Nail Fungus *Poronia punctata* in Norfolk, and possible factors affecting its appearance at Hockwold Heath and Cranwich Camp

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The Nail Fungus *Poronia punctata* is a distinctive species that occurs on the dung of horses and, much less commonly, that of other herbivores. It may have been quite widespread before the middle of the 20th century but since then has been almost restricted to the New Forest. Recently, however, it has been recorded more widely in southern England, including Norfolk, probably as a consequence of the increasing use of ponies for conservation grazing where they are used to prevent scrub encroachment**.** *P. punctata* is designated as Near Threatened on the UK Fungi Red Data List (Evans et al. 2006) and is a Biodiversity Action Plan species.

In this study, factors affecting its appearance on horse dung, including the nature of the vegetation and the condition of the dung, were investigated.

The fungus and its lifecycle

P. punctata is an ascomycete in the family Xylariaceae. Its 'fruiting bodies'(ascomata) appear as buff-coloured discs (stromata), typically 5-15 mm in diameter, on the surface of the dung. Each discarises from a dark stalk embedded in the dung, giving the whole fungus the appearance of a clout or nail when extracted from the dung. Within the upper surface are numerous perithecia appearing as black dots (Figs. 1 & 2. p. 121). Each is a spherical cavity lined with asci, each of which contains eight spores.The ripe spores pass out of the perithecium via a small pore in its apex. These spores are dispersed by the wind and may land on vegetation which is ingested by a horse and thereby pass through its gut to arrive in fresh dung. Here it develops into a mycelium which colonises the dung and feeds on the organic matter present.

Spores produced in this way are the product of a sexual process but it is likely that *Poronia* is homothallic, that is, self-fertilizing. In addition, the young ascomata produce asexual spores visible as a powdery covering before the perithecia are visible.

Occurrence and distribution

There is anecdotal evidence that *P. punctata* was formerly much commoner in the past. Although this distinctive fungus is readily seen when present (but see *P. erici*, below), it is generally the case for fungi that their recording is patchy and greatly influenced by variable 'recorder effort'. Examination of the 272 records (December 2015) for this species on the Fungal Record Database of Britain and Ireland (FRDBI) showed that 22% of these records were made before 1945 and that none was made between 1945 and 1970. To determine whether this pattern is unusual it was compared with records for Field Mushroom *Agaricus campestris*, a species often associated with non-intensive agriculture, and for Fly Agaric *Amanita muscaria*, a well-recorded species associated with birch trees (Table 1). These data do suggest that disappearance of *P. punctata* after the Second World War and its subsequent increase in abundance are real.

Only seven of the pre-1945 records which had locality data were from Hampshire whereas virtually all of the post-1970 records were from that county. Exceptions were from Dorset (1984), Surrey (1985), Oxfordshire (1995, 1996) and Anglesey (2001), but since 2001 nearly half of the 40 records have been from counties other

Table 1. Number of records in FRDBI for different periods. Percentages are those of total records .

Period	Nail Fungus	Field Mushroom	Fly Agaric
<1945	60 (22%)	196 (6.8%)	240 (3.3%)
1945-1970	0	108 (3.7%)	119 (1.6%)
>1970	212 (78%)	2592 (89.5%)	6951 (95.1%)

than Hampshire (including Sussex, Kent, Berkshire, Herefordshire and Norfolk). This supports the hypothesis that this once widespread fungus became rare when horses ceased to be extensively used in agriculture but persisted in New Forest ponies and is now spreading again as ponies are used for conservation grazing.

Norfolk records:

- 1873 near Hunstanton TF6740. CB Plowright, 1 December.
- 1941 Wacton Common TM1890. EA Ellis, 4 May.
- 1944 Horsford Heath (Woods) TG1818. EA Ellis, 30 July.
- 2012 Hockwold Heath TL7590. J Spencer, March.
- 2013 Roydon Common and Grimstone Warren J Preston, November.

The only record from Suffolk is 1874 from Yarmouth, on the Norfolk /Suffolk Border.

Site Manager Andrew Palles-Clarke has commented that when *Poronia* was first found at Hockwold it only occurred at Hockwold Heath on a couple of droppings but over last two to three years seems to have flourished and is now at Weeting Heath too.

Poronia erici

In 1988, *Poronia erici,* a smaller species but with larger spores, was described from specimens collected on rabbit dung in Germany (Lohmeyer & Benkert 1988) and was later found at Holme (TF7145) by Ray Purser in 2010 (Leech *et al*. 2011)). This species has been recorded from horse dung both in Australia and, in 2010, from Snape Warren in Suffolk. This makes it possible, even likely, that the record for *P. punctata* on the Norfolk Fungus Databases as '1982 Holme, Broad Water TF714504. PC Holland, 5 June', is actually for *P. erici* as it is likely to be within one km (and possibly much less) of the 2010 discovery. The grid reference given for the 1982 record (TF714504) must be incorrect as it specifies a point five km north of Holme beach! The name Broad Water, however, and the easting, suggest that it is within one km, and possibly much less, of the 2010 record.

Conditions for 'fruiting'

A large number of studies have reported somewhat contradictory findings concerning the ecological requirements for *P. punctata* (for references see Edwards 2015) but as the fungus is only visible when it is fruiting, these may be requirements either for growth or for fruiting *per se*.

Fruiting is most commonly observed in the autumn or winter months but there is some fruiting in summer months. The fungus can withstand being frozen.

All British records in FRDBI in which the origin of the dung is specified are from horse (or pony) dung, with the exception of one 19th century record from cow dung. In North America it occurs more commonly on cow dung.

In most studies, more fruiting was seen on dung that was still in lumps but it also occurred on 'collapsed' dung (due to activities of insect larvae) and on desiccated dung.

P. punctata is typically found on dung where grazing is of low quality, mainly on acidic habitats, the most likely conditions under which conservation grazing is taking place. Whether this is due to the effect of diet (for

example on the lower nitrogen content of vegetation on unimproved grassland), on dung properties or on environmental factors pertaining after defaecation is not clear. It has been suggested that the failure to find fruiting bodies on dung deposited in woodland could be due to increased beetle activity in dung deposited there.

The greater abundance of fruiting on habitats where the vegetation is shorter rather than longer may reflect variation in transmission of the fungus.

To enable *P. punctata* populations to persist in a herd of horses, either fruiting must be continuous throughout the year or the spores must be viable for sufficiently long periods. Although ascospores are viable for long periods, the slender vegetation they adhere to is not and must be grazed within its lifecycle or before non-vector species consume it.

Sites and ponies

The study areas

Hockwold Heath (TL7589/7590) is approximately 2 km north-west of Weeting and consists of two areas. A 40 hectare area (Site A) and an adjacent 30 hectare area (Site B). Cranwich Camp (TL7794) is approximately 2 km west of Mundford and consists of 22 hectares of reversion land. Both sites were clear-felled in approximately 2000 and are parts of the once extensive Breckland heaths.

Soil inversion has recently been carried out on part of the Hockwold Heath. In this technique the invasive plant species are turned underneath the nutrient-poor chalky mineral layer which is brought to the surface in order to encourage species, some either unique to Breckland or with very few UK habitats, to colonise the newly created bare ground.

Both sites are owned by the Forestry Commission and managed by Norfolk Wildlife Trust (NWT).

Ponies

Dartmoor ponies are used to prevent scrub invasion and to maintain an open sward. It is intended that this will maintain a suitable breeding habitat for Stone Curlew. Hockwold Heath has been grazed by ponies since 2007.Ponies are grazed on site A from April to October and on site B from October to March. Cranwich Camp has been grazed continuously since 2014. The ponies are mixed age mares at a low stocking rate.

Methods

A series of belt transects were created by walking predetermined routes across each of the three sites. The transect lines were chosen to include the different habitats present. The position of dung piles within two metres either side of the line walked were recorded by GPS and the presence or absence of *P. punctata* fruiting bodies noted. If fungi were present, the total number and maximum diameter were recorded. Fieldwork was carried out between December 2014 and February 2015.

Around each dung pile, a 2 x 2 metre quadrat was used to survey the height and composition of the vegetation surrounding the dung.The structure of each pile of dung was assessed to be normal, disturbed, collapsing or flat, and its condition (related to moisture content) to be normal, normal ageing, saturated or desiccated. Moisture, pH and nitrogen levels in soil (Ellenberg factors) were also predicted from the vegetation composition.

Results and discussion

Grazing regime

Although Table 2 shows there to be much higher incidence of *P. punctata* in areas in which summer grazing had been carried out (so was not occurring at the time of study), it could not be concluded that this is causally related as dung structure was different for the older dung.

In this study it was not possible to ascertain whether *Poronia* mycelium was present in all

Table 2. Number of dung piles examined and the incidence of the fungus.

	Hockwold A	Hockwold B	Cranwich Camp
Grazing regime	Summer	Winter	Year round
No. of transects	4	6	3
Total transect length (m)	1198	974	445
No. of dung piles	80	87	97
% piles with fungus	27.5	6.9	3.1
Maximum no./pile	496	140	21

dung or whether only certain ponies were responsible for dispersing the fungus.

Vegetation type

Table 3 shows that the highest incidence of *P. punctata* was observed in Group 1 habitats. However, χ^2 tests showed that none of the associations was statistically significant. This suggests that the overall habitat type does not strongly influence the development of fruiting bodies. Similar negative conclusions were apparent for vegetation height and Ellenberg factors.

However, vegetation composition immediately surrounding dung was found to significantly influence the appearance of fruiting bodies through a multivariate MANOVA test. Microclimates created by local vegetation are predicted to influence dung suitability through determination of coprophilous invertebrate assemblage (Masunga *et al*. 2006; Klein 1989).

Dung structure and condition

The dung condition and structure characteristics correlated strongly with number and size of fruiting bodies. Fruiting bodies were observed on all structures, but not all moisture conditions.

'Disturbed' dung was able to support fruiting bodies across the moisture gradient and generally had the highest percentage

Table 3. Incidence of fungus on dung in different vegetation types.

Group	NVC community	% dung piles with *P. punctata*	Total no. fruiting bodies
1	U4, U4b, U16 and W11. These communities all have a similar field layer, with fine leaved grasses and few dicotyledons.	19	640
2	MG1, a mainly coarse grassland with many dicotyledons, characteristic of little grazing.	4	35
3	MG5 and MG5a, mainly fine-leaved grasslands, characteristic of grazed land with some dicotyledons	13	525
4	OV27, an open community, characteristic of disturbed ground with stands of Rosebay Willowherb (*Epilobium angustifolium*).	12.5	310
5	W16a, English Oak (*Quercus robur*) woodland with a species-poor field layer (no canopy layer was recorded – field layer thought to be represented)	**15**	**145**

occurrences and number of fruiting bodies. Moderate disturbance of dung can improve aeration by opening air pockets and channels (Dix & Webster 1995). However, if 'disturbed' dung is greatly picked apart, by dung beetles, birds or mechanical means, it becomes less likely to support fruiting, possibly because the mycelium is disrupted (Bignell & King 2011; Dix & Webster 1995).

Dung with 'normal' structure supported fewest colonies, possibly because this generally tended to be younger so that mycelia had not developed sufficiently. 'Collapsing' dung also supported little fruiting – such dung is older and has a lower water-retaining ability and fewer air pockets (Dix & Webster 1995; Isaac 1998). The single dung pile supporting the most fruiting bodies (496) was characterised as 'desiccated-normal'.

Desiccated dung occurred rarely, probably due to weather conditions. Saturated dung was more common and supported the largest fruiting bodies when disturbed or collapsing.

These different combinations of condition and structure are thought to regulate nutrient uptake and consequent mycelium growth, leading to appearance of fruiting bodies when dung edges are reached (Tudor *et al.* 2012; Engh *et al*. 2010; Jennings & Lysek 1996).

Effect of worming treatment

The use of vermifuges is known to reduce insect numbers and diversity in herbivore dung, and a negative effect on coprophilous fungi has been suggested. Any effect would be difficult to investigate as it could be manifested directly or via an influence on insects which could alter dung properties in ways which could harm or benefit *Poronia*.

The only anthelmintic used since at least 2012 is pyrantel (a tetrahydropyrimidine), although avermectins had been used previously. Whereas these avermectins could delay dung decomposition by arthropods for up to six months, pyrantel photodegrades and rapidly loses insecticidal activity when excreted (Horvat *et al.* 2012). Pyrantel is used biannually on both sites with the last dose administered in November 2014 (Andrew Palles-Clarke, pers. com.).

As no untreated controls were available, it was not possible to investigate the effect of the worming treatment on the appearance of *Poronia*. It is simply noted that the treatment does not prevent appearance of the fungus.

Management

This study has confirmed that *Poronia* is flourishing at these sites but because of the duration of the study and the complexity of the possible interactions between ponies, insects and vegetation, it has not been possible to define precisely the conditions required for *Poronia* fruiting.

The suggestion (Spooner & Roberts 2005) that year-round grazing is required to support *P. punctata* is not supported in this study as Cranwich Camp, with its lower incidence of *Poronia,* is grazed constantly and Hockwold Heath ponies interchange between site A and B. It is possible, however, that air-borne spores could be blown between these sites which are adjacent.

Even if all factors were known it would be unrealistic to tailor management to the benefit of *Poronia* if this jeopardised other priorities on the site, including restoration of a diverse Breckland flora and arthropod fauna, and maintaining suitable habitat for nesting Stone Curlew. Nevertheless, it is encouraging to know that current management is consistent with maintaining a population of *Poronia.*

Origin

A final unanswered question is how the fungus was introduced to the site. The presumption must be that it was in the gut of one or more of the introduced ponies. All of the ponies have come from the Dartmoor

area but it is curious that the only record from South Devon on FRDBI is from 1923. Since this distinctive fungus has only been seen recently at Hockwold and Cranwich it may have been introduced with recent stock, rather than there with original ponies (Andrew Palles-Clarke, pers. com.).

This investigation was successfully submitted for an MSc degree at the University of Hertfordshire and awarded the John Houghton prize.

Acknowledgements

Thanks to Andrew Palles-Clark (Norfolk Wildlife Trust) for invaluable assistance during the project. A final thanks goes to my family (NE) for extensive support throughout the project.

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Figure 1. Nail Fungus *Poronia punctata* on horse dung at Grimston Warren. *Photo:* Robert Smith.

Figure 2. Conidial stage of Nail Fungus *Poronia punctata* on horse dung at Hockwold Heath. *Photo:* Nicola Edwards.