both located on Second Ridge within the drier heath. Myrmecological research has recorded *L. alienus* as being commonly suppressed by the presence of *L. niger* which forces the former to forage underground losing the opportunity to acquire protein nutrition through the ingestion of ground-dwelling invertebrates (Brian 1977).

While both *L. niger* and *Myrmica* spp. have been recorded in damper, boggier areas than the other three ant species found on the heathland, it does not appear that exclusive, interspecific competition with *Myrmica* spp. is having an impact on *L. niger* distribution. *M. scabrinodis* was found in just five plots (numbers 16, 26, 24, 25 and 33), four of which were open woodland areas where *L. niger* are not commonly found (Casacci et al. 2013). *M. ruginodis* was found in four plots (numbers 11, 21, 39 and 40) which are located on Curlew Heath, close to Pipley Bridge, on Third Ridge north and on First Ridge south. Like *M. scabrinodis*, the species is known to inhabit woodland clearings and forests as well as boggy areas and grasslands but its habitat requirements do not appear to be in competition with those of *L. niger*. Results from the Spearman's Rank Correlation Coefficient found this species to have a significant, positive correlation with rush and sedge percentage cover (r= .451, p=.006) and height (r=.452, p=.006) but this was not seen as a requirement for *L. niger*. Neither *M. scabrinodis* nor *L. alienus* were found to have a significant correlation with any of the vegetation variables tested in the Spearman's Rank Correlation Coefficient. Additionally, *Myrmica* ants are not considered to be territorial (Czechowski 1979) unlike *L. niger* who will actively defend their foraging grounds.

Overall, it would appear the distribution of *L. niger* (and hence *P. argus*) is affected by *F. rufa* only in terms of interspecific competition. The next section looks at another key ecological factor, soil chemistry, and considers whether it could be influencing the distribution of *P. argus* at Studland.

6.5 How important are soil components to the distribution of *P. argus*?

To assess whether *P. argus* has specific habitat requirements, the components extracted from the PCA analysis were regressed against the results for *P. argus* abundance to determine if any significant relationships could be predicted. Multiple Linear Regression was carried out with the soil chemistry samples in Sections 5.2 and Section 5.3 and with the Resources variables in Section 5.4

6.5.1 **The relationship between *P. argus* with soil chemistry and resources**

6.5.1.1 Soil Chemistry

Results from Multiple Linear Regression in Section 5 showed that none of the PCA soil components at the 3cm and 10cm depth had a significant relationship with *P. argus* numbers. Soil chemistry does not therefore appear to be a factor in the butterfly's habitat requirements. While research on *P. argus* habitat requirements frequently discusses the importance of host plants for egg laying and larval feeding (Emmet & Heath 1990, Thomas 2007), there is very little discussion of any Lycaenid soil preference other than by reference to habitat type (i.e. the presence of heathland or grasslands) which are associated with particular subspecies, as defined in Section 1.1.4.

Component 1 in the 3cm soil PCA, did however show a significant, positive regression equation with *L. niger* abundance (Results 5.3.5). Component 1 had the highest loadings for magnesium (.905) and organic matter (.839) plus high loadings (over .7) for cadmium, zinc, total nitrogen, manganese and potassium. The association in Component 1 between magnesium and organic matter is not surprising as organic matter is made up of decomposing animal and plant residues and substances synthesized by organisms which inhabit the soil (Steila and Pond 1989). Magnesium is an essential plant nutrient facilitating photosynthesis through absorption in chlorophyll cells (Promix 2016, Patterson 2017) hence a high loading score for organic matter is very likely to be reflected by a high loading score for magnesium. Plants are used by ants in several ways; they may be used in the construction of nests (especially in *Formica* spp.), as shelter; as foraging grounds for the attainment of herbivorous prey (Skinner and Allen 1996) and they can be used as a food source. As omnivorous insects, ants feed on a variety of plants, seeds, small fruits and the plant exudate, aphid honeydew. *L. niger* is known to feed from the extrafloral nectaries found on *Pteridium aquilinum* (Skinner and Allen

1996), on *E. tetralix* and *C.vulgaris* which provide protein and flavionoids (Panda 2006) and on the stalks and caruncle of primula seeds. In addition, both *Lasius* and *Formica* species have been observed carrying *Viola* spp. and *Ulex* spp. seeds into nests aiding their dispersal (Brian 1977). Skinner and Allen (1996) discuss the impact that ants can have on soil pH, phosphorous, potassium and carbon readings which increase due to elevated levels of organic matter brought into ants' nests. In addition, Horvitz and Schemske (1986) found in their research on myrmecochory in a forest in Laguna Encantada, Mexico, that the nests of seed-dispersing ants had elevated concentrations of magnesium, nitrate-nitrogen, iron and cadmium compared to control samples without ant nests.

Ants have been described as key soil engineers (Nkem et al. 2000, Cammeraat and Risch 2008) who have a significant impact on soil properties. Frouz and Jilková (2008) discuss the physical changes in soil porosity, bioturbation and soil cohesion which ants can bring about through the excavation of underground galleries and chambers. Their paper considers the increase in nitrogen content found in soil close to ants' nests due to the accumulation of food, excreta and sodium levels which were higher than in the surrounding soil. An interesting paper by Grzes (2009) looked at cadmium and zinc accumulation in *L. niger* ants. While it is believed that ants store both metals in an inactive form for long periods of time (Vijver at al. 2004 cited by Grzes 2009), eventually elimination of metals takes place when maximum storage levels within the body are reached. This would result in increased levels of cadmium and zinc within the soil. Frouz and Jilkova (2008) point out that it is unlikely that ants choose particular sites due to their exiting soil components as foraging and nest establishment are more pressing concerns. This is supported by their research which shows that changes in soil occur slowly over time and that ants' nests follow similar patterns regardless of the original soil components the nests were built in. It would seem logical therefore to infer that the relationship between Component 1 with *L. niger* is a result of ant activity within the soil which has influenced its chemical components.

6.6 How has the distribution of *P. argus* changed since the 1930s?

The final section in this discussion focuses on sub-question three which considers whether there has been a change in the distribution of *P. argus* since Diver's surveys in the 1930s. Although it is not possible to compare the persistence of *P. argus* populations across the peninsula since Diver's work, it is possible to compare the two snapshots of *P. argus* distribution by looking at the two distribution maps from the 1930s (Figure 58) and this 2015 research (Figure 59).

Figure 58 shows *P. argus* presence, in the 1930s, at a site on the junction of Dyke Bay, on the western side of Plateau Heath, at Curlew Cottages, at two sites in Twelve Acre Wood and at Wood Heath and Aspen Wood. In this research *P. argus* was recorded on Plateau Heath and at Curlew Cottages but there were no sightings in plots located in any of the woodland areas. The butterfly was however recorded in many sites not noted by Diver; namely in plot one on Second Ridge in addition to Spur Bog and Spur Heath, Jerry's Point and Plateau Heath central, Brands Heath, Western Arms Heath and Plateau Enclosure. It appears that the distribution of the species has changed and evidence from this research demonstrates the loss of *P. argus* in the woodland areas and in Wood Heath in 2015. It is not possible however to ascertain when this distribution change occurred as Diver's records of *P. argus* sites (held by the Cyril Diver Project) are limited or if this is a permanent change in distribution. It would be inaccurate to state that there has been a long-term shift in distribution based on research from just one summer in this research or even from the results of UKBMS as the two transects carried out at Studland focus solely on the land on either side of the Ferry Road. It is however possible to discuss the apparent requirements of *P. argus*, based on results in this research, and to compare the vegetation composition of sites where *P. argus* was found in 2015 with Diver's botanical records from the 1930s.

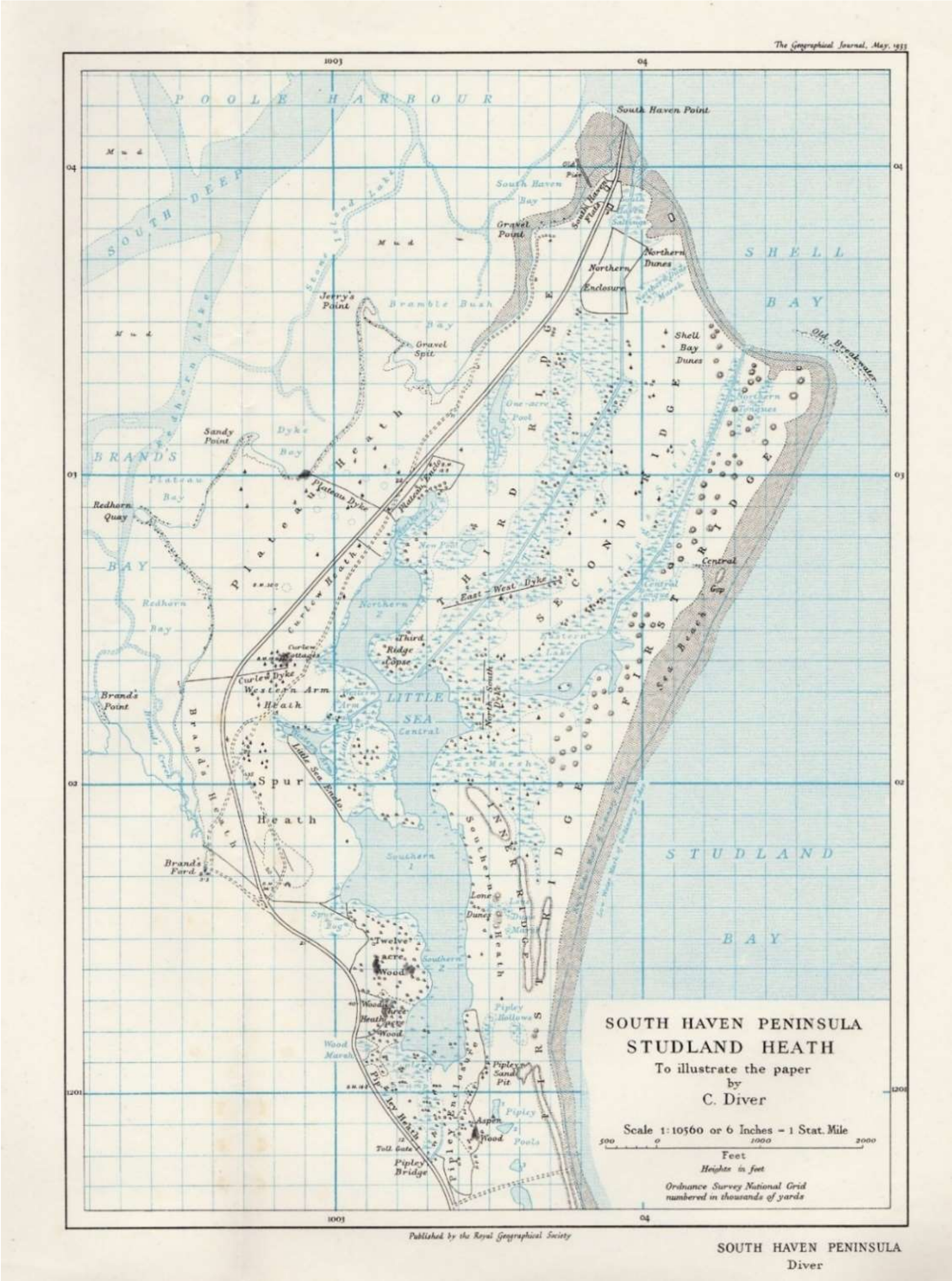

Figure 58 : The distribution of *P. argus* across Studland peninsula in the 1930s (Diver 1933)

Archive map from Dorset History Centre, Dorchester

Figure 59 : *P. argus* plots and plots in Group - + where *P. argus* was recorded by Diver in 1930s but not found in 2015 (Amended from EDINA Digimap Ordinance Survey Service : Studland Peninsula -using ArcMap 10.2.2.)

6.6.1 The possible loss of *P. argus* from Twelve Acre Wood, Wood Heath and Aspen Wood (plots 21-25)

It is possible that the loss of the butterfly in the woodland sites is due to vegetation changes which have occurred since the 1930s. Research carried out by the Cyril Diver Project (2013-2015) has noted the loss of open habitat and the growth of scrub, willow carr and woodland in many areas across the peninsula. This is due partly to natural successional processes and hydrological change but also perhaps due to changes in land use with the consequent loss of the traditional land management strategies of coppicing, grazing and burning which has taken place across the UK since the 1930s (Price 2003).

If the plant communities recorded by Diver (1936) and by the Cyril Diver Project at plots 21-25 (Figure 59) are compared, the extent of vegetation change is shown. Diver's botanical records (Cyril Diver Project 2013-15) of Aspen Wood (plot 21) reveal the presence of many grassland and heathland species which are no longer present. Diver noted: *Jasione montana* (sheep's bit); *Bellis perennis* (daisy); *Rumex acetosa* and *Rumex acetosella* (sorrel); *C. vulgaris* (ling) and *Dactylorhiza maculata* (heath spotted-orchid) in addition to several grasses including *Aira praecox* (early hair grass) and several *Agrostis* spp. While *E. tetralix* was not recorded, it is possible that the *P. argus* populations which Diver recorded, were feeding on *C. vulgaris* which can be used by the butterfly in the absence of preferred host plants (Dennis et al. 2006).

A very different picture has emerged in 2015. The vegetation recorded at plot 21 (Figure 59) in this study found very little bare or open ground with sparse grass cover. The percentage of ground cover in plot 21 was 83.4% while leaf litter % cover was 36% with *Kindbergis praelonga* (common feather moss), which is frequently found in woodland, recorded at 21%. In addition, ground cover was comprised of an abundant layer of *Glechoma hederacea* (ground ivy) with abundant woodland species notably *Hyancinthoides non-scripta* (bluebell) at 23.4%. *Pteridium aquilinum* (bracken), both dead and alive, was also abundant and the tree canopy (*Betula* spp.) was extensive at

73%. Similarly, Pipley Hollow Enclosure (site 22) had ground cover at 84% with bryophyte cover (*C. introflexus* and *D. scoparium*) at 29%.

Vegetation records taken at plots 24 and 25 (Twelve Acre Wood) also show how much change has occurred over the last 80 years. Diver recorded both *E. tetralix* and *C. vulgaris* in Twelve Acre Wood along with *Ajuga reptans* (bugle), several willow herb species (*Epilobrium obscurum*, *E.palustre and E.tetragonum*) in addition to *Veronia* spp. (speedwell); notably *V.officinalis* (heath speedwell) which grows in woodland clearings; *Rumex* spp. and *Ranunculus bulbosus* /*R. repens* (buttercup). In the wetter areas, Diver recorded the presence of many rushes including *Luzula campestris* (heath wood-rush) with *Juncus acutiflorus* (sharp-flowered rush) and *J. articulatus* (jointed rush). None of these species were found at plots 24 and 25 in this study which instead showed an abundance *of Betula* spp.*, Pteridium* spp. and an absence of large clearings of bare ground. At plot 24, 43% of ground cover was comprised of leaf litter and dead wood with *H. jutlandicum* at 23.6% while plot 25 was noted to have *sphagnum*/bryophyte cover at 27.6% and leaf litter/dead wood at 70%. Plot 23 (Wood Heath) showed a similar picture with 27% cover of dead wood, leaf litter and dead bracken with 30% bryophyte cover and little bare ground. There was no presence of *C. vulgaris* or *E. tetralix* and grass cover made up just 9%.

In the past, it is likely that these woodland sites would have been coppiced or grazed which would have opened up clearings suitable for grassland and heathland vegetation. Names such as Pipley Enclosure suggest that grazing took place at some point and the occasional presence of *Lolium multiflorum* (perennial rye grass) outside of the sample area at plot 21, suggest some degree of past cultivation. This would perhaps explain the presence of *P. argus* in the 1930s which, as discussed in Section 3, requires the presence of larval food plants such as *E. tetralix* for feeding and egg laying and warm soil where insolation is possible for larval development (Emmet & Heath 1990). Significantly, Diver's map of *L. niger* distribution in the 1930s (Figure 51) shows the species to be present in all the areas where *P. argus* was also recorded. In this research, no *L. niger* ants were found in Group **- +** (where Diver found the butterfly in the 1930s but the butterfly was not found in 2015).

Given the findings of this research, the next section considers current management strategies of heathland areas and looks at possible initiatives which could be carried out to enhance the long-term survival of *P. argus*.

7 Conclusion

7.1 The Importance of Conservation Management Strategies

Over the past 40 years, 76% of the UK's resident and migrant butterfly species have decreased in number (Fox et al. 2015) due to agricultural intensification and land conversion (Schirmel & Fartmann 2014) and so far, there is little evidence to suggest that this will change. The habitats used by butterflies for breeding have been radically altered by humans and most species are now reliant on the ways in which humans manage land for their continued existence (Warren 1993). The management of land for butterfly conservation is of vital importance if the decline in butterfly abundance is to be reversed. For *P. argus*, at Studland, the management of heathland to ensure that early successional stages are always available is of vital importance; the next sub-section looks at some of the ways in which succession may be arrested.

7.1.1 **Successional processes and the threat to *P. argus* and *L. niger***

Succession to the climax community of trees, dominant grasses and tall shrubs is a real threat to heathland butterflies. If successional processes continue unchecked, nutrients begin to accumulate within the soil encouraging the growth of competitive, dominant species which shade out other plants and reduce diversity. As grasses, shrubs and trees begin to encroach on the heath, they change the microclimate of the soil by blocking out sunlight and reduce the abundance of host plants (Schirmel & Fartmann 2014). Warm soil temperatures are important for the development of *P. argus* larvae improving their chances of surviving winter hibernation while *L. niger* needs warm soil for larval growth, for the restoration of glandular activity, to enable the sex organs to develop (Brian 1977) and to activate foraging behaviour. Woodland growth is often associated with an increase in plant species such as *Pteridium aquilinum*, *Rubus fruticosus* and *Hedera helix* which undermine the growth of ericaceous species used by *P. argus* as a nectar source, for egg laying and are key food plants for larvae hatching in the spring. Unarrested succession also leads to the loss of bare ground as dense, dominant vegetation begins to colonise. Ravenscroft & Warren (1996) state that *P. argus* frequently deposits eggs directly onto bare soil or on the stems of food plants which fringe bare soil while bare ground is equally important for *L. niger* enabling nesting opportunities.

7.1.2 **Creating a disturbance**

A number of management strategies are used within conservation to halt successional processes which threaten lowland heathland areas and to retain structural diversity in vegetation on the heath. It is important to create a disturbance in the successional process which enables threatened plants to survive.

7.1.2.1 Grazing by livestock cattle

Summer grazing by Red Devon cattle has been introduced at Studland Heath in several areas including Plateau Heath and Curlew Heath. This docile breed is frequently used in conservation management as it is relatively hardy and can thrive on unimproved pastures as well as on marginal grassland. The aim is to maintain the delicate structure of the heath by controlling the growth of dominating species and prevent the encroachment of scrub (English Nature 2005). For *P. argus*, the creation of a structurally varied resource with a mosaic of heather standing at different heights and at different age phases (Thomas 1993) is needed to fulfil all life stages. Cattle grazing is effective as the animals' disturbance of the ground aids the rejuvenation of Ericaceous plants (Ravenscroft and Warren 1996) while reducing the dominance of colonising shrubs and tree saplings and keeping the grass short. Grazing removes vegetation in a gradual way which allows species in the habitat to move away and cattle create their own access routes even over rough terrain. As cattle use their tongues to break off vegetation tufts, they do not graze too closely to the ground where butterfly eggs may have been deposited. They leave tufts behind which can provide refuge for many invertebrates and do not graze selectively (English Nature 2005). The impact of the Red Devon cattle at Studland is being monitored to ensure that sensitive habitats are not excessively disturbed and to keep the delicate balance between stable and open vegetation with areas of bare ground.

7.1.2.2 Burning

Burning can be problematic on heathland where it occurs in close proximity to urban areas (MAFF 1992) but it is an effective method of disposing of litter layer thus decreasing nutrient levels in the soil, while encouraging regrowth of Ericaceous species and *Ulex* spp. Controlled burning (on a rotational basis) has taken place on Plateau Heath in the last 5 years (Peters et al. 2011) and, interestingly, this is a stronghold for *P. argus* habitation where most of the sightings in this study were recorded. Controlled burning can increase biodiversity as it reduces the abundance of shrub/ tree sapling vegetation and opens new areas for cattle grazing (Chatters 2015) which, in turn, helps to maintain the early successional stage community needed by *P. argus*. Management of heathland, after burning, is considered essential by Chatters (2015) as fire can stimulate the growth of *M. caerulea* which dominates wet heathland. The combination of burning and grazing is considered to be most effective in maintaining conditions.

7.1.2.3 Cutting and Mowing

A key initiative designed to encourage open, ericaceous heathland is the removal of encroaching scrub and trees, notably *Pinus sylvestris* and *Betula* spp. (Peters et al. 2011) and dominant grasses in addition to the felling of trees where fragmentation of heathland has occurred. While the presence of some tall shrubs and bushes close to larval food plants is a possible requirement for *P. argus* as a shelter in unseasonable weather and for mating and brooding (Dennis 2003), open, unshaded areas are important for host plant growth. A balance between the two is required to enhance the survival of *P. argus* through all life stages. In addition, *P. aquilinum* (bracken), while having some benefits for *L. niger* who feed on the extra-floral nectaries at the base of the bracken frond (Brian 1977), is a dominant plant which can form a dense canopy up to two metres in height. During the autumn, the canopy drops creating a thick mat of litter which smothers low growing plant species (Forestry Commission 2014). Where *P. aquilinum* is beginning to encroach on the open heathland, cutting and removal is effective before the canopy collapses.

7.1.3 **Habitat fragmentation and isolation**

The provision of linking, heathland corridors is particularly important for sedentary species like *P. argus* which needs connected habitat patches at different stages of development, to avoid local extinction. Within a heathland, a hub of local breeding areas exist which are connected by dispersal and while disturbances occur, suitable habitat for *P. argus* continues to be available for emigrating butterflies (Thomas et al. 1998). Metapopulations have however, now become confined to large heathlands in the south of England (Thomas et al. 1998). As the butterfly lives in discrete areas and is a sedentary species, in small heathland fragments, breeding pockets can be found close together which can have an impact on genetic fitness (Thomas 1985). A study by Thomas et al. (1998) considered the effect of habitat fragmentation on the costs and benefits of migration and ultimately on any consequent evolutionary alteration in dispersal traits. The research looked at five morphological characters of *P. argus* individuals from both limestone and heathland sites and related this to the level of fragmentation in both types of site. The results showed that landscape spatial structure does indeed effect morphological characteristics, specifically those associated with flight ability. It was found that individual butterflies total mass increased while heathland areas decreased suggesting that larger *P. argus* individuals are more successful in smaller heathland areas possibly because larger individuals are worse at flying. The research suggested that changes in life history traits which appear to be occurring in response to landscape fragmentation, may in *P. argus* be linked to mate-location strategies on emigration rates and could consequently change dispersal traits in the future.

Heathland fragmentation can also lead to population fragmentation and consequently reduced genetic diversity and fitness. As threatened, sedentary species become confined to small areas of land, bottlenecks are more likely to occur influencing the genetic profile of the remnant population. Research on genetic diversity by Brookes et al. (1997) on *P. argus* sample butterflies taken from several sites in North Wales in 1992 and 1994, found a loss of genetic diversity in rare allozyme alleles in descendant populations. The research states that although this loss is unlikely to cause population instability due to the rarity of the alleles identified and due to the large *P. argus* population in N. Wales, it does indicate vulnerabilities for the butterfly living within smaller populations.

7.1.4 **Other management methods**

7.1.4.1 Digging scapes for L. niger

In addition to the management of successional processes, *L. niger* presence can be actively encouraged in an area by the digging out of scapes to provide ant habitat. At Broadcroft Quarry, Isle of Portland, Dorset, in 2003, 0.2 ha of earth were dug to create ant scapes for *L. alienus* (the symbiotic ant on this site) and the excavated soil was then used to create effective windbreaks (de Whalley et al. 2006). This is an exposed, calcareous quarry site hence shelter from the wind was needed for both the ants and *P. argus*. By 2005, *L. alienus* had recolonised the bare ground on the scapes and the numbers of *P. argus* have continuously increased. Similar methods could be used to maintain *L. niger* presence or in areas of the heathland where *L. niger* and *P. argus* are not currently found but where host plants and other conditions seem suitable. The creation of bare ground (without scapes) across the heath would benefit both *L. niger* and *P. argus* as a warm microclimate is created for larval development and foraging activity. It is also possible that this modification to the environment, in conjunction with tree felling and succession control, may deter *F. rufa* presence as conditions become unfavourable for nesting.

7.1.5 **Monitoring numbers**

Continual monitoring of the vegetation communities on the heathland and of *P. argus* abundance and distribution would highlight any changes in the butterfly's population density. Butterfly Conservation carry out two transects at Studland and Ferry Road each summer and the changes in numbers are meticulously recorded and fed into nationwide results. It would be beneficial to include additional transects at the southern edge of Second Ridge where three *P. argus* imagines were recorded in 2015 and on the western edge of Plateau Heath where the butterfly has hotspot areas.

Management planning also needs to take the impact of climate change into account as it is possible that there will be shifts in species distribution as temperatures change. Hodgson et al. (2015) have recorded a rise of 1°C in the regional May temperature at Great Orme and the Dulas Valley in North Wales which is populated by *P. a. caernensis* and is close to the species' northwest range. The linear model constructed to assess butterfly numbers, based on past and present records, predicted that *P. a. caernensis* is likely to undergo small shifts in distribution especially in response to higher May temperatures when larvae and pupae are present. The study pointed out that *P. argus* might respond to a warming climate by expanding its range but that there is a great deal of uncertainty at present over what kind of an impact climate change will have for butterflies as there are many climatic variables governing their daily lives. The paper advises conservation managers to adopt robust strategies which can incorporate uncertainty due to the unpredictable, idiosyncratic response of many species to change.

7.1.5.1 Monitoring ant communities

To protect *P. argus* populations in the future, more research needs to be carried out on the interspecific relationships between *Lasius* spp. with other ant species, particularly *Formica rufa* which was listed on the UK's Biodiversity Action Plan in 2004 (absent from the UK post 2010 Biodiversity Framework). Regular surveying of ant abundance and distribution across the peninsula would enable conservation managers to see how the community is changing. This research did not find *L. niger* and *F. rufa* coexisting at any of the sample points and this has been substantiated by many studies which show that interspecific competition between the two is likely to lead to a reduction in *L. niger* nesting sites. This is determined by vegetation to some extent; if current *L. niger* sites succumb to successional processes losing bare ground and short grass, *F. rufa* will be able to expand its distribution as shrubs and trees encroach on to open heathland areas. Given the close symbiotic relationship between *P. argus* and *L. niger*, any change in the distribution of the latter will have an impact on the spatial occurrence and abundance of the butterfly.

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9 Appendices

9.1 GPS Site References

Table 17 : GPS references for each sample plot

Plot No.	**Easting**	**Northing**
Group + -		
1	403223	85101
4	402884	85722
6	402615	85469
7	402341	84981
9	402679	84463
11	402385	84606
19	402850	86083
31	402704	84268
33	402671	84612
Group - +		
21	403157	83668
22	403248	83907
23	402961	84065
24	403010	84158
25	402928	84273
Group - -		
2	403471	85356
3	403552	85470
8	402741	84977
12	403188	85395
13	403250	84800
14	403608	85189
15	403823	85620
16	403592	85640
17	403452	85810
18	403300	85759
20	403787	85105
32	402969	83808
34	403145	85648
35	403420	86047
36	403753	85884
37	404059	85752
38	403765	85213
39	403344	85982
40	403400	84105
Group + +		
5	402640	85515
10	402835	85393

Plot No.	**Easting**	**Northing**
26	402651	84944
27	402653	85098
28	402870	85411
29	402700	85600
30	402366	85482

9.2 Habitat Type

Table 18 : Brief description of habitat type at each sample point

Sample Plot Number	Habitat Type
1	Humid/wet heath
2	*Molinia* bog
3	Dune heathland
4	Wet heath
5	Wet heath
6	Wet heath
7	Wet heath/ edge gorse scrub
8	Near Little Sea
9	Transitional damp-wet heath
10	Wet woodland
11	Dry heath
12	*Molinia* bog
13	Wet heath
14	Dry woodland
15	Wet heath
16	Woodland
17	Wet heath
18	Dune heath/gorse scrub
19	Gorse scrub
20	Dune heath
21	Dry woodland
22	Gorse scrub
23	Wet woodland
24	Wet woodland
25	Woodland clearing
26	Dry/wet transitional heath
27	Dry/wet transitional heath
28	Wet heath
29	Dry/wet transitional heath
30	Grassland
31	Bog land
32	bog land
33	Dry Heath
34	Woodland scrub
35	Woodland scrub
36	Dry heath
37	Dune heath
38	Dune heath
39	Dry heath
40	Dry heath

9.3 Morphology of the butterfly

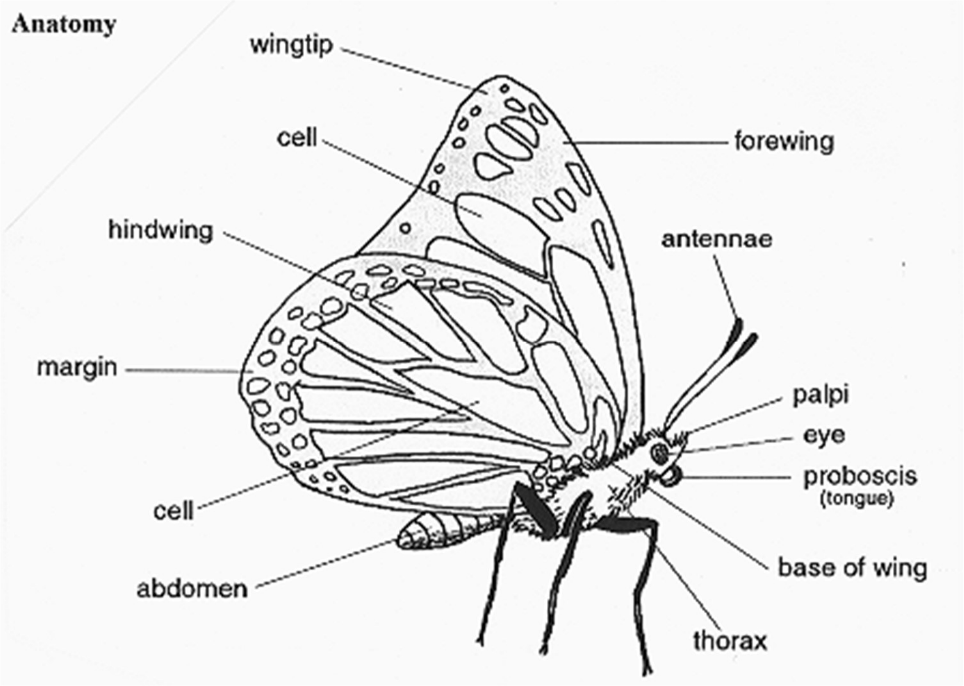

Figure 60 : Lepidoptera anatomy

(Welcomewildlife 2017)

9.4 Morphology of the ant

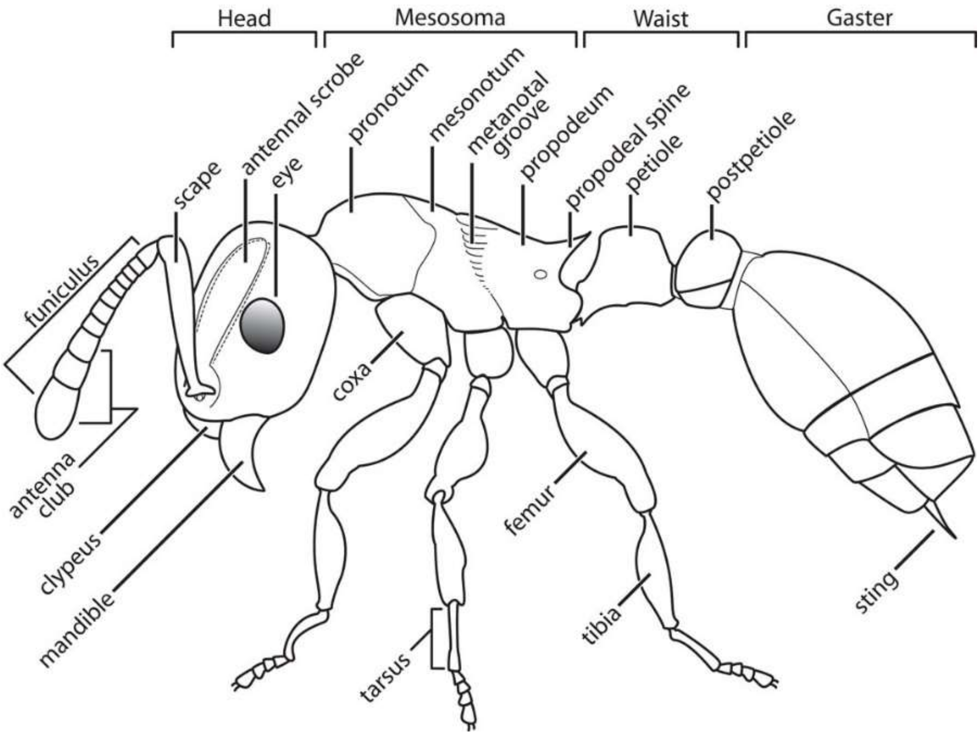

Figure 61 : External anatomy of the worker ant (antennal club/Antark)

9.5 Mann Whitney U test comparing *P. argus* abundance in Groups + - and + +.

Differences not shown to be significant as p= .314.

Table 19 : Ranks for Groups **+ -** and **+ +**

Ranks

	group	N	Mean Rank	Sum of Ranks
P_argus	1000.00	9	7.44	67.00
	4000.00	7	9.86	69.00
	Total	16		

Table 20 : No significant difference found between *P. argus* abundance in Group **+ -** and **+ +**

Test Statistics[a]

	P_argus
Mann-Whitney U	22.000
Wilcoxon W	67.000
Z	-1.007
Asymp. Sig. (2-tailed)	**.314**
Exact Sig. [2*(1-tailed Sig.)]	.351[b]

a. Grouping Variable: group

b. Not corrected for ties.

9.6 Resource Variables PCA

Resources PCA compiled using independent vegetation variables used plus *L. niger*

Table 21 : Kaiser-Meyer Olkin Measure of Sampling Adequacy and Extraction amounts for each variable

Kaiser-Meyer-Olkin Measure of Sampling Adequacy.		**.557**
Bartlett's Test of Sphericity	Approx. Chi-Square	131.473
	df	120
	Sig.	.223

	Initial	Extraction
Cal_vul_cover	1.000	.745
E_tetralix_cover	1.000	.823
bryo_spagnum	1.000	.608
c_introflex	1.000	.813
trees_cover	1.000	.640
bare_ground	1.000	.737
leaf_lit_deadwd	1.000	.645
grd_plant_cover	1.000	.777
grass_under2cm	1.000	.863
grass_2_5cover	1.000	.676
grass_5_10cm	1.000	.750
E_cinereacver	1.000	.630
L_niger_ab	1.000	.730
cladonia	1.000	.678
flowering_cover	1.000	.753
shrub_cover	1.000	.710

Extraction Method: Principal Component Analysis.

9.7 MLR : *P. argus* and Resources

Model Summary: A significant regression equation found between *P. argus* abundance and Component 5 of Resources PCA

Table 22 : R value for MLR

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.664[a]	.441	.319	32.52658

Table 23 : A significant p value (.006) seen when *P. argus* regressed against Resources PCA and p value of .001 when regressed against Component 5.

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	26709.089	7	3815.584	3.606	**.006[b]**
	Residual	33855.311	32	1057.978		
	Total	60564.400	39			

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	15.300	5.143		2.975	.006
	REGR factor score 1 for analysis 1	-8.435	5.208	-.214	-1.619	.115
	REGR factor score 2 for analysis 1	-2.580	5.208	-.065	-.495	.624
	REGR factor score 3 for analysis 1	1.805	5.208	.046	.347	.731
	REGR factor score 4 for analysis 1	-9.823	5.208	-.249	-1.886	.068
	REGR factor score 5 for analysis 1	**-19.701**	**5.208**	**-.500**	**-3.782**	**.001**
	REGR factor score 6 for analysis 1	9.603	5.208	.244	1.844	.074
	REGR factor score 7 for analysis 1	5.192	5.208	.132	.997	.326

a. Dependent Variable: *P_argus*

9.8 Binary Regression Logistics

Table 24 : Model Summary

Step	-2 Log likelihood	Cox & Snell R Square	Nagelkerke R Square
1	39.910[a]	.294	.398

a. Estimation terminated at iteration number 4 because parameter estimates changed by less than .001.

Table 25 : Contingency Table for Hosmer and Lemeshow Test

		*P_argus*_recode = .00		*P_argus*_recode = 1.00		Total
		Observed	Expected	Observed	Expected	
Step 1	1	22	22.000	6	6.000	28
	2	2	2.000	10	10.000	12

Table 26 : **Classification Table[a]**

Observed			Predicted		
			P_argus_recode		Percentage Correct
			.00	1.00	
Step 1	*P_argus*_recode	.00	22	2	91.7
		1.00	6	10	62.5
	Overall Percentage				80.0

a. The cut value is .500

Table 27 : **Variables in the Equation**

		B	S.E.	Wald	df	Sig.	Exp(B)	95% C.I.for EXP(B)	
								Lower	Upper
Step 1[a]	*L_niger*_recode	2.909	.901	10.418	1	.001	**18.333**	3.134	107.232
	Constant	-1.299	.461	7.958	1	.005	**.273**		

a. Variable(s) entered on step 1: *L_niger*_recode.

9.9 Vegetation PCA using ant plots only

Table 28 : KMO and Bartlett's Test Results

Kaiser-Meyer-Olkin Measure of Sampling Adequacy.		.517
Bartlett's Test of Sphericity	Approx. Chi-Square	121.294
	df	105
	Sig.	.132

Table 29 : Communalities Extraction Figures

	Initial	Extraction
bare_ground	1.000	.694
bryo_spagnum	1.000	.577
c_introflex	1.000	.611
Cal_vul_cover	1.000	.743
E_cinereacver	1.000	.624
E_tetralix_cover	1.000	.783
flowering_cover	1.000	.774
grass_under2cm	1.000	.569
grass_2_5cover	1.000	.731
grass_5_10cm	1.000	.709
grd_plant_cover	1.000	.835
leaf_lit_deadwd	1.000	.615
rush_sedge_cov	1.000	.324
shrub_cover	1.000	.766
trees_cover	1.000	.787

Extraction Method: Principal Component Analysis.

9.10 MLR of ants with Vegetation PCA using ant plots only

9.10.1 *L. niger* results

Table 30 : Model Summary, ANOVA and Coefficient results for *L. niger*

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.514[a]	.265	.112	87.93395

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	80697.017	6	13449.503	1.739	.147[b]
	Residual	224238.983	29	7732.379		
	Total	304936.000	35			

a. Dependent Variable: *L_niger*_ab

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	54.333	14.656		3.707	.001
	REGR factor score 1 for analysis 1	-13.239	14.864	-.142	-.891	.380
	REGR factor score 2 for analysis 1	-24.627	14.864	-.264	-1.657	.108
	REGR factor score 3 for analysis 1	-29.168	14.864	-.312	-1.962	.059
	REGR factor score 4 for analysis 1	-24.212	14.864	-.259	-1.629	.114
	REGR factor score 5 for analysis 1	-1.328	14.864	-.014	-.089	.929
	REGR factor score 6 for analysis 1	-9.225	14.864	-.099	-.621	.540

9.10.2 **Kruskal Wallis H test : *L. niger* across groups**

Table 31: Results from Kruskal Wallis H Test and *L. niger* across all groups

Ranks

	group	N	Mean Rank
*L. niger*_ab	1000.00	9	22.78
	2000.00	5	12.50
	3000.00	15	14.57
	4000.00	7	25.71
	Total	36	

Test Statistics[a,b]

	L_niger_ab
Chi-Square	12.043
df	3
Asymp. Sig.	.007

a. Kruskal Wallis Test

9.10.3 ***F. rufa* results**

Table 32 : Model Summary, ANOVA and Coefficients for *F. rufa*

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.589[a]	.347	.212	133.14391

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	272937.054	6	45489.509	2.566	.041[b]
	Residual	514091.696	29	17727.300		
	Total	787028.750	35			

a. Dependent Variable: *F_rufa*_ab

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	79.583	22.191		3.586	.001
	REGR factor score 1 for analysis 1	1.946	22.505	.013	.086	.932
	REGR factor score 2 for analysis 1	1.605	22.505	.011	.071	.944
	REGR factor score 3 for analysis 1	-15.648	22.505	-.104	-.695	.492
	REGR factor score 4 for analysis 1	27.286	22.505	.182	1.212	.235
	REGR factor score 5 for analysis 1	20.802	22.505	.139	.924	.363
	REGR factor score 6 for analysis 1	79.810	22.505	.532	3.546	.001

a. Dependent Variable: F_rufa_ab

9.10.4 ***F. fusca***

Table 33 : Model Summary, ANOVA and Coefficients for *F. fusca*

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.387[a]	.149	-.027	22.99081

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	2692.262	6	448.710	.849	.543[b]
	Residual	15328.738	29	528.577		
	Total	18021.000	35			

a. Dependent Variable: *F_fusca*_ab

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	15.833	3.832		4.132	.000
	REGR factor score 1 for analysis 1	-1.058	3.886	-.047	-.272	.787
	REGR factor score 2 for analysis 1	-.819	3.886	-.036	-.211	.834
	REGR factor score 3 for analysis 1	-4.091	3.886	-.180	-1.053	.301
	REGR factor score 4 for analysis 1	-4.177	3.886	-.184	-1.075	.291
	REGR factor score 5 for analysis 1	4.274	3.886	.188	1.100	.280
	REGR factor score 6 for analysis 1	-4.763	3.886	-.210	-1.226	.230

a. Dependent Variable: *F_fusca*_ab

9.10.5 ***L. alienus* results**

Table 34 : Model Summary, ANOVA and Coefficients for *L. alienus*

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.252[a]	.064	-.130	30.36091

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	1820.234	6	303.372	.329	.916[b]
	Residual	26731.766	29	921.785		
	Total	28552.000	35			

a. Dependent Variable: *L_alienus*_ab

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	6.667	5.060		1.317	.198
	REGR factor score 1 for analysis 1	-3.466	5.132	-.121	-.675	.505
	REGR factor score 2 for analysis 1	-2.263	5.132	-.079	-.441	.662
	REGR factor score 3 for analysis 1	4.065	5.132	.142	.792	.435
	REGR factor score 4 for analysis 1	2.830	5.132	.099	.551	.586
	REGR factor score 5 for analysis 1	-3.187	5.132	-.112	-.621	.539
	REGR factor score 6 for analysis 1	-.426	5.132	-.015	-.083	.934

a. Dependent Variable*: L_alienus*_ab

9.10.6 *M. scabrinodis* results

Table 35 : Model Summary, ANOVA and Coefficients for *M.scabrinodis*

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.464[a]	.215	.053	40.12553

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	12796.212	6	2132.702	1.325	.278[b]
	Residual	46691.677	29	1610.058		
	Total	59487.889	35			

a. Dependent Variable: *M_scabrinodis*_ab

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	15.056	6.688		2.251	.032
	REGR factor score 1 for analysis 1	17.771	6.782	.431	2.620	.014
	REGR factor score 2 for analysis 1	-1.260	6.782	-.031	-.186	.854
	REGR factor score 3 for analysis 1	4.022	6.782	.098	.593	.558
	REGR factor score 4 for analysis 1	1.062	6.782	.026	.157	.877
	REGR factor score 5 for analysis 1	.766	6.782	.019	.113	.911
	REGR factor score 6 for analysis 1	-5.506	6.782	-.134	-.812	.424

a. Dependent Variable: *M. scabrinodis*_ab

9.10.7 ***M. ruginodis* results**

Table 36 : Model Summary, ANOVA and Coefficients for *M. ruginodis*

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.553[a]	.305	.162	68.57285

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	59918.796	6	9986.466	2.124	.081[b]
	Residual	136364.843	29	4702.236		
	Total	196283.639	35			

a. Dependent Variable: *M_ruginodis*_ab

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	25.694	11.429		2.248	.032
	REGR factor score 1 for analysis 1	-10.348	11.591	-.138	-.893	.379
	REGR factor score 2 for analysis 1	29.639	11.591	.396	2.557	.016
	REGR factor score 3 for analysis 1	10.403	11.591	.139	.898	.377
	REGR factor score 4 for analysis 1	24.707	11.591	.330	2.132	.042
	REGR factor score 5 for analysis 1	-2.784	11.591	-.037	-.240	.812
	REGR factor score 6 for analysis 1	-.153	11.591	-.002	-.013	.990

a. Dependent Variable: *M_ruginodis*_ab

9.11 Results from PCA in 3cm Soil Samples: 3 main components extracted from PCA

Table 37: KMO and Bartlett's Test and Component Score Coefficient Matrix Results

Kaiser-Meyer-Olkin Measure of Sampling Adequacy.		.713
Bartlett's Test of Sphericity	Approx. Chi-Square	471.526
	df	78
	Sig.	.000

Component Score Coefficient Matrix

	Component		
	1	2	3
pH_a	-.152	-.056	.587
conductivity_a	-.039	.004	.389
total_N_a	.133	.085	-.087
OM_a	.213	-.024	-.153
K_a	.109	-.004	.108
Ca_a	.158	-.122	.139
Mg_a	.214	-.101	-.007
Fe_a	.008	.183	.109
Mn_a	.169	-.098	.035
P_a	.067	.171	-.060
Cu_a	-.180	.456	-.002
Pb_a	-.094	.418	-.081
Zn_a	.248	-.124	-.199

9.12 Multiple Linear Regression of *P. argus* abundance with 3cm soil PCA

Table 38 : ANOVA and Coefficients for *P. argus* showing p =.480

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	3969.467	3	1323.156	.842	.480[b]
	Residual	56594.933	36	1572.081		
	Total	60564.400	39			

a. Dependent Variable: *P. argus*

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	15.300	6.269		2.441	.020
	REGR factor score 3 for analysis 1	-1.908	6.349	-.048	-.301	.765
	REGR factor score 2 for analysis 1	.922	6.349	.023	.145	.885
	REGR factor score 1 for analysis 1	9.864	6.349	.250	1.554	.129

9.13 Multiple Linear Regression of *L. niger* abundance with 3cm soil PCA

Table 39 : Model Summary, ANOVA and Coefficients Results

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.381[a]	.145	.074	86.58031

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	45702.212	3	15234.071	2.032	.127[b]
	Residual	269861.388	36	7496.150		
	Total	315563.600	39			

a. Dependent Variable: *L_niger*_abundance

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	48.900	13.690		3.572	.001
	REGR factor score 3 for analysis 1	-2.540	13.864	-.028	-.183	.856
	REGR factor score 2 for analysis 1	1.006	13.864	.011	.073	.943
	REGR factor score 1 for analysis 1	34.123	13.864	.379	2.461	.019

a. Dependent Variable: *L._niger* abundance

9.13.1 **Component 1 in 3cm soil PCA and *L. niger***

Results showing significant, positive regression equation between *L. niger* with Component 1.

Table 40 : Model Summary, ANOVA and Coefficients for *L. niger* and Component 1 of 3cm soil PCA

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.379[a]	.144	.121	84.31653

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	45411.041	1	45411.041	6.388	.016[b]
	Residual	270152.559	38	7109.278		
	Total	315563.600	39			

a. Dependent Variable: *L_niger*_ab

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	48.900	13.332		3.668	.001
	REGR factor score 1 for analysis 1	34.123	13.501	.379	2.527	.016

a. Dependent Variable: *L. niger*_ab

9.14 Results from PCA in 10cm soil samples: 3 components extracted

3 components extracted accounting for 72.31% of variance

Table 41 : KMO and Bartlett's Test and Communalities Extraction results

Kaiser-Meyer-Olkin Measure of Sampling Adequacy.		.805
Bartlett's Test of Sphericity	Approx. Chi-Square	378.003
	df	66
	Sig.	.000

	Initial	Extraction
pH_b	1.000	.787
conductivity_b	1.000	.691
total_N_b	1.000	.742
OM_b	1.000	.779
K_b	1.000	.766
Mg_b	1.000	.924
Fe_b	1.000	.298
Mn_b	1.000	.623
Cu_b	1.000	.807
Pb_b	1.000	.686
Zn_b	1.000	.647
Na_b	1.000	.927

9.15 MLR : *P. argus* with 3 components extracted from soil 10 cm samples PCA

No significant regression found

Table 42 : Model Summary, AMOVA and Residuals Statistics for *P. argus* when regressed against 10cm soil sample PCA

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	580.255	3	193.418	.116	.950[b]
	Residual	59984.145	36	1666.226		
	Total	60564.400	39			

a. Dependent Variable: *P_argus*

Model Summary[b]

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.098[a]	.010	-.073	40.81943

Residuals Statistics[a]

	Minimum	Maximum	Mean	Std. Deviation	N
Predicted Value	-1.7586	26.7727	15.3000	3.85724	40
Residual	-20.55257	198.43709	.00000	39.21804	40
Std. Predicted Value	-4.422	2.974	.000	1.000	40
Std. Residual	-.503	4.861	.000	.961	40

a. Dependent Variable: *P. argus*

9.16 MLR : *L. niger* with 3 components extracted from soil 10 cm samples PCA

No significant regression equation found.

Table 43 : Model Summary, ANOVA and Residuals Statistics for *L. niger* in 10 cm soil PCA

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	23871.913	3	7957.304	.982	.412[b]
	Residual	291691.687	36	8102.547		
	Total	315563.600	39			

a. Dependent Variable: *L. niger*_ab

Model Summary[b]

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.275[a]	.076	-.001	90.01415

b. Dependent Variable: *L. niger*_ab

Residuals Statistics[a]

	Minimum	Maximum	Mean	Std. Deviation	N
Predicted Value	-25.1034	113.9503	48.9000	24.74066	40
Residual	-98.07225	249.57524	.00000	86.48280	40
Std. Predicted Value	-2.991	2.629	.000	1.000	40
Std. Residual	-1.090	2.773	.000	.961	40

a. Dependent Variable: *L. niger*_ab