An experimental test of the capacity for long-distance dispersal of freshwater diatoms adhering to waterfowl plumage

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Abstract

Waterfowl are potential long-distance dispersal vectors for aquatic microbes such as diatoms, but experimental evidence is scarce. We conducted an experiment designed to emulate diatom dispersal via adherence to waterfowl, and to evaluate the effects of humidity and transport duration on potential dispersal success. We dipped individual mallard breast feathers in a pure benthic diatom culture (*Nitzschia pusilla* Grunow), then subjected them to one of four relative humidity levels (RH; from ca. 8% to 88%) crossed with one of four transport durations (10, 60, 120, 240 minutes) within a chamber through which air was passed continuously, mimicking light wind. We then placed the feather on sterile growth medium. After two weeks we used spectrofluorometry to detect diatom growth and thus diatom viability. A logistic regression on viability revealed a significant interaction between transport duration and RH: the negative effect of duration was strongest under lower RH conditions, but under high RH (88%) the probability of being viable was moderate to high regardless of transport duration. Importantly, even after 4 hours, the probability of being viable was predicted to be 0.45 (95% confidence interval: 0.18 to 0.75). We then placed our findings in the geographic context of the central waterfowl migration flyway in North America, and specifically Nebraska, South Dakota, and North Dakota, for which sufficient data were available to enable geospatial predictions of potential mallard-borne diatom dispersal. Combined with published data about (i) mallard flight speeds, (ii) the geographic distribution of surface waters and of *N. pusilla*, and (iii) daytime RH during the months of April through June, our model predicted high probabilities of potential dispersal among the region's suitable water bodies.

Introduction

Dispersal is a fundamental ecological process that connects populations and communities and moderates how diversity is distributed across the landscape. Consequently, data describing dispersal have been key to gaining a more complete understanding of diversity patterns and their origins among a variety of taxonomic groups (Cadotte, 2006; Heino *et al.*, 2015). However, such data have proved challenging to obtain for microbial organisms, and this shortfall has fuelled debate about the frequency and scales over which microbes disperse (Baas-Becking, 1934; Finlay, 2002; Fenchel & Finlay, 2004; Pither, 2007; Soininen, 2007; Heino, 2011), and about the ways dispersal influences diversity patterns among microbes (Martiny *et al.*, 2006). A case in point is provided by freshwater diatoms (Pither, 2007; Telford, Vandvik & Birks, 2007; Vyverman *et al.*, 2007; Verleyen *et al.*, 2009): despite their prevalence and importance to the functioning of inland aquatic ecosystems, little is known about the frequency and mechanisms of dispersal among waterbodies, particularly among isolated lakes and ponds (i.e., those unconnected to other waterbodies by overland streams or rivers).

Pioneering work by Maguire (1963) and others demonstrated the potential for substantive dispersal of freshwater diatoms among waterbodies (reviewed in Kristiansen, 1996), and the roles that animal vectors, especially waterbirds, could potentially play have been highlighted repeatedly (Schlichting, 1960; Figuerola & Green, 2002; Stoyneva, 2016; Kleyheeg *et al.*, 2019). To date, most experimental research has focused on endozoochory, testing whether the propagules of plants (including diatoms) remain viable after ingestion and internal transport (Proctor, 1959; Atkinson, 1972; Sides, 1973; Soons *et al.*, 2008; Viana *et al.*, 2013c; Tesson *et al.*, 2018). These efforts have revealed mixed findings, but do suggest the potential for successful diatom dispersal via internal transport. For instance, Atkinson (1972) sampled the

hind guts of several waterbird species captured on Lake Windermere, and successfully cultured several diatom taxa including species of *Melosira* and *Fragilaria*. Using samples from the esophagus and colons of gulls collected in Texas and North Carolina, Sides (1973) successfully cultured a variety of diatom taxa including species of *Fragilaria*, *Navicula*, and several species of *Nitzschia*. However, these authors did not know the length of time the diatoms had been present in the sampled birds.

Although diatoms have been observed on waterbird plumage (Schlichting, 1960; Kristiansen, 1996; Figuerola & Green, 2002), we are unaware of any suitably designed experiments evaluating adherence to feathers as a potential means for diatom long-distance dispersal. Such experiments would need to evaluate desiccation, as this is considered the limiting factor (Kristiansen, 1996). For instance, Souffreau *et al.* (2010, 2013) experimentally tested the tolerance of 69 strains (34 species) of diatom to desiccation, and found only 5 to exhibit some tolerance to desiccation. Combined with their findings of limited tolerance to freezing and heating, the authors concluded that the physiological sensitivities of vegetative diatom cells to harsh conditions are likely to severely limit dispersal capacity. However, desiccation risk could be lessened if sufficiently high humidity is maintained around the diatom cells during transport, as might be the case within waterfowl plumage, owing to its strong insulating properties (Coughlan *et al.* 2015).

Here, we present the results of a novel experiment designed to emulate diatom dispersal via adherence to waterfowl feathers. We used breast feathers from mallard ducks, which are the most abundant waterfowl species in the world (Kleyheeg *et al.*, 2017), with estimates in North America at almost 10 million (U.S. Fish & Wildlife Service, 2019). The mallard duck is an omnivorous, widely dispersed migratory species that frequents a broad range of aquatic habitats

(Kleyheeg *et al.*, 2019), and thus has the capacity to transport diatoms to a broad range of environments. As waterfowl such as mallards dabble in productive littoral zones, it is reasonable to expect their plumage – especially breast plumage – comes into contact with large numbers of benthic and epiphytic diatoms, which can reach very high densities in favourable conditions (Patrick, 1977; Wehr & Sheath, 2004). Barbed feathers provide enormous surface area for potential adherence, especially for micro-algae such as diatoms. Our experiment mimicked this encounter process by dragging individual breast feathers through solution droplets containing relatively high densities of the benthic diatom *Nitzschia pusilla* Grunow. We examined how relative humidity (RH) and duration individually and interactively affected the viability of the diatoms adhered to feathers. We found that under high RH, diatoms remained viable for a prolonged period (4 hours), suggesting ample potential for long-distance dispersal via ectozoochory. To place our experimental results in real-world contexts, we combined them with data about mallard flight speeds, and geospatial data describing waterbody distribution and RH within the central migration flyway of North America, specifically the states of North Dakota, South Dakota, and Nebraska. This spatial modeling revealed high potential connectivity among suitable waterbodies.

Materials & Methods

Diatom culture

We obtained a pure culture of a strain of *Nitzschia sp.* (CPCC 499) from the Canadian Phycological Culture Centre (Waterloo, Ontario). Among the genera available from the centre, we chose *Nitzschia* because it is a common benthic genus found throughout inland waters in North America, and we chose this particular strain because it is readily maintained in culture. According to the centre, the original material for strain CPCC 499 was sourced in 1999 from an

effluent pond at St. Mary's River pulp and paper mill in Sault Ste. Marie, Ontario, Canada. Upon completion of our study, this strain was identified to be *Nitzschia pusilla* Grunow (pers. comm. Kathryn Thomas, Stillwater Environmental) using standard taxonomic references (Krammer & Lange-Bertalot, 1988; Cox, 1996) and morphological assessments of preserved and live material under 1000x magnification (Supplemental Material, Fig. A1).

Throughout the duration of our study, we grew and maintained the diatom culture in its exponential growth phase at approximately 20-23° C and 21-24 $\mu E/m^2/s$ using serial dilutions in 125mL Erlenmeyer flasks capped with tinfoil.

Feathers

We collected mallard (*Anas platyrhynchos*) feathers under Environment Canada Scientific Permit No. BC-18-0005 and adhered to the *Migratory Birds Convention Act*, (Government of Canada, 1994). In response to a request communicated by a local wildlife biologist / waterfowl hunter, waterfowl hunters in the Okanagan Valley region of southern British Columbia, Canada, donated mallard skin patches from the breast/abdominal section (i.e., the section typically immersed when the duck is in water) with the feathers attached. We provided hunters a video depicting the skinning method alongside written instructions for reference, to standardize the collections as much as possible. Each bird yielded a single feather patch. Once removed, the patch was placed in a Ziploc bag, labelled with the sex, collection date, site, and hunting context (over water or field) and stored in a freezer as soon as possible. We obtained a total of twelve suitable mallard feather patches (six male and six female). By the time the experiment began, the patches had been stored in the freezer for at least 38 weeks. Any diatoms that may have been on the feathers would have been killed by the prolonged freezing (cf. Souffreau *et al.*, 2010, 2013). This was confirmed by the results of control trials (see below).

Using latex gloves we plucked fifteen feathers haphazardly from different parts of each feather patch and trimmed at the shaft to remove the downy portion of the feather to isolate the part of the feather that is normally exposed. Feathers from this pile were then haphazardly selected for each experimental run.

Experimental apparatus

A photograph of two experimental apparatuses is provided in the Supplemental Material (Supplemental Material, Fig. A2). A single experimental apparatus consisted of the following: one plastic filter holder that served as the makeshift airflow chamber (henceforth "chamber") within which the feather was placed (see below); three lengths of inline tubing; one 250mL Erlenmeyer flask filled with a glycerol and water solution (see below); a WhirlPak bag, and a hygrometer. The first length of inline tubing connected the benchtop air valve to the Erlenmeyer flask, which in turn was connected to the top of the chamber with the second length of tubing. The third and final length of tubing was used to direct outflow air from the bottom of the chamber into the WhirlPak bag, in which the hygrometer measured RH. The Erlenmeyer flask contained one of four ratios of glycerol:water (approximately 100:0, 90:10, 60:30, or 0:100), which were adjusted as needed to maintain one of four different RH levels within the main chamber (Forney & Brandl, 1992): near 0%, 35%, 70%, and near 100%, respectively. Thus, the air flowed first through the flask to adjust the RH of the air, which then entered the top of the chamber where it passed over the feather (see below), then exited the bottom of the chamber into the WhirlPak bag containing the hygrometer. The bag was twist-tied shut around the tubing. We constructed four apparatuses in total.

The air valves were turned on 30 minutes before the start of each experimental run. We did not have the means to directly measure the rate of airflow inside the chamber, but we strove

157 to ensure airflow rate was consistent across experimental runs: the airflow valve was opened just
158 enough so air could be felt moving through the system and would ruffle the feather slightly. We
159 envision this small rate of airflow simulating the internal areas of the plumage that are likely
160 protected from direct wind (sensu Coughlan *et al.* 2015).

161 We connected the hygrometer to an Em50® Series Data Collection System Data Logger,
162 and took RH readings every minute. We assume that the RH measured in the WhirlPak bag
163 reliably estimated the RH in the chamber proper. We also monitored the RH of the room using a
164 hygrometer and the same data logger.

165 *Experimental procedure*

166 The procedure for a single experimental run consisted of the following steps: 1mL of
167 culture (a droplet roughly the size of a feather) was pipetted into a petri dish (60mm diameter x
168 15mm depth). Using forceps, each side of a single feather was dragged through the droplet,
169 ensuring that the entire surface of the feather made contact. We verified in preliminary trials that
170 this procedure resulted in diatoms readily adhering to feather barbs, as seen in Figure 1. The
171 feather was then placed in the chamber with the base of the feather shaft compressed in the seal,
172 to hold the feather in place. The chamber containing the feather was maintained near the desired
173 RH for the desired time (10, 60, 120, or 240 minutes). The feather was then transferred to a new
174 petri dish containing 7mL of sterile growth medium (enough to cover the bottom). The feather
175 was gently placed on the liquid medium, but not submerged or pressed downward. The dish was
176 maintained under the same growing conditions as the pure culture for fourteen days (determined
177 during preliminary trials as sufficient time for growth). The medium was then assessed for
178 evidence of diatom growth using spectrofluorometry (see below). Pre-experiment trials

179 confirmed that this procedure of placing an inoculated feather on the growth medium resulted in
180 the successful establishment of diatoms.
181 During the procedure the RH occasionally varied beyond desired ranges immediately
182 following sample changeovers. We ignored fluctuations to a higher humidity immediately
183 following sample changeovers, regardless of how long the higher humidity persisted, as the
184 sensor was likely detecting the water evaporating off the feather as it exited the system. When
185 the RH varied beyond the desired range at any other point during the procedure, the sample was
186 discarded, and the procedure started over. The mean RH that we achieved for each of the four
187 RH levels (calculated using 32 samples per group) was 8.3% (± one standard error: 0.38), 36.0 (±
188 0.56), 71.0 (± 0.33), and 88.4 (± 0.44) (Supplemental Material, Fig. A3). For our statistical
189 analyses (below) we used the average RH value, calculated using the 1-minute interval readings
190 taken during the given experimental run.
191 We used 8 replicates (feathers) for each pairwise combination of target RH (4 levels) and
192 duration (4 durations), for a total of N = 128. We conducted the experiment over two four-day
193 intervals, separated by one day. One replicate from each unique treatment (16 unique
194 combinations of target RH level and duration) was completed each day, and the order of
195 treatments was randomized within each day, as was the assignment of a target humidity level to
196 one of the four chambers. At the end of each experimental day, the volume drawn from the
197 culture was replaced with an equal volume of sterile media. This ultimately diluted the culture
198 over the course of the experiment, but concentrations remained very high ($> 1.7 \times 10^5$ cells/mL)
199 and were thus suitable for ensuring feather inoculation (Supplemental Material, Fig. A4).
200 We conducted all assessments of diatom growth using an RF-1501 Shimadzu
201 spectrofluorophotometer, which yields a fluorometer intensity reading as an indicator of cell

concentration. We set the excitation and emittance wavelengths for the in vivo fluorometry to 530nm and 680nm, respectively, to target chlorophyll a and fucoxanthin (Vincent, 1983; Watras & Baker, 1988; Beutler *et al.*, 2002), with gap length set to 10nm (5nm per side). Fucoxanthin is unique to brown algae including diatoms (Beutler *et al.*, 2002), and thus our estimates of cell density are unlikely to be affected by any other microbial organisms, if present. We equated evidence of diatom growth with the survival of viable cells. To ensure the reliability of the measurement, we first established the limit of detection (LOD) for our spectrofluorometer intensity readings, as described below.

Experimental controls and Limit of Detection

On each of the 8 days, we ran one control replicate in which a feather was dipped in sterile growth medium instead of diatom culture, placed in the chamber as described above, then removed after 2 seconds (thus it did not undergo humidity and duration treatments), and subsequently placed in a petri dish with growth medium. The spectrofluorometer intensity readings obtained after 14 days from these 8 controls served to establish our LOD. We additionally ran 8 controls for which we used a $2cm^2$ square of filter paper instead of feathers as the experimental unit. The spectrofluorometer intensity readings for the feather and paper controls were statistically indistinguishable (Supplemental Material, Fig. A5). We are thus confident that the feathers used in our experiment were not pre-contaminated with organisms producing fucoxanthin or chlorophyll. We equated the LOD with the mean intensity reading from the feather controls plus three times the standard deviation of the readings (Shrivastava & Gupta, 2011; Choo *et al.*, 2018).

Statistical analyses

For our experiment, our response variable of interest was binary: viable or inviable. We equated evidence of diatom growth (i.e. intensity readings > LOD) after 14 days with evidence of viability. We analyzed this binary response variable in relation to RH (%) and duration (minutes), and their interaction, using a generalized linear model (GLM) with a binomial link (i.e., logistic regression). We coded RH and duration as continuous variables. Coding them as ordinal factors yielded qualitatively similar results (see "Ordinal Factor GLM" section of Supplemental Material). We computed the McFadden pseudo-R^2 value (McFadden, 1974), which, for logistic regression, is analogous to the coefficient of determination for general linear models.

Extrapolating our experimental results to real landscapes

To help place the results of our experiment within real-world contexts, we considered the states of North Dakota, South Dakota, and Nebraska as an example study region, and the period between April 1 and June 30, which corresponds with high frequencies of mallard occurrences in the region (see the eBird website: https://ebird.org/science/status-and-trends/mallar3) (eBird, 2020), and also diatom growth. These three states are located along the central flyway for waterfowl migration, and host the Prairie Potholes region, a crucial breeding ground for North American waterfowl including mallard ducks. We could not extend our geospatial analyses north into Canada due to a lack of data. We first used our GLM in combination with the average flight speed of 69 km/h for mallards (the middle value between average breeding time flight speeds and migration flight speeds; McDuie *et al.*, 2019) to estimate the probability of remaining viable across a range of distances and RH values typical of the study region and time period (Supplemental Material, Fig. A6). We present these predictions alongside (i) data about within-

246 day dispersal distances of mallard ducks, based on banding observations (Viana *et al.*, 2013a,
247 2013b), (data accessed from Dryad here:
248 https://datadryad.org/resource/doi:10.5061/dryad.619gd), and (ii) distances between each surface
249 water body (> 0.1 km^2 area) in the study region and its nearest neighbour water body, calculated
250 using the Global Lakes and Wetlands Database (GLWD), downloaded from the World Wildlife
251 Fund (https://www.worldwildlife.org/pages/global-lakes-and-wetlands-database). Finally, we
252 generated a map of probability of potential dispersal, akin to potential connectivity, among the
253 region's water bodies, using *N. pusilla* Grunow as the focal diatom taxon. To do this, we used
254 our GLM in combination with (i) the average flight speed of mallards (as above), (ii) spatially
255 interpolated RH for the region, using average daytime values for the last week of May,
256 calculated using data from the ASOS Network online database
257 (https://mesonet.agron.iastate.edu/request/download.phtml?network=IA_ASOS), (iii) distances
258 between surface water bodies (using GLWD data), and (iv) data about the distribution of
259 *Nitzschia* taxa, including *N. pusilla*, acquired from the 2007 and 2012 National Lakes
260 Assessment (https://www.epa.gov/national-aquatic-resource-surveys/data-national-aquatic-
261 resource-surveys). We used lakes hosting *N. pusilla* Grunow as the focal "source" lakes for
262 diatoms, and generated a raster layer of probability of potential dispersal based on distance from
263 these source lakes combined with the spatially-interpolated RH values. Then for each waterbody
264 hosting diatoms of the *Nitzschia* genus, we extracted the probability of potential dispersal values
265 from the resulting raster layer. This procedure (i) equates probability of potential dispersal with
266 the probability of diatoms remaining viable after the given transport duration (based on distance
267 and flight speed) and RH (based on spatially interpolated RH), and (ii) assumes that waterbodies
268 hosting the genus *Nitzschia* are suitable for colonization by *N. pusilla* Grunow. We acknowledge

that mallards often forage on land, and this may reduce the probability of successful dispersal. We do not account for this in our predictions. Additional details of these methods are provided in the Supplemental Material.

Ensuring computational reproducibility

All our analyses were performed using R version 3.6.1 (R Core Team, 2019) within the RStudio IDE (RStudio Team, 2019). To ensure computational reproducibility we used the `packrat` package (Ushey *et al.*, 2018) that manages R and R package versions, and we generated an annotated R Markdown script that, along with all data, are freely available for download (including in HTML format) from the Open Science Framework (https://osf.io/ujnw2). We encourage readers to contact the corresponding author (JP) if any part of our study is unclear and / or irreproducible. In addition to `packrat` we used the following R packages within our analyses: `BH` (Eddelbuettel, Emerson & Kane, 2020), `cowplot` (Wilke, 2019), `dplyr` (Wickham *et al.*, 2020), `ggeffects` (Lüdecke, 2018), `ggplot2` (Wickham, 2016), `ggmap` (Kahle & Wickham, 2013), `ggsn` (Santos Baquero, 2019), `ggspatial` (Dunnington, 2018), `gstat` (Pebesma, 2004; Gräler, Pebesma & Heuvelink, 2016), `investr` (Greenwell & Schubert Kabban, 2014), `lubridate` (Grolemund & Wickham, 2011), `minpack.lm` (Elzhov *et al.*, 2016), `plogr` (Müller, 2018), `plotrix` (Lemon, 2006), `pscl` (Jackman, 2020), `riem` (Salmon, 2016), `raster` (Hijmans, 2020), `rdryad` (Chamberlain & Boettiger, 2018), `rmapshaper` (Teucher & Russell, 2020), `sf` (Pebesma, 2018), `sp` (Pebesma & Bivand, 2005; Bivand, Pebesma & Gomez-Rubio, 2013), `spatstat` (Baddeley, Rubak & Turner, 2015), `testthat` (Wickham, 2011).

290 **Results**

291 The full logistic regression model from our experiment yielded a McFadden pseudo-R^2
292 value of 0.51, indicating a significant amount of the deviance was accounted for by the full
293 model (Table 1). Transport duration and RH interacted significantly to affect diatom viability: at
294 high RH, the probability of being viable was moderate to high across all transport durations,
295 including the maximum four-hour period (Fig. 2). For instance, at an average RH value of 88%
296 and a duration of four hours, the 95% confidence interval for the probability of being viable was
297 0.18 to 0.75, and for a sixty-minute duration, the interval ranged from 0.61 to 0.89. At lower
298 values of RH, the probability of being viable declined more rapidly with increasing duration, and
299 at the lowest RH values (8 and 36%), viability was predicted to be possible (between 0 and 0.40
300 probability) over only the shortest duration (10 min).

301 Based on predictions from the GLM, Figure 3A depicts probability of remaining viable
302 across a range of travel distances using an estimated average mallard flight speed of 69 km/h,
303 and RH values typical of the study region from April to June inclusive (70 – 90%). Figure 3B
304 shows the frequency distribution of distance travelled by mallards within a single day, and
305 Figure 3C shows the frequency distribution of distance to nearest neighbouring water body
306 among lakes and wetlands (those > 0.1 km^2 area) in the study region. Collectively, these figures
307 suggest strong potential for long-distance diatom dispersal by mallard vectors, especially over
308 distances up to 100 km.

309 The U.S. National Lakes Assessment survey revealed that diatoms of the genus *Nitzschia*
310 were observed in 85 waterbodies in the study region in the years 2007 and 2012, with 5 of these
311 locations hosting *N. pusilla* in 2007 (species-level identifications were only available for the
312 2007 surveys) (Fig. 4). Using these 5 waterbodies as potential sources of mallard-borne *N.*

313 *pusilla* diatoms, Figure 4A shows how the probability of potential dispersal varies
314 geographically in the study region, based on distance from the source lake, average mallard flight
315 speed (69 km/h), and RH interpolated throughout the study region (using late May as an example
316 time period). The map shows considerable potential for dispersal throughout the study region.
317 Figure 4B shows the probability of potential dispersal from the 5 source lakes into each of the 80
318 other waterbodies that host *Nitzschia* diatoms. More than 46% of these lakes are associated with
319 a greater than 0.5 probability of potential dispersal.

320 **Discussion**

321 Diatoms play key roles in the structure and functioning of freshwater ecosystems (Wehr
322 & Sheath, 2004). Yet, as is the case with microbial organisms in general, we have limited
323 knowledge about how freshwater diatoms disperse among geographically isolated habitats. This
324 knowledge gap limits our understanding of diatom diversity patterns (Soininen & Teittinen,
325 2019), specifically how dispersal moderates alpha and beta diversity among the Earth's inland
326 waters (Potapova & Charles, 2002; Pither & Aarssen, 2005; Soininen *et al.*, 2007; Vyverman *et*
327 *al.*, 2007; Vanormelingen, Verleyen & Vyverman, 2008). Our objective was to shed light on the
328 potential role of waterfowl as long-distance dispersal vectors, and to focus on adherence to
329 feathers, which has received limited attention to date.

330 In the real world the probability of successful diatom dispersal via adherence to
331 waterfowl feathers is a function of (i) the number of diatoms that successfully adhere to the
332 feathers, (ii) the number that survive transport, (iii) the number that successfully dislodge into the
333 new habitat, and (iv) the suitability of the new habitat with respect to establishment. We
334 designed our experiment to emulate the process of adherence, transport, dislodgement
335 (specifically an entire feather), and establishment in favourable growth conditions, and to

estimate the individual and interactive effects that humidity and transport duration have on potential dispersal success. Using the mallard duck (*Anas platyrhynchos*) as a representative vector, and a benthic *Nitzschia* species as a test case, we showed that diatoms readily adhered to feathers, could remain viable on feathers for at least four hours under realistic levels of relative humidity, and could successfully establish in favourable growth medium by way of the feather being placed in the medium. We then used our experimental findings to model potential connectivity among waterbodies within the central migration flyway of North America. These spatial models revealed high potential connectivity between source lakes hosting *N. pusilla* and other lakes hosting members of the genus *Nitzschia*, which we assumed provide suitable habitat. Below we discuss our findings with reference to each of the four points listed above.

(i): As waterfowl such as mallards dabble in productive littoral zones, their breast plumage likely comes into contact with numerous benthic and epiphytic diatoms. In our experiment we aimed to emulate this process by dragging individual breast feathers through solution droplets containing relatively high densities of diatom (see Supplemental Material Figure A4). We can't be sure whether using an entire patch of breast feathers as the experimental unit would have affected the adherence process, but it certainly would have provided more surface area of feather barbs for the diatoms to adhere to. While designing the experiment we tested the procedure of dragging a single feather through a droplet of diatom solution, and each time large numbers of diatoms readily adhered to the feather barbs (as seen in Figure 1). We speculate, therefore, that in productive littoral zones, large numbers of diatom may adhere to mallard breast plumage. If correct, dispersal success is unlikely to be limited by the number of diatoms that successfully adhere to mallard feathers, especially when one

358 considers the vast numbers of diatoms and mallards involved – almost 10 million mallards in
359 North America alone, (U.S. Fish & Wildlife Service, 2019).

360 The apparent ease with which diatoms were observed to adhere to feathers could be due
361 in part to properties of diatom frustules. Specifically, diatom frustules contain polysaccharides
362 (Gélabert *et al.*, 2004; Le Costaouëc *et al.*, 2017) that are typically attracted to hydrophobic
363 substances, like those waterfowl use to keep warm and dry (Bakken *et al.*, 2006). Secreted from
364 a gland on their lower backs (uropygial gland), the hydrophobic substance on waterfowl has been
365 found to contain chemical derivatives found in waxes (wax esters) (Stenhagen & Odham, 1971).
366 Additionally, the polysaccharides in diatom frustules can reduce the surface tension of water
367 (Ozkan & Berberoglu, 2013), which could further facilitate contact between diatoms and
368 feathers, despite the hydrophobic substance on the waterfowl.

369 (ii): For diatoms adhering to feathers during flight, desiccation is thought to be the key
370 limiting process, and previous experiments that exposed diatom cultures to desiccation showed
371 that diatoms are broadly intolerant of this form of stress (Souffreau *et al.*, 2010, 2013). Our
372 findings are consistent with this: we directly manipulated ambient relative humidity and found a
373 dramatic decrease in viability with decreasing relative humidity (Figure 2). However, we did not
374 examine the physiological status of the diatoms in our experiment, so cannot be certain that
375 inviability was due to desiccation per se. Importantly, we found that under levels of relative
376 humidity that are representative of real-world conditions in the midwest (e.g. 71 and 88%;
377 Supplemental Material Figure A6), diatoms remained viable after prolonged periods adhered to
378 feathers, including 4 hours at 88% relative humidity. Considering that mallard breast plumage
379 appear to maintain a humid microclimate (Coughlan *et al.*, 2015), the conditions experienced by

380 the diatoms in our experiment may have been more harsh than would be experienced in transit
381 within duck plumage.
382 Flight duration is another factor that will influence the number of diatoms that survive
383 transport. Our study explored this in three ways. In our experiment we directly manipulated
384 duration and found that increasing it from 10 minutes to 4 hours decreased viability, but only
385 moderately so under high relative humidity (Fig. 2). Then, assuming an average flight speed of
386 69 km/h (McDuie *et al.*, 2019), we used the statistical model from our experimental results to
387 predict the probability of remaining viable across a range of relative humidity levels and flight
388 distances. For example, our model predicts a greater than 50% chance of diatoms remaining
389 viable over 80 km, and a 68% chance of remaining viable over 40 km (Fig. 3A). In many parts
390 of the North American range of mallards, especially in Canada, these distances more than span
391 the distances among neighbouring water bodies. Lastly, and again assuming an average flight
392 speed of 69 km/h, we made spatially-explicit predictions of the potential connectivity
393 (probability of potential dispersal) in a geographic region within North America's central
394 migration flyway that hosts enormous numbers of mallard ducks. These predictions indicated
395 moderate to high potential connectivity among the region's waterbodies (Fig. 4).
396 During flight the external surface of the duck's plumage would be subjected to strong
397 airflow. However, below the plumage surface, it is possible that feathers are protected from
398 strong airflow due to the insulating properties of the plumage and its microstructure (Coughlan *et*
399 *al.*, 2015). It is these conditions that were approximated in our experiment: the individual
400 feathers were subjected to a light breeze rather than a strong wind. Wind increases evaporation
401 when RH is less than 100%. If in nature strong airflow typically occurred under conditions of
402 low ambient humidity and high temperature, the probability of desiccation would be high. The

403 results from our low humidity treatments may thus be most representative of this scenario.
404 However, relative humidity is typically high during spring and summer in the midwest
405 (Supplemental Material Figure A6), so this is likely to ameliorate conditions for the diatoms.
406 Future experiments could explore this by simultaneously manipulating wind speed, relative
407 humidity, and temperature.

408 (iii): Diatoms that withstand transport could be dislodged from feathers during landing,
409 by preening, and/or could remain adhered to feathers that themselves become dislodged. Our
410 experiment mimicked the latter scenario in which feathers detach from the bird (due to molting,
411 for example), and rest on the water surface. We suggest this scenario is highly plausible; during
412 early spring and late summer molting seasons, the surfaces of ponds and lakes hosting large
413 numbers of waterfowl are often littered with detached feathers. Although this process clearly
414 yielded successful colonization in our experiment, we don't know how effective it is in the real
415 world at enabling diatoms to successfully colonize the new habitat. Future experiments could
416 compare alternative dislodgment scenarios, including dragging the feather through sterile media.

417 (iv) The final step of establishing a local population is clearly dependent on the suitability
418 of local conditions (e.g., water chemistry) for the given diatom taxon. In our experiment we
419 aimed to ensure that diatoms surviving transport would not be limited by subsequent growth
420 conditions, and therefore provided algal growth medium as the receiving habitat. The diatom
421 strain we used was sourced from an effluent pond at St. Mary's River pulp and paper mill in
422 Sault Ste. Marie, Ontario, Canada, and was identified using standard morphological techniques
423 to be *Nitzschia pusilla* Grunow. According to algaebase.org (accessed March 10, 2020), this
424 freshwater taxon has been recorded throughout North America and Europe, and in the 2007
425 National Lakes Assessment it was observed in 23 lakes in 13 different states. It occurred in 5

lakes within our study region (Fig. 4A). There is some uncertainty surrounding the taxonomy of the genus *Nitzschia* (Rimet *et al.*, 2011), so the available data about the distribution of *N. pusilla* should be interpreted with caution. We simply emphasize that the likelihood of successful dispersal is clearly limited by the suitability of the receiving habitat, which itself depends on the taxon. It is also important to note that the available survey and geospatial data (e.g. the lakes and wetlands data used for Figures 3 and 4) likely underestimate the distribution and abundance of suitable diatom habitat, because they do not include very small and ephemeral waterbodies. Given that the mallard duck frequents a broad range of aquatic habitats (Kleyheeg *et al.*, 2019), it has the capacity to transport diatoms to a broad range of environments. Future research should modify our experimental design to explore multiple species of diatom simultaneously, using receiving solutions with contrasting conditions.

One characteristic of our study taxon that could be important is its size: *Nitzschia pusilla* Grunow is a comparatively small diatom, with reference material suggesting lengths between 8 and 33 μm, and widths of 2.5 to 5 μm (Krammer & Lange-Bertalot, 1988; Cox, 1996). Body size has long been linked to dispersal capacity among microorganisms (Finlay, 2002). Smaller body size is associated with larger population size, which will promote dispersal capacity, and with respect to microbes, smaller organisms are more efficiently dispersed. We hypothesize that the small size of *N. pusilla* Grunow may facilitate adherence to feather barbs. Specifically, we propose that its small size enables it to better "fit" within the barb structure (see Fig. 1), and promotes interaction over a greater surface area (for a given volume) between the polysaccharides on the diatom frustules and the hydrophobic substance in waterfowl plumage (see above). Future experiments could test these ideas by using diatoms of contrasting body size, and by using feathers that have and have not been washed of its hydrophobic substance.

We have provided novel evidence consistent with the idea that adherence to waterfowl feathers is an effective mode of ectozoochory for freshwater diatoms. More generally, our study adds to a growing body of evidence that waterfowl are potentially effective long-distance dispersal vectors for aquatic organisms (Figuerola & Green, 2002; Viana *et al*., 2013c). Considering (i) the vast numbers of waterfowl that migrate annually and visit numerous waterbodies en route, and (ii) the high densities of diatom that many aquatic habitats host, it is possible that the number of diatoms that successfully disperse adhered to waterfowl feathers is extremely large. Nevertheless, the efficacy of this mode of dispersal is likely to vary among diatom taxa; it may favour, for example, benthic and epiphytic taxa whose times of peak abundance match peak flight activities of waterfowl. Effective waterfowl-mediated dispersal could serve to homogenize taxonomic composition (i.e., decreases beta diversity) among waterbodies with suitable abiotic conditions, particularly among taxa for which waterfowl vectors are effective.

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Data Availability Statement

All data and scripts are freely available for download from the OSF (https://osf.io/ujnw2), and are citable as: Pither et al. (2020). Data about within-day dispersal distances of mallard ducks are available on Dryad (https://datadryad.org/resource/doi:10.5061/dryad.619gd). The Global Lakes and Wetlands Database (GLWD) is available from the World Wildlife Fund (https://www.worldwildlife.org/pages/global-lakes-and-wetlands-database). The ASOS Network online database is freely accessible (https://mesonet.agron.iastate.edu/request/download.phtml?network=IA_ASOS). Data about the distribution of *Nitzschia* taxa, including *N. pusilla*, are freely accessible within the 2007 and 2012 National Lakes Assessment online repository (https://www.epa.gov/national-aquatic-resource-surveys/data-national-aquatic-resource-surveys).

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693

694

695 **Tables**

696

697 **Table 1.** Logistic regression (generalized linear model with binomial link) of the effects of
698 relative humidity (RH) (%), transport time (TIME) (minutes), and their interaction (RH × TIME) on
699 dispersal success. Shown are the coefficient estimates, lower and upper 95% confidence limits
700 (CL), *Z*-values, and associated probability values (*P*-value). The null and residual deviance was
701 146.1 and 71.9 respectively, on 127 and 124 degrees of freedom respectively). The McFadden
702 pseudo-R^2 was 0.51.

Coefficient	Estimate	Lower 95% CL	Upper 95% CL	*Z*-value	*P*-value
Intercept	–3.355	-7.200	–0.682	–2.092	0.036
RH	0.058	0.022	0.109	2.701	0.007
TIME	–0.091	–0.186	–0.024	–2.238	0.025
RH × TIME	9.391×10^{-4}	1.552×10^{-4}	20.187×10^{-4}	2.024	0.043

703

704

Figures

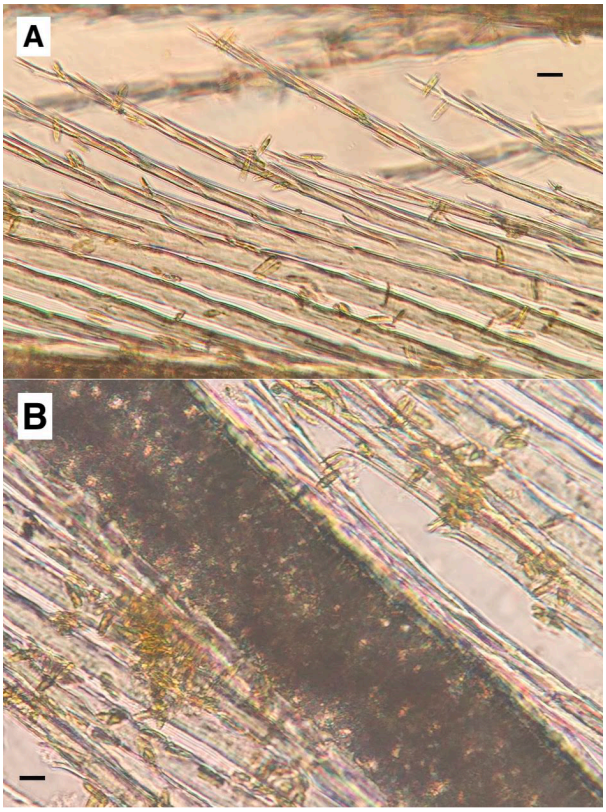


Figure 1. Two views (panels A and B) of *Nitzschia pusilla* Grunow diatoms embedded within a mallard breast feather. The black scale bar in each panel is approximately 1 μm. For reference, individual diatoms examined during identification work were, on average, 13.7 μm long (see Figure A1).

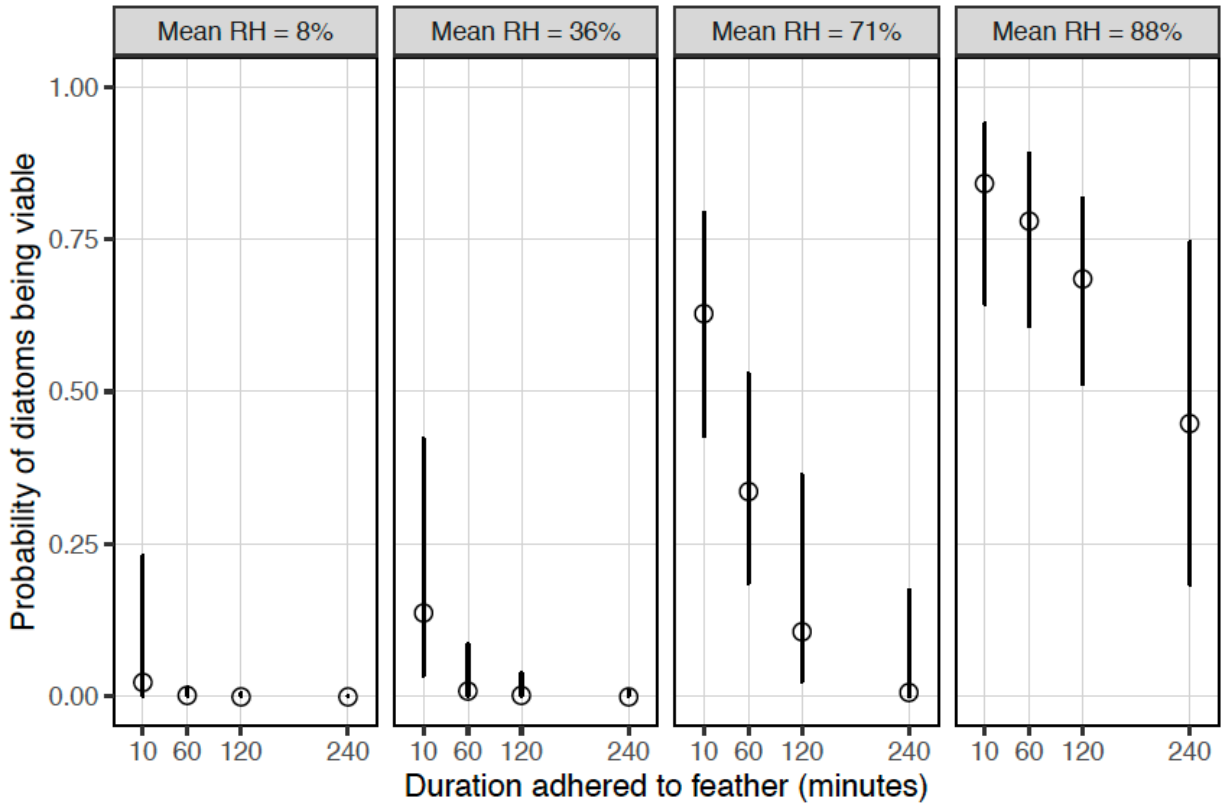


Figure 2. Predicted probability of *Nitzschia pusilla* Grunow diatoms being viable as a function of relative humidity and transport duration. Panels display results grouped by target relative humidity (from lowest to highest, left to right), and panel labels show the mean relative humidity measured across replicates of the corresponding group. Predictions are based on the GLM from the main experiment, and use marginal responses, the default approach in the R package `ggeffects` (Lüdecke, 2018).

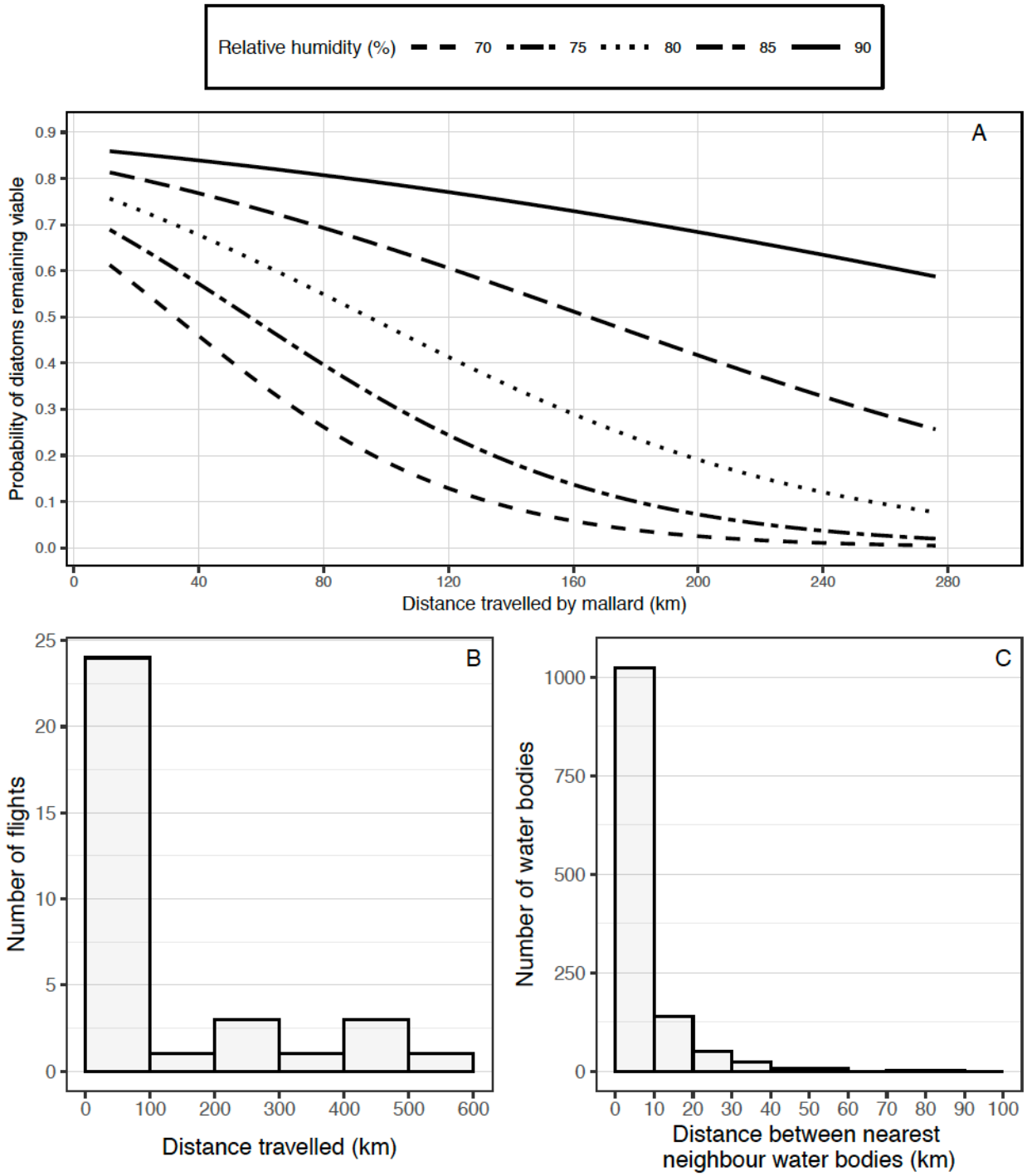
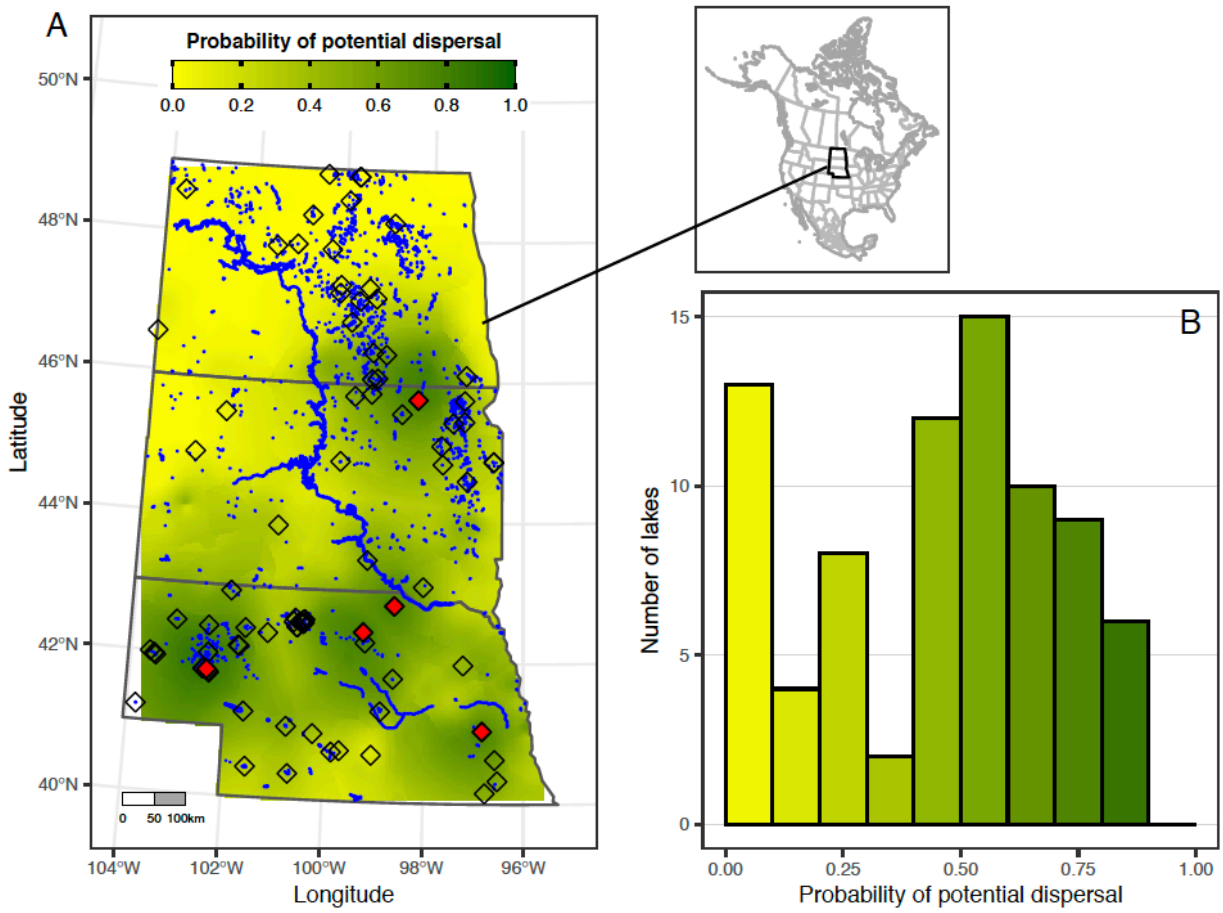


Figure 3. Panel (A): Predicted probability of *Nitzschia pusilla* Grunow diatoms remaining viable as a function of relative humidity and travel distance. Predictions are based on our full

GLM, and assume average flight speed of 69 km/h. (B) Histogram of distances travelled by mallards in North America in a single day (N = 33) (Viana *et al.*, 2013a, 2013b). (C) Histogram of distances between surface water bodies and their nearest neighbours within North Dakota, South Dakota, and Nebraska (N = 1252 water bodies).



732

733 **Figure 4.** Panel (A): Geographical predictions of the probability of potential diatom dispersal
734 (omnidirectional) from 5 source lakes hosting *Nitzschia pusilla* Grunow (red diamonds).
735 Predictions are derived from the GLM from our experiment, using estimates of mallard flight
736 duration (based on average flight speed of 69 km/h and geographic distances) and spatially
737 interpolated relative humidity values. See Materials and Methods for details. All surface
738 waterbodies are indicated in blue. Hollow black diamonds denote the 80 waterbodies that in
739 2007 or 2012 hosted diatoms of the genus *Nitzschia* (aside from the 5 hosting *N. pusilla*). The
740 map projection is North American Equidistant Conic. Inset map shows the study region outlined
741 in black within North America (North American Lambert Conformal Conic projection). For the
742 80 *Nitzschia* waterbodies shown in Panel A, Panel (B) shows a histogram of their associated

probabilities of potential dispersal from source lakes, corresponding to the raster values from the prediction map. The colour scale corresponds to that shown in the map.