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Endozoochory of large bryophyte fragments by waterbirds

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Abstract – We provide observations confirming that viable fragments of bryophytes are dispersed by migratory birds after surviving transit through the alimentary canal. A specimen of *Didymodon insulanus* was cultured from a large fragment extracted from faeces of Mallard *Anas platyrhynchos* at Lake Windermere in Cumbria, England. Similar fragments were recorded elsewhere in England in faeces of Mallard and Lapwing *Vanellus vanellus*. Endozoochory is likely to be an important dispersal mechanism for bryophyte fragments as well as spores.

Didymodon / dispersal / endozoochory / waterbirds

INTRODUCTION

Dispersal is a fundamental requirement for all organisms; indeed theoretical arguments show that dispersal is still required even in a uniform and predictable environment, and it is a key mechanism by which plants respond to climate change (Hamilton & May, 1977; Huntley & Webb, 1989). In bryophytes, spores provide an especially important means of dispersal (Glime, 2014; Porley & Hodgetts, 2005), and are often small enough to potentially move between continents in the atmosphere (Wilkinson *et al.*, 2012). However, waterbirds are also major vectors for a broad range of plant types (Green *et al.*, 2016), and it is likely that bryophyte spores are dispersed by migratory waterbirds, both by epizoochory (external dispersal on

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plumage or feet) and endozoochory (internal dispersal after ingestion and survival of transit through the gut). Indeed, Proctor (1961) showed experimentally that spores of the liverwort *Riella americana* survive gut passage through Mallards (*Anas platyrhynchos*).

Spores are not the only potential units of dispersal. As bryophytes are totipotent, in principle a new plant can arise from any small fragment – even from a single cell (Porley & Hodgetts, 2005). The potential for dispersal of such fragments was illustrated by Parsons *et al.* (2007), who extracted and cultured numerous viable bryophyte fragments (including *Acroprium sp.*) from the faeces of the spectacled flying fox (*Pteropus conspicillatus*). These fragments were possibly ingested by the bats while grooming (Parsons *et al,* 2007). However, many migratory bird species could potentially move bryophyte fragments far greater distances than fruit bats. There is already some evidence for ectozoochory of fragments by waterbirds that undergo long-distance migrations. Potentially viable *Sphagnum* leaf fragments and a bryopsid leaf fragment were recovered from the plumage of American Golden Plover (*Pluvialis dominica*), Semipalmated Sandipiper (*Calidris pusilla*), and Red Phalarope (*Phalaropus fulicarius*; Lewis *et al.*, 2014a), although their viability was not confirmed. Long-distance dispersal by these trans-equatorial migratory shorebirds has been proposed as the explanation for the bipolar biogeographical distributions of many bryophytes (Lewis *et al.*, 2014a, 2014b).This is a potentially important mechanism given the difficulty in moving between hemispheres if relying on wind dispersal (Wilkinson *et al.*, 2012). Recently, Behling *et al.* (2016) reported the presence of bryophyte fragments in the faeces of the White-bellied Seedsnipe *Attagis malouinus* from Cape Horn in Chile, but did not confirm their viability. To our knowledge, there is no other record in the literature of endozoochory of bryophyte fragments by birds. Here we describe evidence of endozoochory from a study of the role of waterbirds in plant dispersal in North West England during 2016. Unlike Lewis *et al.* (2014a) and Behling *et al.* (2016), we confirm fragment viability.

MATERIALS AND METHODS

Fresh faeces were collected after flushing monospecific groups of waterbirds that were resting on the land, with each sample corresponding to a different individual. Faecal samples were visually inspected in the field, removing any soil or plant fragments adhering to the outside of the faeces, before being placed in a zip-lock bag then transported back to the laboratory, where they were kept in a fridge until processing (with a delay of up to one week). All the moss fragments described below looked potentially viable (*i.e.* they were still green), and came from *within* the dropping. They were extracted after sieving using deionised water and a 125 µm mesh, followed by examination of material under a binocular microscope (7-40×). The sieve size and low magnification means that small propagules < 100 µm in diameter (such as those recorded by Lewis *et al.*, 2014a) were not quantified. Large moss fragments were placed on plain, non-nutrient agar in Petri dishes to attempt to confirm viability.

Here we report on moss fragments recovered from three different locations. Five samples of Mallard faeces were collected from Sefton Park, Liverpool on 21.06.2016 (53°22’45”N, 2°56’17”W). Twelve samples of Mallard faeces were collected from Fell Foot, Lake Windermere (Fig. 1), in the English Lake District on

Fig. 1. The site at Fell Foot on Lake Windermere from which the Mallard faeces containing *D. insulanus* were collected. Several moss species, including this one, are growing on the wall at the edge of the lake. Windermere is the most human influenced of the many lakes in the English Lake District and one of the most well-studied lakes in the world, with a freshwater biology laboratory that was opened on the lake shore in 1931 (Moss, 2015).

26.06.2016 (54°16’32”N, 2°57’9”W). In addition, 37 samples of Lapwing (*Vanellus vanellus*) faeces were collected from Budworth Mere, Cheshire (53°17’21”N, 2°31’11”W), on 14.07.2016. In total, over this time period in our wider study we looked at 430 faecal samples, collected from 10 species of waterbirds, but no other samples were observed to contain large moss fragments.

RESULTS

A vegetative fragment of moss was extracted from one sample of Mallard faeces from Sefton Park, Liverpool but it failed to grow on agar. Vegetative fragments of moss were extracted from 4 samples of Mallard faeces (with a total of 5 fragments) collected from Lake Windermere. All the fragments were placed on agar on 30.06.2016. One of these fragments grew, proving viability, with protonemata appearing after 11 days. This moss was maintained for six weeks and was identified as *Didymodon insulanus* (Fig. 2), a common moss in the region and one often found at lowland lake margins, especially on concrete and brickwork, as was observed at Fell Foot. One moss fragment was extracted from one sample of Lapwing faeces from Budworth Mere, but failed to grow on agar.

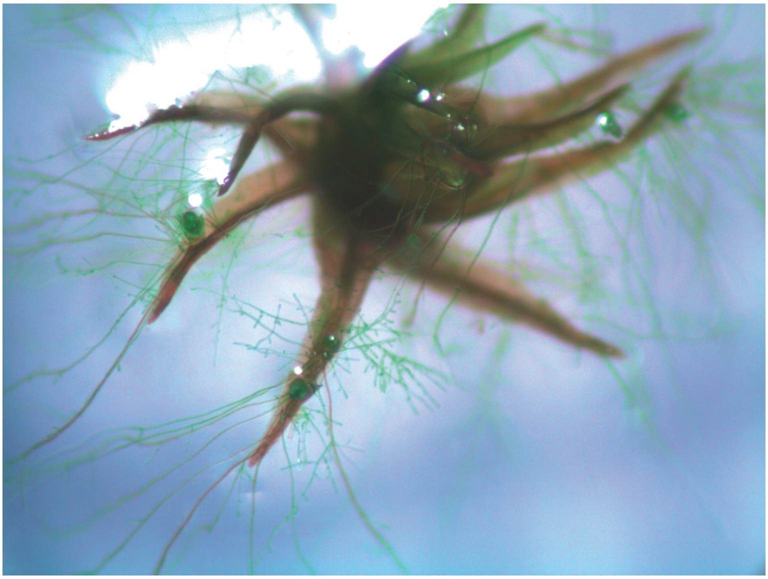

Fig. 2. *Didymondon insulanus* fragment from Fell Foot growing on non-nutrient agar.

DISCUSSION

Our results demonstrate that viable-looking fragments can readily be recovered from waterbird faeces, and that at least some of these fragments are able to grow into new plants. While the Mallards may have deliberately ingested moss while feeding, in the case of the Lapwing the moss may have been accidently ingested, as this species mainly feeds on ground-living invertebrates (Cramp & Simmons, 1983). Bryophytes have previously been recorded as food items for dabbling ducks (Owens, 1972), diving ducks (Bartonek & Murdy, 1970) and especially migratory geese (Fox *et al.*, 2006; Stech *et al.*, 2011). Mallards can be seen feeding on terrestrial mosses close to the edge of lakes when an increase in water levels after rainfall inundates the mosses (personal observation).

It is likely that we underestimated the rate of dispersal because the delay between faecal collection and processing is likely to have reduced viability, and because we did not search for fragments of < 100 µm. Our casual observations in the laboratory suggested that, the longer the delay between sample collection and processing, the more likely that any moss fragments recorded had already lost their green colour. These discoloured fragments were not considered potentially viable and therefore were not quantified. Given that birds in general, and waterbirds in particular, can fly long distances and often have high population sizes (e.g. there are around 4,500,000 Mallards in North-West Europe, Wetlands International, 2016) they are important dispersers of bryophyte propagules both by endozoochory and ectozoochory – be these spores or vegetative fragments as described in this paper. Birds that migrate long distances in a short time, such as geese and other waterbirds which breed in the Arctic but overwinter in northern and central Europe – are likely to be particularly important bryophyte vectors. Given the high abundance of waterbirds it is likely that large numbers of viable vegetative fragments are moved around in this way, as previously estimated for dispersal of angiosperm seeds (Soons *et al.*, 2016). Co-dispersal of other organisms by vertebrates is an area in need of far greater investigation (Tesson *et al.*, 2016), as indicated by the fact that these first records of endozoochory of bryophyte fragments by birds were not published until the 21st Century!

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