

## Endozoochory of large bryophyte fragments by waterbirds.

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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 obviously 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 be moved 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 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 some evidence for ectozoochory of fragments by migratory waterbirds. Potentially viable *Sphagnum* leaf fragments and a Bryopsid leaf fragment were recovered from the plumage of American golden plover (*Pluvialis dominica*), semipalmated sandpiper (*Calidris pusilla*), and red phalarope (*Phalaropus fulicarius*; Lewis et al. 2014a), although importantly 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). However, we are unaware of any record in the literature of

endozoochory of bryophyte fragments being internally transported in birds. Here we describe evidence of endozoochory from a study of the role of waterbirds in plant dispersal in North West England during 2016.

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. The sieve size and low magnification means that propagules < 100 µm in diameter (such as those recorded by Lewis et al. 2014a) were not quantified. Large moss fragments were placed on non-nutrient agar in Petri dishes to attempt to confirm viability.

Fragments were 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). A vegetative fragment of moss was extracted from one sample but it failed to grow on agar. Twelve samples of mallard faeces were collected from Fell Foot, Lake Windermere (Fig. 1), in the English Lake District on 26.06.2016 (54°16'32"N, 2°57'9"W). Vegetative fragments of moss were extracted from 4 samples (with a total of 5 fragments). All the fragments were placed on agar on 30.06.2016. One of these fragments grew, proving viability, and was maintained for six weeks. This moss was identified as *Didymodon insulanus* (Fig. 1), a common moss in the region and one often found at lowland lake margins, especially on concrete and brickwork, as indeed was the case at this site. This demonstrates not only that viable-looking fragments can be recovered from bird faeces, but that at least some of these fragments are able to grow into new plants.

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. A moss fragment was extracted from one sample, but failed to grow on agar. 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. While the mallards may have deliberately ingested moss while feeding, in the case of the lapwing the moss may have been accidentally 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).

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 potentially 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 Britain – 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 the first record of endozoochory of bryophyte fragments by birds has not been published until the 21<sup>st</sup> Century!

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**Fig caption.**

Fig 1a. *Didymodon insulanus* fragment from Fell Foot growing on agar.

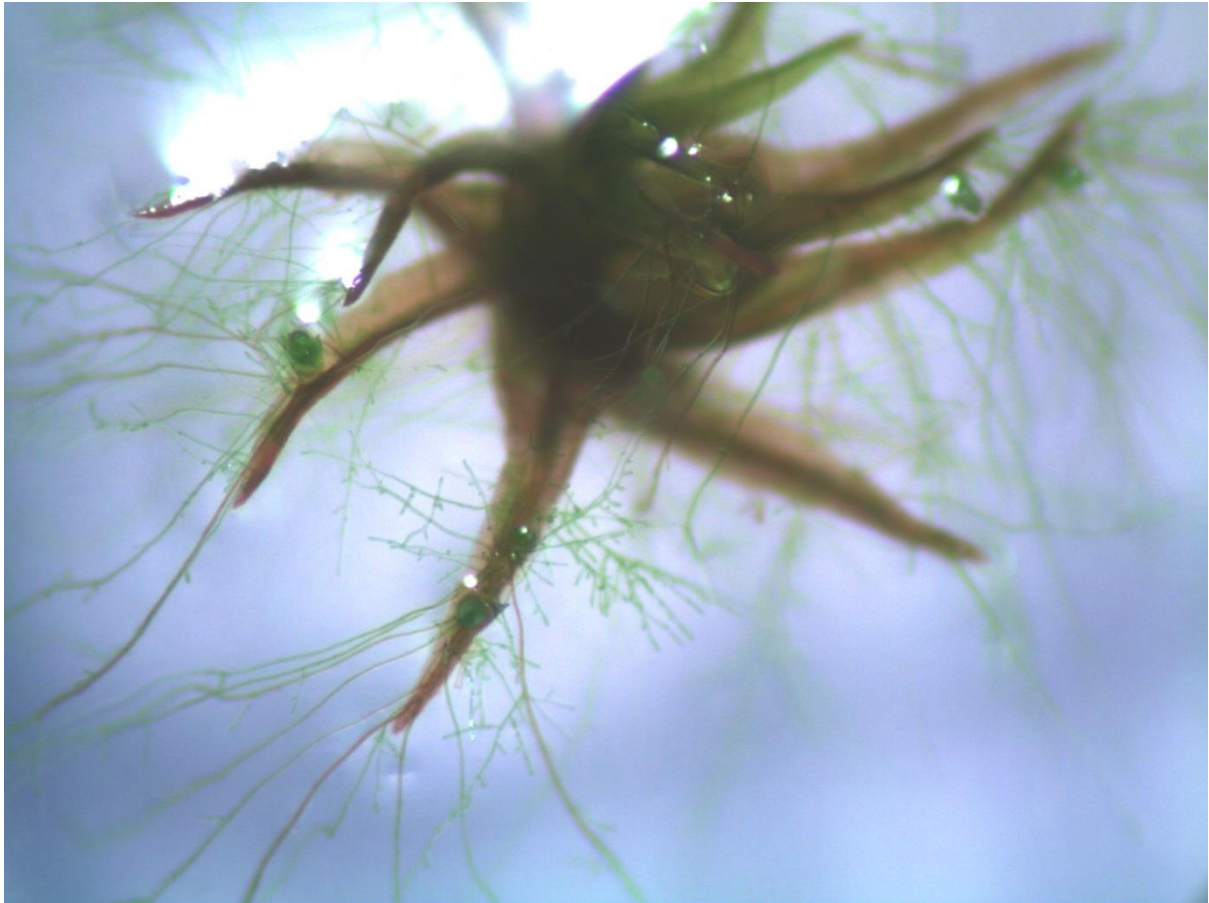


Fig 1b. The site at Fell Foot on Lake Windermere from which the mallard faeces containing *D. insulanus* were collected. Several moss species, including this one, are growing on the wall at the edge of the lake. Windermere is the most human influenced of the many lakes in the English Lake District and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

