

How far can the freshwater bryozoan *Cristatella mucedo* disperse in duck guts?

Iris Charalambidou^{1*}, Luis Santamaría^{1,2} and Jordi Figuerola³

With 1 figure and 1 table

Abstract: Statoblasts of *Cristatella mucedo* were fed to two duck species, pintail (*Anas acuta*) and shoveler (*A. clypeata*), to assess whether endozoochorous dispersal is responsible for the metapopulation structure of this bryozoan. Eight individuals (four per duck species) were force-fed 500 statoblasts each. The number of intact statoblasts retrieved from the ducks faeces up to 48 hours after ingestion and their retention times, i.e. the time spent in the gut from ingestion to defecation, were recorded. Retrieval of intact statoblasts did not differ significantly between duck species ($37 \pm 29\%$ for pintail and $13 \pm 21\%$ for shoveler, average \pm SE) and the pattern of retrieval over time was identical. Most statoblasts (79–96% for pintail and 51–96% for shoveler) were recovered during the first four hours after ingestion. Maximum retention times were 44 hours for pintail and 32 hours for shoveler. A few statoblasts retrieved two hours following gut passage germinated, but none in the control group did. We provide evidence that the potential for waterfowl dispersal of *C. mucedo* statoblasts is higher for short distances up to 300 kilometres, but still possible over longer distances.

Key words: *Cristatella mucedo*, *Anas acuta*, *Anas clypeata*, dispersal, endozoochory.

Introduction

The dormant stages of aquatic organisms are believed to disperse long distances in the digestive tract (endozoochory) or stuck to the plumage and feet (epi-

¹ **Authors' addresses:** Netherlands Institute of Ecology – KNAW, P.O. Box 1299, 3600 BG Maarssen, The Netherlands. E-mail: i.charalambidou@nioo.knaw.nl

² Present address: Laboratory of Terrestrial Ecology, Mediterranean Institute for Advanced Studies (IMEDEA, CSIC-UIB), C/Miquel Marquès 21, 07190 Esporles, Mallorca, Islas Baleares, Spain.

³ Department of Applied Biology, Estación Biológica de Doñana, CSIC, Avda. de María Luisa s/n, 41013 Sevilla, Spain.

* Corresponding author: i.charalambidou@nioo.knaw.nl

zoochory) of birds (FIGUEROLA & GREEN 2002). *Cristatella mucedo* CUVIER 1798 (Phylum Bryozoa, Class Phylactolaemata) is a freshwater bryozoan distributed throughout the Holarctic region. Overwintering occurs as statoblasts which are small (<1 mm), multicellular, amictic dormant stages (OKAMURA 1997) with highly resistant chitinous valves (FREELAND et al. 2000). Microsatellite studies of populations of *C. mucedo* collected along a major waterfowl migratory route in North West Europe provide evidence for a large scale metapopulation structure (FREELAND et al. 2000). Transport of statoblasts by waterfowl has been proposed to determine the metapopulation dynamics within this region.

Statoblasts of *Fredericella sultana*, *Plumatella repens*, *P. emarginata* and *Pectinatella magnifica* germinate after ingestion by mallard (*Anas platyrhynchos*; BROWN 1933). Although survival after gut passage indicates that endozoochorous dispersal is possible, detailed information on the rates of defecation of viable statoblasts over time is necessary to estimate potential dispersal distances. Nevertheless, ecological studies addressing and quantifying animal-mediated dispersal of freshwater invertebrates are rare (BOHONAK & WHITEMAN 1999). Moreover, experimental work with captive waterbirds has usually provided basic and non-conclusive data. For example, only two studies cite the numbers of invertebrate dormant stages ingested and excreted by the birds (BROWN 1933, MELLORS 1975). In these, a low number was administered (BROWN 1933, MELLORS 1975), the methodology of retrieval from droppings was not accurate (BROWN 1933) and the number of experimental animals was low (N = 1 and 2, BROWN 1933, N = 1, MELLORS 1975). Detailed information on the rates of excretion over time is also lacking although some basic data have been collected for the brine shrimp (*Artemia salina*; PROCTOR et al. 1967, MACDONALD 1980). Furthermore, few water bird species have been utilized in such studies (MELLORS 1975, MACDONALD 1980) among which was one duck species, the mallard (e.g. BROWN 1933).

In this study, we fed statoblasts of *C. mucedo* to pintail (*Anas acuta*) and shoveler (*A. clypeata*), two species specialized in different diets: shoveler feed mainly on zooplankton, while pintail consume a wide variety of plant and animal materials (CRAMP & SIMMONS 1977). We then retrieved the intact statoblasts defecated by the ducks. Our objectives were: 1. to estimate the proportion of statoblasts that resist digestion in relation to retention time, i.e. the time spent in the gut from ingestion to defecation, and establish whether they are consistent with long-distance dispersal and, 2. to compare the outcome of gut passage in the two waterfowl species, pintail and shoveler, that differ in their feeding habits.

Materials and methods

C. mucedo statoblasts were collected from lakes in Southern England (Hinksey Lake, Oxfordshire; Tufty's Corner and White Swan Lake, Berkshire) in autumn 1998. Aliquots of 50 statoblasts were placed in 1.5 ml Eppendorf tubes filled with distilled water and stored frozen at -20°C throughout winter. One day before the experiment, they were left to thaw in a refrigerator (temperature: 4°C) and on the day of the experiment were kept at room temperature (20°C) for 3 to 4 hours.

Four pintail and four shoveler, all female one-year-old birds born in captivity, were used in this study. Prior to the experiment, they were housed in outdoor facilities and fed a stable diet of commercial pellets (Anseres 3 @ Kasper Faunafood) and mixed grain (HAVENS Voeders) for one year. Although they had access to the same diet, the shoveler preferentially consumed more pellets, which are animal based, while the pintail tended to favor the seed-based mixed-grain diet. During the experiment (18 to 20 April 1999), they were kept individually in wooden cages ($0.60\text{ m} \times 0.50\text{ m} \times 0.50\text{ m}$) with a mesh floor (mesh size 12 mm) and removable plastic trays placed under each cage. Birds were placed in the cages the night before the experiment. Food pellets and water were available ad libitum.

Each duck was force-fed 500 statoblasts, to ensure an exact amount of statoblasts and the time of ingestion were known. To facilitate force-feeding, the statoblasts were placed inside pellets made with *Anseres* soaked in water. Each pellet contained 100 statoblasts. Duck faeces were collected in the removable trays every four hours up to 48 hours after ingestion. They were immediately sieved (sieve size $150\text{ }\mu\text{m}$) and intact statoblasts were retrieved, counted, placed in water in closed plastic containers and stored at 4°C in a refrigerator. The experiment was terminated when no intact statoblasts were retrieved from any of the experimental animals after one collection period of four hours. The efficiency of the retrieval of statoblasts from the faeces was initially tested with $0.5\text{ }\mu\text{m}$ plastic beads fed and retrieved from mallard up to 36 hours after ingestion, which resulted in recoveries of 80–90% of the beads.

We assessed the germination of statoblasts retrieved from pintail ($N = 736$) and shoveler ($N = 265$), and of non-ingested control ones ($N = 100$). All statoblasts were positioned in microtitre plastic trays with each 3.5 ml cell filled with 2 ml of distilled water (OKAMURA, pers. comm.). Individual samples were placed in separate cells with a maximum number of 5 statoblasts per cell. On 6 May 1999, the trays were positioned in a climate room set at 15 hours light/9 hours dark and 15°C to simulate the light and temperature conditions of mid spring in Southern England and Central Europe. Germination was checked every two days and was scored when an ancestrula was observed. The germination trial was terminated on 31 August 1999.

The number of statoblasts retrieved from the faeces was analysed with Generalized Linear Models, using binomial error distribution and a logit link function. 'Duck species' was introduced as a fixed factor and 'duck individual' as a random factor. The analysis was performed using the GLIMMIX procedure in SAS (SAS Institute INC. 1996, 1997). Differences in the retention time of statoblasts between the duck species were tested by fitting a Cox proportional hazards regression model (e.g. ALLISON 1995) to data consisting of the number of hours between ingestion of the statoblasts

and their recovery in faeces. As in the previous analysis, species was introduced as a fixed effect, and to account for the effects of digestion by different duck individuals, a replicate effect was added to the model as a random, or 'failry' effect. Ties were managed using the exact method, and survival analysis was computed using S-Plus 2000 (MathSoft 1999).

Results

The model to analyse the number of statoblasts retrieved from the faeces fitted the data well (Deviance = 3587, $df = 3998$, $P > 0.9$) and no evidence of over- or under-dispersion was found ($\phi = 0.99$). The proportion of retrieved statoblasts did not differ significantly between duck species ($F_{1,3} = 2.55$, $P = 0.21$, Table 1). Average retrieval estimated by the GLIMMIX procedure was $37 \pm 29\%$ for pintail and $13 \pm 21\%$ for shoveler (average \pm standard error, following back-transformation from the logit link and application of the delta method; SAS Institute Inc. 1996, 1997).

Most statoblasts were retrieved within 4 hours after ingestion (79–96% of those retrieved from pintail and 51–96% from shoveler), less between 4 and 8 hours (3–16% from pintail and 4–49% from shoveler) and few after 8 hours (0.3–10% from pintail and 0–12% from shoveler; Fig. 1). The retrieval of statoblasts over time did not differ significantly between duck species (Cox's Regression Model, $R^2 = 0.001$, $\chi^2 = 1.42$, $df = 1$, $P = 0.23$). Maximum retention times recorded were 44 hours for pintail and 32 hours for shoveler (Table 1).

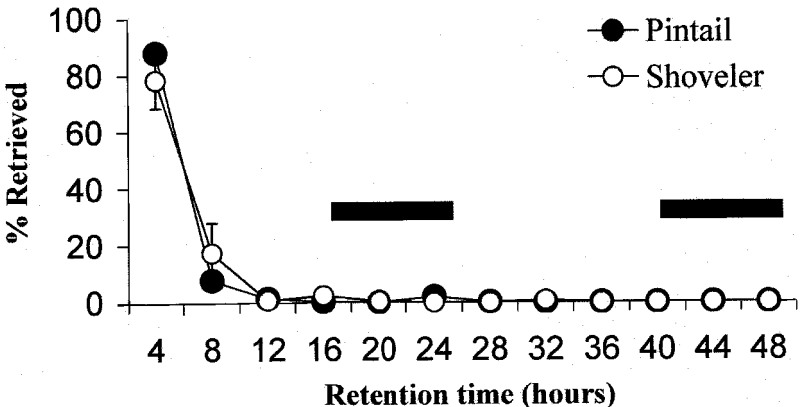


Fig. 1. Percentage and standard error values (\pm SE) of intact statoblasts of *Cristatella mucedo* retrieved from the faeces of the pintail and shoveler over retention time (i.e. time retained in the gut, from ingestion to defecation). The black bars represent night period collections (from 22:00 to 06:00 hours).

Table 1. Results of force-feeding and germination experiments. N = number of individual ducks (= replicates) per species. Maximum retention time = maximum time after ingestion at which at least one intact statoblast was recovered from the faeces.

Treatment	Survival to gut passage			Maximum retention time (h)	Germination	
	N	Ingested	Retrieved (average \pm SE)		Set to hatch	Hatched
Pintail	4	500	183 \pm 70	44	736	2
Shoveler	4	500	66 \pm 11	32	265	1
Control					100	0

Only three statoblasts, two retrieved from pintail and one from shoveler, germinated. They were all obtained from faeces collected two hours after ingestion. None of the controls germinated (Table 1).

Discussion

In this study we provide evidence that the pintail and shoveler, and probably other duck species, are possible dispersal agents of *C. mucedo*. The average proportion of *C. mucedo* statoblasts retrieved from the faeces did not differ significantly between duck species, owing to high individual variation among replicate ducks. Measured ranges of statoblast retrieval were 4–70 % for pintail and 7–17 % for shoveler. The pattern of retrieval of statoblasts over time was also similar in both duck species. To obtain reliable estimates of the resistance of statoblasts to digestion and their viability after gut passage, we fed a high number of statoblasts to each duck. Average retrieval of intact statoblasts from duck faeces was lower (13 to 37%) than the retrieval of plastic beads (80 to 90 %) ingested by mallard. It is likely that the majority of statoblasts were either digested or damaged during gut passage in contrast to the beads that could not be digested.

Our results indicate a distinction between the possibilities for short and long distance dispersal. Most statoblasts were recovered by 4 hours and a small fraction (<12 %) after 8 hours following ingestion (Fig. 1). Maximum records of 44 hours for pintail and 32 hours for shoveler are proof that a few statoblasts remain intact for long retention times. Retrieval of the plastic beads also peaked at 4 hours and decreased sharply afterwards, with maximum records of 36 hours. Since the majority of statoblasts are defecated within few hours after ingestion, they stand higher chances of being dispersed over shorter rather than longer distances. Similar experiments with the brine shrimp support this observation. Although data were not complete, PROCTOR et al. (1967) found that the first intact cysts fed to killdeer (*Charadrius vociferous*) were retrieved 5–15 minutes following ingestion, the peak number was

reached at 1½ hours, 'a small number' was retained as long as 8 hours and 'an even lower proportion' was retrieved up to 24–26 hours following ingestion. Similarly, MACDONALD (1980) found that faeces of one shelduck (*Tadorna tadorna*) and one flamingo (*Phoenicopterus ruber roseus*) contained large numbers of cysts until 2 to 3 hours after ingestion with few recovered up to 38 hours later.

Extrapolating from a flight speed of 60 to 78 km/h for *Anas* ducks (WELHAM 1994), our results indicate that endozoochorous dispersal of statoblasts by ducks will most likely occur at distances less than 250 to 300 kilometers, and extremely unlikely but possible at distances longer than 500 to 600 kilometers. Of critical importance for this assessment is the likelihood of statoblast ingestion by ducks. Bryozoan statoblasts of unknown species, consumed either on purpose or accidentally together with other foodstuffs, have been found in the gizzards of a number of duck species including pintail and shoveler (ANDERSON 1959). However, there is no published information about the ingestion of any invertebrate dormant stages by ducks in the field (GREEN et al. 2002). How far a statoblast is carried inside the gut of a duck also depends on whether, and how often, ducks defecate during flight. Although such information is lacking, studies of a passerine bird regularly flying for 12 hours in a wind-tunnel showed that at least some birds produce droppings while flying, and not only shortly after take-off (KLAASSEN et al. 2000). Mechanisms for delaying the passage of food during bird flight may also play a part in endozoochorous dispersal (CLENCH & MATHIAS 1992). Even though the extent to which studies on captive ducks correspond to the situation in the wild also warrants further research, owing to the distances involved in this process, feeding experiments of captive birds still represent one of the best tools for the testing of hypotheses of endozoochorous dispersal of aquatic organisms (CHARALAMBIDOU & SANTAMARIA 2002).

Only three (0.3 %) of the statoblasts recovered after ingestion germinated, while none of the controls did. Although statoblasts remain viable after freezing (BUSHNELL & RAO 1974, WOOD 1989), few germination data are accessible for *C. mucedo* that is known to have a low germination success (SMYTH & REYNOLDS 1995). Probably our inability to successfully germinate controls reflects the use of inadequate germination conditions. However, our results show that *C. mucedo* statoblasts are able to germinate following duck gut passage, and although most of them will be dispersed over short to medium distances (<300 km), waterfowl provide a vehicle for less frequent phenomena of transport over long distances (>1000 km).

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