# Potential long-distance dispersal of freshwater diatoms adhering to waterfowl plumage

**Faye S. Manning[1,2], P. Jeff Curtis[3], Ian R. Walker[1,2,3], and Jason Pither[1,2,3]**

[1]Okanagan Institute for Biodiversity, Resilience, and Ecosystem Services (BRAES), University of British Columbia, Okanagan Campus, Kelowna, British Columbia, Canada, V1V 1V7.

[2]Department of Biology, University of British Columbia, Okanagan Campus, Kelowna, British Columbia, Canada, V1V 1V7.

[3]Department of Earth, Environmental, and Geographic Sciences, University of British Columbia, Okanagan Campus, Kelowna, British Columbia, Canada, V1V 1V7.

Corresponding Author:

Jason Pither

Email address: jason.pither@ubc.ca

OrcID: 0000-0002-7490-6839

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**Abstract**

1. Waterfowl are potential long-distance dispersal vectors for aquatic microbes such as diatoms, but supporting empirical data are scarce, especially concerning external transport on feathers.

2. We conducted an experiment designed to partially emulate diatom dispersal via adherence to waterfowl, and to evaluate the effects of relative humidity (RH) and exposure time on viability. Using eight replicates per treatment combination, we dipped individual breast feathers from mallard ducks (*Anas platyrhynchos*) in a pure culture of the freshwater diatom *Nitzschia pusilla* Grunow, then subjected them to one of four contrasting levels of RH (ca. 8, 35, 70, 88%) crossed with one of four exposure times (10, 60, 120, 240 minutes) within a chamber through which air was passed continuously, mimicking light wind that might be experienced by diatoms adhered to subsurface feathers. All treatments occurred at room temperature, and thus our four RH treatments corresponded to the following values of vapour pressure deficit (VPD): 2.5, 1.8, 0.8, and 0.3 kPa respectively. We then gently placed the feather on sterile growth medium. After two weeks we used spectrofluorometry to detect diatom growth and thus diatom viability. Finally, we combined our experimental findings with geospatial data to predict the probability of potential dispersal via adherence to mallards throughout Nebraska, South Dakota, and North Dakota, which are situated within the central waterfowl migration flyway in North America, and host important mallard breeding grounds.

3. We found that exposure time and RH interacted significantly to affect diatom viability: the negative effect of exposure time was strongest under low RH conditions, but under high RH (88%) the probability of being viable was 0.84 for a ten minute exposure (95% confidence interval: 0.64 to 0.94), and 0.45 for four hours of exposure (95% confidence interval: 0.18 to 0.75). Combining these results with published data about (i) mallard flight speeds, (ii) the

geographic distribution of surface waters and of *N. pusilla*, and (iii) vapour pressure deficit during the months of April and May, our geospatial model predicted high probabilities of potential dispersal, over tens to hundreds of kilometres, among ~~the suitable~~ water bodies of the central migration flyway.

4. Taken together, the results of our experiment and geospatial models suggest that long-distance dispersal of diatoms via adherence to waterfowl feathers is highly plausible, particularly during the near-dawn hours when waterfowl flight activity peaks and VPD is low. Considered alongside previous evidence suggesting successful internal transport by waterfowl, we conclude that for freshwater diatoms ectozoochory is likely commonplace ~~high~~ among waterbodies frequented by waterfowl.

## Introduction

Dispersal is a fundamental ecological process that connects populations and communities and moderates how diversity is distributed across the landscape (Leibold *et al.*, 2004; Vellend, 2016). 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 (e.g. Heino, 2011; Tesson *et al.*, 2015). 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 numerous researchers have highlighted the roles that animal vectors, especially waterbirds, could potentially play (Schlichting, 1960; Figuerola & Green, 2002; Green *et al.*, 2016; Stoyneva, 2016; Coughlan *et al.*, 2017; 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; Coughlan *et al.*, 2017; Tesson *et al.*, 2018). These studies have revealed mixed findings, but do suggest strong potential for successful diatom dispersal via endozoochory. 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.

The degree to which diatoms successfully disperse via ectozoochory (external transport) by waterbirds remains an open question. To be successful, the diatom propagules would need to (i) come into contact with the bird, (ii) adhere or attach to the bird, (iii) survive and remain attached during transport, (iv) detach in the new habitat, and (v) successfully colonize and persist in the new habitat (Coughlan *et al.*, 2017). Diatoms have been observed on waterbird plumage (e.g. Schlichting, 1960; Kristiansen, 1996; Figuerola & Green, 2002), but we are unaware of any quantitative data about diatom survival and viability following ectozoochory. A key limiting step is surviving exposure to desiccation (Kristiansen, 1996; Coughlan *et al.*, 2018), which is governed by flight time and the humidity experienced by the propagules during transport. 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 for cells berried within waterfowl plumage, which has strong insulating properties (Coughlan *et al.* 2015). Considering that waterfowl such as mallard ducks (*Anas platyrhynchos*) fly at 60-70km/h (McDuie *et al.*, 2019), diatoms that survive transport could conceivably cover considerable

distances in short periods of time. For reference, we use the phrase "long distance dispersal" to simply refer to dispersal among disjunct waterbodies separated by tens to hundreds of kilometres.

Here, we present the results of a novel experiment designed to partially emulate diatom dispersal via adherence to waterfowl feathers. We used breast feathers from mallard ducks, which are common throughout temperate regions of the world (Wetlands International, 2021), 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 forage in productive littoral zones, it is reasonable to expect their plumage – especially breast plumage – comes into contact with large numbers of benthic, epiphytic and epipelic diatoms, which can reach very high densities in favourable conditions (Patrick, 1977; Wehr & Sheath, 2004). Barbed feathers provide relatively large 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. Under near-constant room temperature, we examined how relative humidity (RH) and exposure time individually and interactively affected the viability of the diatoms adhered to feathers. We predicted that viability would decrease with decreasing RH and increasing exposure time. 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 vapour pressure deficit (VPD) (based on RH and air temperature) within the states of North Dakota, South Dakota, and Nebraska. These states are situated in the central migration flyway of

North America, and of the four major north-south flyways in North America (see map at https://www.fws.gov/birds/management/flyways.php), the central flyway hosts the largest numbers of mallards (U.S. Fish & Wildlife Service, 2019).

## 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 relatively common, primarily benthic genus found throughout inland waters in North America (Potapova & Charles, 2002), 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 (Supporting Information, Fig. S1).

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 (video available at https://osf.io/ujnw2/), 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 at -18°C 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 randomly selected for each experimental run.

*Experimental apparatus*

We constructed four apparatuses in total, and 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; three lengths of inline tubing; one 250mL Erlenmeyer flask filled with a glycerol and water solution; a WhirlPak® bag, and a hygrometer (Supporting Information, Fig. S2). 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 sensor (Hobo 12-bit smart sensor, model S-THB-M002) simultaneously measured RH and temperature. The bag was twist-tied shut around the tubing. We connected the sensor to a Decagon Em50® Series Data Collection System Data Logger, and took RH and temperature readings every minute. We assume that the RH and temperature measured in the WhirlPak® bag reliably estimated the RH and temperature in the chamber proper. We also monitored the RH and temperature of the room using another identical sensor, attached to the same datalogger.

The RH within the chamber was manipulated using glycerol-water solutions. Specifically, increasing the glycerol:water ratio in the flask reduces the equilibrium relative humidity in the air within the sealed chamber, as the glycerol directly affects the water activity of the solution (Forney & Brandl, 1992). 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 target RH levels within the main chamber: near 0%, 35%, 70%, and near 100%, respectively. The mean RH that we achieved for each of the four target RH levels (calculated using 32 samples per group; see below) was 8.1% (± one standard error: 0.35), 35.9 (± 0.54), 71.1 (± 0.35), and 88.4 (± 0.44), and the corresponding mean temperatures were 22.7 (± 0.04), 22.8 (± 0.04), 22.9 (± 0.04), and 22.7 (± 0.04) °C (Supporting Information, Fig. S3). For our statistical analyses (below) we used the average RH value calculated using the 1-minute interval readings taken during the given experimental run.

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 to ensure airflow rate was consistent across experimental runs: the airflow valve was opened just enough so air could be felt moving through the system and would ruffle the feather slightly. We

were not attempting to mimic the rapid airflow that outermost feathers might experience during flight. Rather, we envision this small rate of airflow simulating the conditions that might be experienced by subsurface plumage (*sensu* Coughlan *et al.* 2015).

*Experimental procedure*

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

During the procedure the RH occasionally varied beyond desired ranges immediately following sample changeovers. We ignored fluctuations to a higher humidity immediately following sample changeovers, regardless of how long the higher humidity persisted, as the sensor was likely detecting the water evaporating off the feather as it exited the system. When

the RH varied beyond the desired range at any other point during the procedure, the sample was discarded, and the procedure started over.

We used eight replicates (feathers) for each combination of target RH (four levels) and exposure time (four times), for a total of N = 128. We conducted the experiment over two four-day intervals, separated by one day. We completed one replicate from each unique treatment (sixteen unique combinations of target RH level and exposure time) each day, and we randomized the order of treatments within each day. We also randomly assigned the target humidity levels to the four chambers. At the end of each experimental day, we replaced the volume drawn from the culture with an equal volume of sterile media. This ultimately diluted the culture over the course of the experiment, but concentrations remained very high ($> 1.7 \times 10^5$ cells/mL) and were thus suitable for ensuring feather inoculation (Supporting Information, Fig. S4).

We conducted all assessments of diatom growth using an RF-1501 Shimadzu 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 eight 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 two seconds (thus it did not undergo RH and exposure time treatments), and subsequently placed in a petri dish with growth medium. The spectrofluorometer intensity readings obtained after fourteen days from these eight controls served to establish our LOD. We additionally ran eight 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 (Supporting Information, Fig. S5). 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 fourteen days with evidence of viability. We analyzed this binary response variable in relation to RH (%) and exposure time (minutes), and their interaction, using a generalized linear model (GLM) with a binomial link (i.e., logistic regression). We coded RH and time as continuous variables. Coding them as ordinal factors yielded qualitatively similar results. 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*

We conducted our experiment at a near-constant air temperature of 22.8ºC, which simplified the interpretation of our RH treatments. In the real world, air temperature varies spatially and temporally, and VPD is a more meaningful measure of desiccation potential because it provides an absolute measure of the atmospheric moisture state independent of temperature (Anderson, 1936). VPD is the difference between the actual moisture content of the air and the saturation point of the air at a given temperature, and it varies as a function of RH and air temperature. Desiccation potential increases with increasing VPD (Anderson, 1936). For example, our experimental treatment of 35% RH at 22.8ºC yields a VPD value of 1.81kPA, but at an air temperature of 10ºC, the same RH yields a VPD value of 0.80kPa. Thus, despite RH being the same, desiccation would occur much faster at 22.8ºC compared to 10ºC. To facilitate extrapolation of our results to the real world, we therefore re-analyzed our experiment data using VPD as a predictor in lieu of RH within the GLM. Specifically, we used the default arguments of the `rH.to.VPD` function within the `bigleaf` R package (Knauer *et al.*, 2018) to estimate VPD in the experimental chambers, using the measured RH and temperature. The average VPD values corresponding to the four RH treatments (from low to high RH), were 2.53 (± 0.01), 1.78 (± 0.02), 0.80 (± 0.01), and 0.32 (± 0.01) (Supporting Information, Fig. S3c). The results of this analysis are provided in Table S1 and Fig. S6 in the Supporting Information.

We extrapolated our results to North Dakota, South Dakota, and Nebraska, and gathered *in situ* VPD data for the period between April 1 and May 31, which corresponds with high frequencies of mallard occurrences in the region (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 (U.S. Fish & Wildlife Service, 2019). We could not extend our geospatial analyses north into Canada due to a lack of data. As we did not manipulate temperature in our experiment, our geographical extrapolations should be interpreted with caution.

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 (with corresponding flight times) and VPD values typical of the study region and time periods (0.25 to 1.25kPa; Supporting Information, Fig. S7). We present these predictions alongside (i) data about within-day dispersal distances of mallard ducks, based on banding observations (Viana *et al.*, 2013a, 2013b), and (ii) distances between each surface water body (> 0.1 $km^2$ area) in the study region and its nearest neighbour water body, calculated using the Global Lakes and Wetlands Database (GLWD), downloaded from the World Wildlife Fund. Finally, we generated a map of probability of potential dispersal among the region's water bodies, using *N. pusilla* Grunow as the focal diatom taxon. To do this, we used our GLM in combination with (i) the average flight speed of mallards (as above), (ii) spatially interpolated VPD for the region, calculated using RH and air temperature data from the ASOS Network online database, and based on the average measurements for three times of day: near dawn (04:00 and 07:00), mid-day (12:00 to 15:00), and dusk (20:00 to 23:00) during the first week of May, (iii) estimated flight times between surface water bodies (using distances from the GLWD data), and (iv) data about the distribution of *Nitzschia* taxa, including *N. pusilla*, acquired from the 2007 and 2012 National Lakes Assessment. All data sources are detailed in the Data Availability Statement. We included the dawn and dusk VPD values because the daily flight activity of ducks typically peaks just before dawn and just after dusk (Bengtsson *et al.*, 2014;

Kleyheeg *et al.*, 2017). We included mid-day VPD values for comparison. We used lakes hosting *N. pusilla* Grunow as the focal "source" lakes for diatoms, and generated a raster layer of probability of potential dispersal based on estimated flight times (based on distances and flight speed) from these source lakes, combined with the spatially-interpolated VPD values. Then for each waterbody hosting diatoms of the *Nitzschia* genus, we extracted the probability of potential dispersal values from the resulting raster layer. This procedure (i) equates probability of potential dispersal with the probability of diatoms remaining viable after the given exposure time and VPD (based on spatially interpolated VPD), and (ii) makes the simplifying assumption that waterbodies hosting the genus *Nitzschia* are suitable for colonization by *N. pusilla* Grunow (see Discussion). Additional details of these methods and of our results are provided in the fully annotated R Markdown script available online (https://osf.io/ujnw2/).

*Ensuring computational reproducibility*

All our analyses were performed using R version 3.6.3 (R Core Team, 2019) within the RStudio IDE (RStudio Team, 2019). To ensure computational reproducibility we used the `renv` package (Ushey, 2020) that manages R package versions, and we generated an annotated R Markdown script that, along with all data, are freely available for download from the Open Science Framework (https://osf.io/ujnw2). We used numerous R packages within our analyses, and these are detailed in the Supporting Information. We encourage readers to contact the corresponding author (JP) if any part of our study is unclear and / or irreproducible.

## Results

The logistic regression from our experiment yielded a McFadden pseudo-$R^2$ value of 0.51, indicating a significant amount of the deviance was accounted for by the full model (Table 1). Exposure time and RH interacted significantly to affect diatom viability: at high RH, the

probability of being viable was moderate to high across all exposure times, including the maximum four-hour period (Fig. 2). For instance, at an average RH value of 88% and an exposure time of four hours, the predicted probability of being viable was 0.45 (95% confidence interval: 0.18 to 0.75), and for a one-hour exposure time it was 0.78 (95% confidence interval: 0.61 to 0.89) (Fig. 2). At lower RH, the probability of being viable declined more rapidly with increasing exposure time, and at the lowest RH values (8 and 36%), viability was predicted to be possible (between 0 and 0.42 probability) over only the shortest duration of exposure (10 min) (Fig. 2).

Using VPD in lieu of RH in a GLM yielded qualitatively identical outcomes (Table S1), including a significant interaction between VPD and exposure time (Table S1; Fig. S6). Based on this GLM, Figure 3A depicts the predicted probability of remaining viable across a range of travel distances (using an estimated average mallard flight speed of 69 km/h) and VPD values that are typical of those observed in the study region during April and May (Fig. S7). Figure 3B shows the frequency distribution of distance travelled by mallards within a single day, and Figure 3C shows the frequency distribution of distance to nearest neighbouring water body among lakes and wetlands (those > 0.1 $km^2$ area) in the study region. Collectively, these figures suggest strong potential for long-distance diatom dispersal by mallard vectors, especially over tens of kilometres, and given the highly favourable VPD conditions (< 0.5kPa) that are typical of dawn hours during April and May (Fig. S7).

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

*pusilla* diatoms, Figure 4 shows how the probability of potential dispersal varies geographically in the study region, based on (i) distance from the source lakes, (ii) average mallard flight speed (69 km/h), and (iii) VPD interpolated throughout the study region (Fig. S8), using data from the first week of May for illustration, and for three different times of day (dawn, mid-day, and dusk). The maps show considerable potential for dispersal throughout the study region, particularly during dawn hours (04:00 – 07:00) when VPD is ubiquitously low (rarely reaching 0.25kPa; Fig. S8) owing to the cool temperatures and high RH that are typical of spring mornings in the region (Fig. S7). Using data extracted from these prediction maps, Figure 5 shows the probability of potential dispersal from the 5 source lakes into each of the other eighty waterbodies in the region that host *Nitzschia* diatoms. It shows the considerable influence that time of day has on the probability of potential dispersal, owing to pronounced variation in VPD that occurs through the day (Fig. S8). During dawn hours, predicted probabilities are generally greater than 0.75, whereas during mid-day hours, most are less than 0.25, though some lakes exhibit higher probabilities owing to their proximity to source lakes (Fig. 4). At dusk, the geographical pattern is more varied, and the probabilities of potential dispersal are more evenly distributed between zero and 0.8 (Fig. 5).

**Discussion**

Consistent with our predictions, we found that the viability of diatoms adhered to feathers declined significantly with increasing exposure time and decreasing RH. However, these treatments interacted significantly: exposure time had diminished negative effects under high RH conditions. For example, the predicted probability of remaining viable after four hours of exposure was near zero at 8 and 36% RH, whereas at 88% RH plausible values for the probability of remaining viable ranged from 0.18 to as high as 0.75. Such interactions between

RH and exposure duration have previously been observed in desiccation experiments involving aquatic macrophytes (e.g. Coughlan *et al.*, 2018), but to our knowledge, ours are the first such findings pertaining to diatoms. Souffreau *et al.* (2010, 2013) examined desiccation tolerance among an impressively long list of diatom taxa (69 strains), but RH was not manipulated directly, and nor was exposure time examined simultaneously. Rather, solutions of diatom culture within well plates were air-dried for five or ten minutes in separate experiments. A factorial experimental design is key for detecting treatment interactions, and in our case revealed the important interaction between RH and exposure time.

Our experiment was also unique in examining desiccation tolerance of diatoms adhered to feathers. Although a number of studies have examined the potential for internal transport of diatoms by waterfowl (reviewed in Coughlan *et al.*, 2017), we are unaware of any that have experimentally evaluated the potential for ectozoochory of diatoms via adherence to feathers. For diatoms to successfully disperse via waterbird-mediated ectozoochory, propagules would need to (i) come into contact with the bird, (ii) adhere or attach to the bird, (iii) survive and remain attached during transport, (iv) detach in the new habitat, and (v) successfully colonize and persist in the new habitat (Coughlan *et al.*, 2017). Below we discuss our findings with reference to each of these five points.

*Contact and adherence of diatoms to waterfowl*

As waterfowl such as mallards forage in productive littoral zones, their breast plumage likely comes into contact with numerous benthic, epiphytic, and epipelic 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 Supporting Information Figure S4). We cannot 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 considers the vast numbers of diatoms and mallards involved – almost 10 million mallards in North America alone, (U.S. Fish & Wildlife Service, 2019).

The apparent ease with which diatoms were observed to adhere to feathers could be due in part to properties of diatom frustules. First, diatom frustules contain polysaccharides (Gélabert *et al.*, 2004; Le Costaouëc *et al.*, 2017) that are typically attracted to hydrophobic substances, potentially including the so-called "preening oil" secreted by waterbirds from the uropygial gland (Bakken *et al.*, 2006), and that has been found to contain chemical derivatives found in waxes (wax esters) (Stenhagen & Odham, 1971). Second, the polysaccharides in diatom frustules can reduce the surface tension of water (Ozkan & Berberoglu, 2013), which could further facilitate contact between diatoms and feathers. Third, *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). We speculate 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. More generally, 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.

*Survival and adherence during transport*

We did not evaluate the extent to which diatoms adhered to feathers might dislodge during flight, and nor did we attempt to mimic the potentially strong wind shear that might be experienced by diatoms adhered to the outermost feathers on a bird. Rather, in our experiment, we exposed the feathers to a light breeze that may be more representative of conditions experienced by diatoms adhering to subsurface plumage, though this remains speculative. Our experimental design could readily be modified to test the effects of wind speed and angle on propagule dislodgement and viability, using a wind-tunnel design, for example.

For any aquatic plants successfully adhering to feathers for the duration of flight, desiccation is thought to be the key limiting process (Green *et al.*, 2016; Coughlan *et al.*, 2017). In the only other studies we are aware of that examined diatom tolerance to desiccation, Souffreau *et al.* (2010, 2013) showed that diatoms are broadly intolerant of this form of stress. Our findings are generally consistent with this: we directly manipulated relative humidity at room temperature and found a dramatic decrease in viability with decreasing relative humidity (Figure 2) and corresponding vapour pressure deficits (Fig. S6). However, we did not examine the physiological status of the diatoms in our experiment, so cannot be certain that inviability was due to desiccation per se. Importantly, we found that under levels of vapour pressure deficit that are representative of the favourable springtime, near-dawn conditions in the central flyway

443 of North America (e.g. < 0.25kPa; Supporting Information Fig. S7 and Fig. S8), diatoms
444 remained viable after prolonged periods adhered to feathers, including 4 hours at a vapour
445 pressure deficit of 0.33kPa. It should be noted that our experiment examined a single
446 temperature regime (between 22 and 23ºC; Fig. S3), and we do not know how diatom viability
447 might respond to the much cooler air temperatures typical of springtime dawns in the region. On
448 one hand, we suspect that air temperatures between 0 and 10ºC are more favourable to diatom
449 viability, as cooler temperatures yield lower vapour pressure deficit (all else being equal), and
450 diatoms are well adapted to cold aquatic environments (Wehr & Sheath, 2004). On the other
451 hand, waterfowl plumage includes microclimates that are buffered to some extent from ambient
452 conditions (Coughlan *et al.*, 2015), and may include warm pockets proximate to the skin. The
453 plumage can also maintain a humid microclimate (Coughlan *et al.*, 2015). Thus, the conditions
454 experienced by the diatoms in our experiment may have been more harsh than would be
455 experienced in transit within duck plumage.
456 It is important to note that the strain of diatom that we used in our experiment was chosen
457 specifically because it is easily maintained in culture. It may therefore be hardier and better
458 adapted to surviving ectozoochory than most diatom taxa. For instance, among the 69 strains (34
459 species) of diatom examined by Souffreau *et al.* (2010, 2013), only 5 exhibited some tolerance to
460 their desiccation treatment, including two strains of *Pinnularia borealis*, one strain of *Hantzschia*
461 *amphioxys*, and two strains of *Navicula radiosa*. It would be beneficial to explore the tolerances
462 of more taxa using our experimental design.
463 Flight duration is another factor that will influence the number of diatoms that remain
464 attached and survive transport. Our study explored this in three ways. In our experiment we
465 directly manipulated exposure time and found that increasing it from 10 minutes to 4 hours

decreased viability, but only moderately so under high relative humidity (Fig. 2) and correspondingly low vapour pressure deficit (Fig. S6). Then, assuming an average flight speed of 69 km/h (McDuie *et al.*, 2019), we used the statistical model from our experimental results to make spatially implicit predictions of the probability of remaining viable across a range of vapour pressure deficits and flight distances. For example, with values of vapour pressure deficit $\leq$ 0.5kPa, which are representative of springtime dawn and dusk conditions (Fig. S7) in the central flyway, our model predicts that diatoms would remain viable over 120 km with a probability of almost 0.5, and this increases to 0.7 for a distance of 40 km (Fig. 3A). In many parts of the North American range of mallards these distances more than span the distances among neighbouring water bodies (e.g. Fig. 3C). Nevertheless, these predictions make a number of key assumptions that should be borne in mind. Most importantly, we assume that diatoms can successfully remain adhered to feathers for the duration of the flight, despite the strong airflow that would impact outermost feathers with flight speeds of 69km/h. Below the plumage surface, it is possible that feathers are protected from strong airflow due to the insulating properties of the plumage and its microstructure (Coughlan *et al.*, 2015). In our experiment we exposed the feathers to a light breeze rather than a strong wind. We assume that this could represent the conditions experienced by diatoms adhered to subsurface feathers, but this requires testing.

The same assumptions apply to our spatially-explicit predictions of the probability of potential dispersal (Fig. 4), which indicated strong potential for dispersal among the region's waterbodies, particularly during dawn and dusk hours when ducks, including mallards, tend to fly between daytime roosting sites and nighttime foraging sites (Bengtsson *et al.*, 2014; Kleyheeg *et al.*, 2017). Indeed, based on air temperature and relative humidity data gathered from ninety-eight ASOS stations distributed throughout the study region, we found that vapour

pressure deficit was quite low ($\leq$ 0.5kPa) and thus favourable in April and May during the hours around dawn (Figs. S7, S8). During mid-day hours (noon to 3pm), higher temperatures combine with lower relative humidity to yield much higher vapour pressure deficits on average (Figs. S7, S8), so feather-borne dispersal during these times are predicted to be much less likely (Figs. 4, 5).

*Detachment in the new habitat*

Diatoms that withstand transport could be dislodged from feathers during landing, by preening, and/or could remain adhered to feathers that themselves become dislodged (Coughlan *et al.*, 2017). Our experiment mimicked the latter scenario in which feathers detach from the bird (due to molting, for example), and rest on the water surface. During early spring and late summer molting seasons, the surfaces of ponds and lakes hosting large numbers of waterfowl are often littered with detached feathers. Although plausible, we do not know how effective this scenario is at enabling diatoms to actually colonize the new habitat. Future experiments could compare alternative dislodgment scenarios, including dragging the feather through sterile media.

*Successful colonization*

The final step of successful colonization and establishing a local population is clearly dependent on the suitability of local conditions (e.g., water chemistry) for the given diatom taxon. In our experiment we aimed to ensure that diatoms surviving the treatments would not be limited by subsequent growth conditions, and therefore provided algal growth medium as the receiving habitat. The diatom strain we used was sourced from an effluent pond at St. Mary's River pulp and paper mill in Sault Ste. Marie, Ontario, Canada, and was identified using standard morphological techniques to be *Nitzschia pusilla* Grunow. According to algaebase.org (accessed March 10, 2020), this freshwater taxon has been recorded at locations throughout North America

and Europe, and in the 2007 National Lakes Assessment it was observed in 23 lakes in 13 different states. It occurred in 5 lakes within our study region (Fig. 4A). Thus, it is not an especially common taxon. However, there is considerable 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. For instance, members of the genus appear to occupy an extremely diverse range of abiotic conditions (Potapova & Charles, 2002), and this can be indicative of a taxonomic group in need of revision. In the case of *N. pusilla*, there is insufficient data upon which to define "suitable habitat". Based on our geospatial predictions, there is considerable potential for dispersal from the five waterbodies that host *N. pusilla* (Fig. 4), but given the small number occurrence records, perhaps "suitable habitat" is comparatively rare in the region.

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) may underestimate the distribution and abundance of potential 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.

*Conclusion*

We have provided novel evidence consistent with the idea that adherence to waterfowl feathers is a potentially 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 via both endozoochory and ectozoochory

(Figuerola & Green, 2002; Viana *et al.*, 2013c; Tesson *et al.*, 2015; Green *et al.*, 2016; Coughlan *et al.*, 2017; Lovas-Kiss *et al.*, 2018). 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, and future research should seek to quantify this variation experimentally.

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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 and Manning (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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## Tables

**Table 1.** Logistic regression (generalized linear model with binomial link) of the effects of relative humidity (RH) (%), exposure time (TIME) (minutes), and their interaction (RH × TIME) on the probability of diatoms remaining viable. Shown are the coefficient estimates, lower and upper 95% confidence limits (CL), *Z*-values, and associated probability values (*P*-value). The null and residual deviance was 146.1 and 71.5 respectively, on 127 and 124 degrees of freedom respectively). The McFadden pseudo-$R^2$ was 0.51.

| Coefficient | Estimate | Lower 95% CL | Upper 95% CL | *Z*-value | *P*-value |
|---|---|---|---|---|---|
| Intercept | –3.420 | -7.355 | –0.717 | –2.099 | 0.036 |
| RH | 0.059 | 0.022 | 0.110 | 2.703 | 0.007 |
| TIME | –0.090 | –0.185 | –0.023 | –2.214 | 0.027 |
| RH × TIME | $9.251 \times 10^{-4}$ | $1.427 \times 10^{-4}$ | $20.044 \times 10^{-4}$ | 2.000 | 0.046 |

## Figures

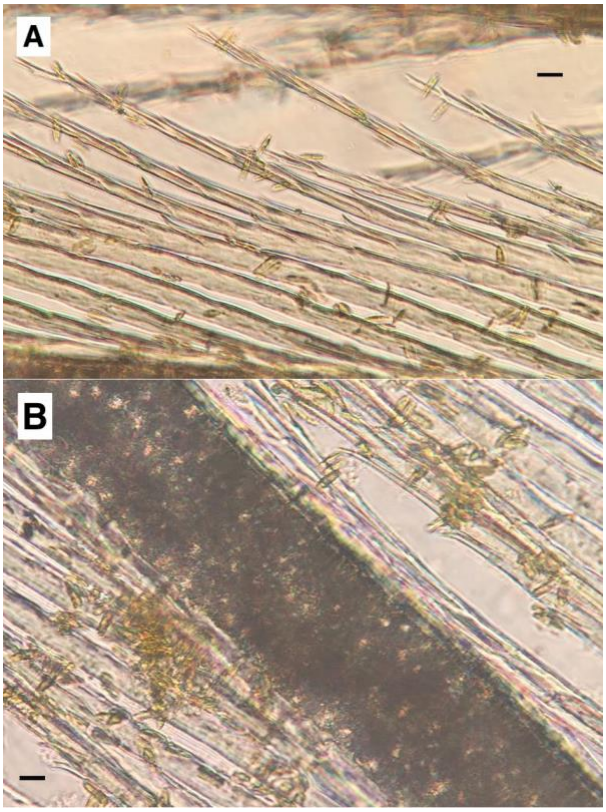



**Figure 1**. Two views (panels A and B) of *Nitzschia pusilla* Grunow diatoms embedded within a mallard breast feather. The black scale bar in each panel is approximately 10 μm. For reference, individual diatoms examined during identification work were, on average, 13.7 μm long (see Figure S1).

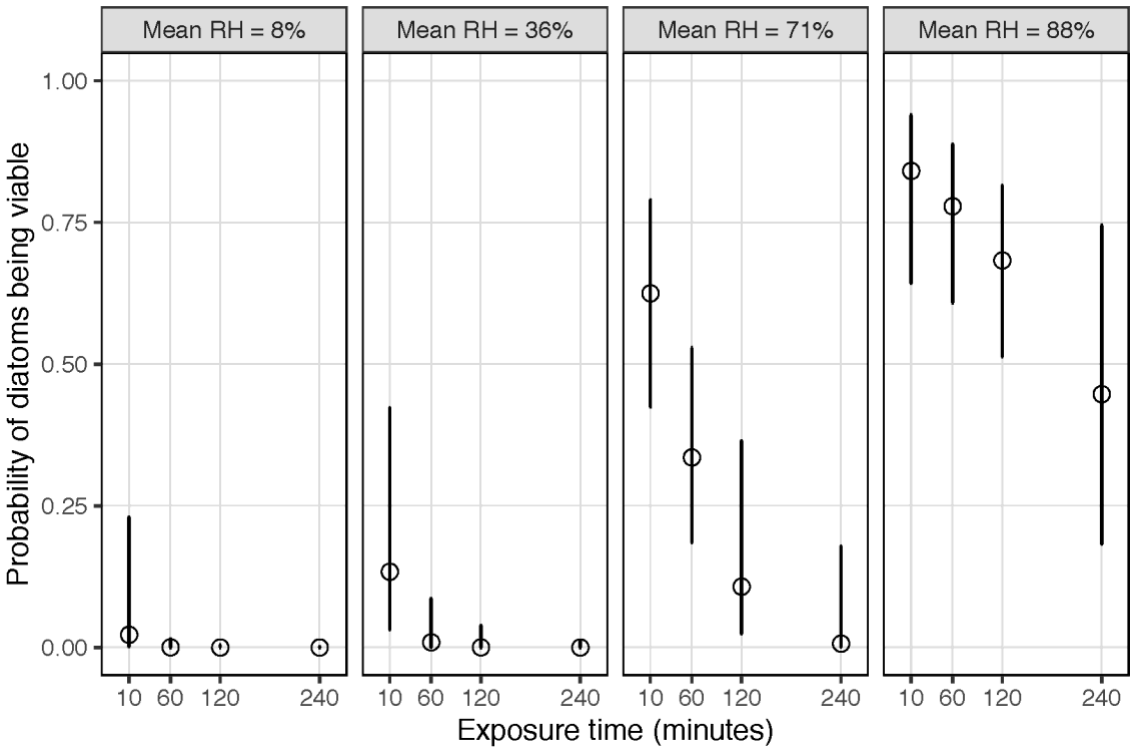



**Figure 2.** Predicted probability (circles) of *Nitzschia pusilla* Grunow diatoms being viable as a function of relative humidity and exposure time. Bars indicate 95% confidence intervals. Panels display results grouped by target relative humidity (from lowest to highest, left to right), and panel labels show the mean relative humidity measured across replicates of the corresponding group. All experimental trials occurred at temperatures between 22.7 and 22.9 °C. Predictions are based on the GLM from the main experiment, and use marginal responses, the default approach in the R package `ggeffects` (Lüdecke, 2018).

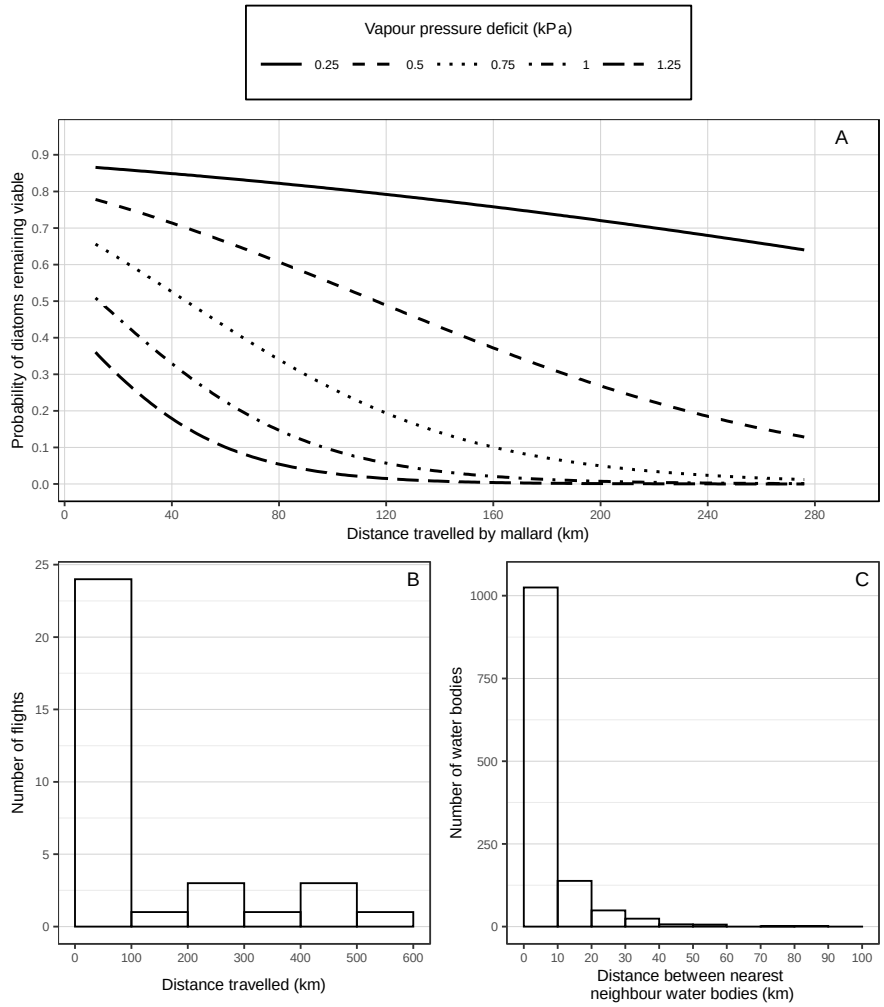
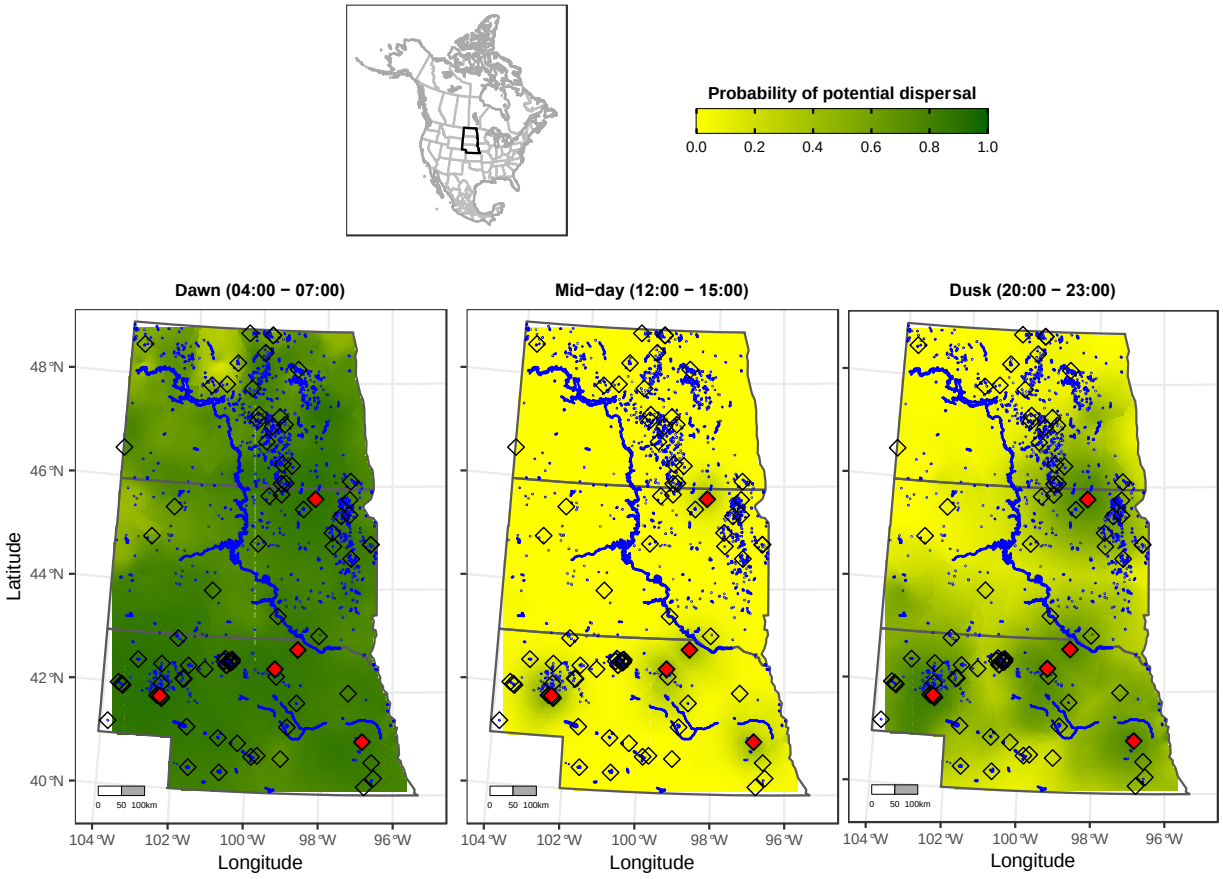



**Figure 3.** Panel (A): Predicted probability of *Nitzschia pusilla* Grunow diatoms remaining viable as a function of vapour pressure deficit and travel distance. Predictions are based on our VPD-based GLM, and assume average flight speed of 69 km/h. (B) Histogram of distances travelled by mallards 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).




779

780 **Figure 4.** Geographical predictions of the probability of potential diatom dispersal
781 (omnidirectional) from 5 source lakes hosting *Nitzschia pusilla* Grunow (red diamonds).
782 Predictions are derived from the VPD-based GLM from our experiment, using estimates of
783 mallard flight duration (based on average flight speed of 69 km/h and geographic distances) and
784 spatially interpolated vapour pressure deficit for the first week of May, averaged over the years
785 2015 through 2020, and for three different times of day (dawn, mid-day, and dusk). See
786 Materials and Methods for details. All surface waterbodies are indicated in blue. Hollow black
787 diamonds denote the 80 waterbodies that in 2007 or 2012 hosted diatoms of the genus *Nitzschia*
788 (aside from the 5 hosting *N. pusilla*). The map projection is North American Equidistant Conic.

789 The map at the top shows the study region outlined in black within North America (North
790 American Lambert Conformal Conic projection).
791
792

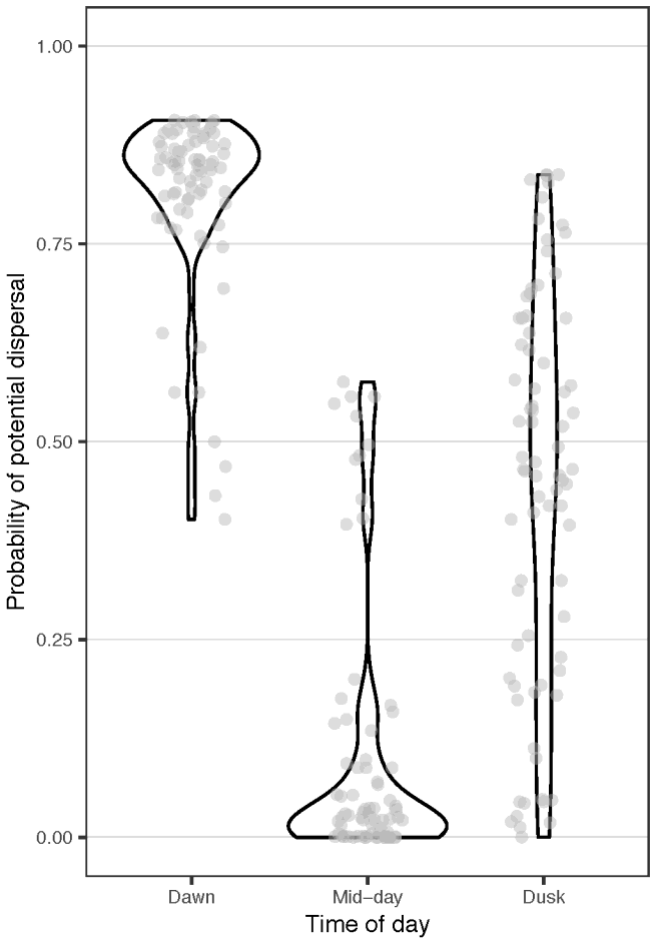



**Figure 5**. For waterbodies in the study region (Fig. 4) known to have hosted members of the diatom genus *Nitzschia*, shown here is a violin plot depicting the probabilities of potential dispersal from five lakes hosting the study species *Nitzschia pusilla* Grunow. The probabilities are extracted from the raster layers depicted in Figure 5. One of the 80 lakes hosting *Nitzschia*, located in the extreme south-west of region, did not overlap with prediction raster, thus the sample size for each of the three time-of-day groups is 79. The width of the black outline shapes reflect the frequency distribution of probabilities, and the grey dots are individual observations.

803

**Supporting Information** for:

# Potential long-distance dispersal of freshwater diatoms adhering to waterfowl plumage

**Faye S. Manning[1,2], P. Jeff Curtis[3], Ian R. Walker[1,2,3], and Jason Pither[1,2,3]**

[1]Okanagan Institute for Biodiversity, Resilience, and Ecosystem Services (BRAES), University of British Columbia, Okanagan Campus, Kelowna, British Columbia, Canada, V1V 1V7.

[2]Department of Biology, University of British Columbia, Okanagan Campus, Kelowna, British Columbia, Canada, V1V 1V7.

[3]Department of Earth, Environmental, and Geographic Sciences, University of British Columbia, Okanagan Campus, Kelowna, British Columbia, Canada, V1V 1V7.

**List of R packages** used in analyses:

`bigleaf` (Knauer *et al.*, 2018), `cowplot` (Wilke, 2019), `dplyr` (Wickham *et al.*, 2020), `ggeffects` (Lüdecke, 2018), `ggplot2` (Wickham, 2016), `ggsn` (Santos Baquero, 2019), `ggspatial` (Dunnington, 2018), `gstat` (Pebesma, 2004; Gräler, Pebesma & Heuvelink, 2016), `investr` (Greenwell & Schubert Kabban, 2014), `kableExtra` (Zhu, 2020), `knitr` (Xie, 2019), `lubridate` (Grolemund & Wickham, 2011), `minpack.lm` (Elzhov *et al.*, 2016), `patchwork` (Pedersen, 2019), `pscl` (Jackman, 2020) `riem` (Salmon, 2016), `raster` (Hijmans, 2020), `rdryad` (Chamberlain & Boettiger, 2018), `rmapshaper` (Teucher & Russell, 2020), `sf` (Pebesma, 2018), `snakecase` (Grosser, 2019), `sp` (Pebesma & Bivand, 2005; Bivand, Pebesma & Gomez-Rubio, 2013), `spatstat` (Baddeley, Rubak & Turner, 2015), `tidyr` (Wickham & Henry, 2019).

**Supplemental Table**

**Table S1.** Logistic regression (generalized linear model with binomial link) of the effects of vapour pressure deficit (VPD) (kPa), exposure time (TIME) (minutes), and their interaction (VPD × TIME) on the probability of diatoms remaining viable. Shown are the coefficient estimates, lower and upper 95% confidence limits (CL), *Z*-values, and associated probability values (*P*-value). The null and residual deviance was 146.1 and 71.7 respectively, on 127 and 124 degrees of freedom respectively). The McFadden pseudo-$R^2$ was 0.51.

| Coefficient | Estimate | Lower 95% CL | Upper 95% CL | *Z*-value | *P*-value |
|---|---|---|---|---|---|
| Intercept | 2.448 | 1.058 | 4.098 | 3.199 | 0.001 |
| VPD | –2.111 | –3.947 | –0.802 | –2.718 | 0.007 |
| TIME | 0.003 | –0.011 | 0.018 | 0.358 | 0.720 |
| VPD × TIME | –0.033 | –0.071 | –0.005 | –1.987 | 0.047 |

**Supplemental Figures**

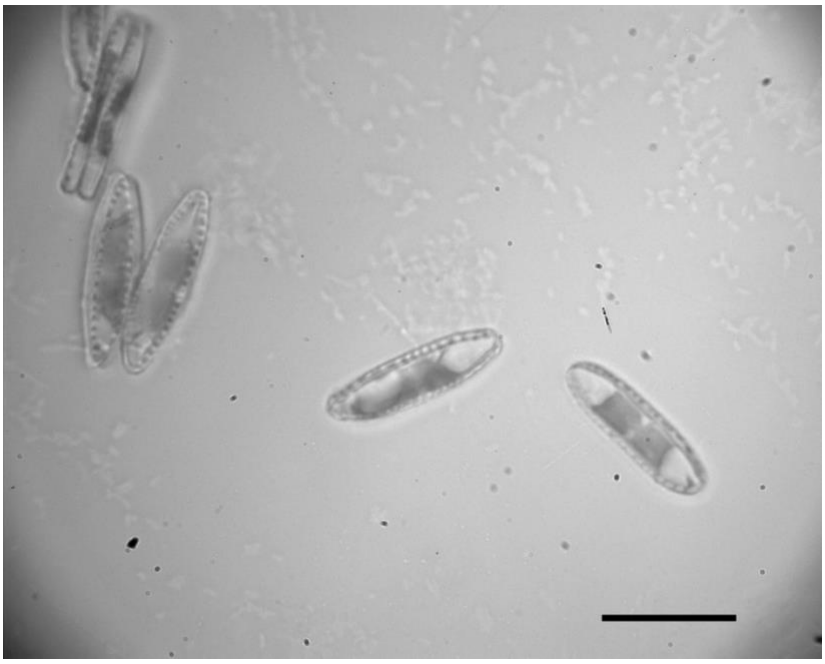


**Figure S1**: Micrograph (1000x) of preserved material provided by Kathryn Thomas, and identified based on morphological characteristics to be *Nitzschia pusilla* Grunow. Scale bar is approximately 10 $\mu$m.

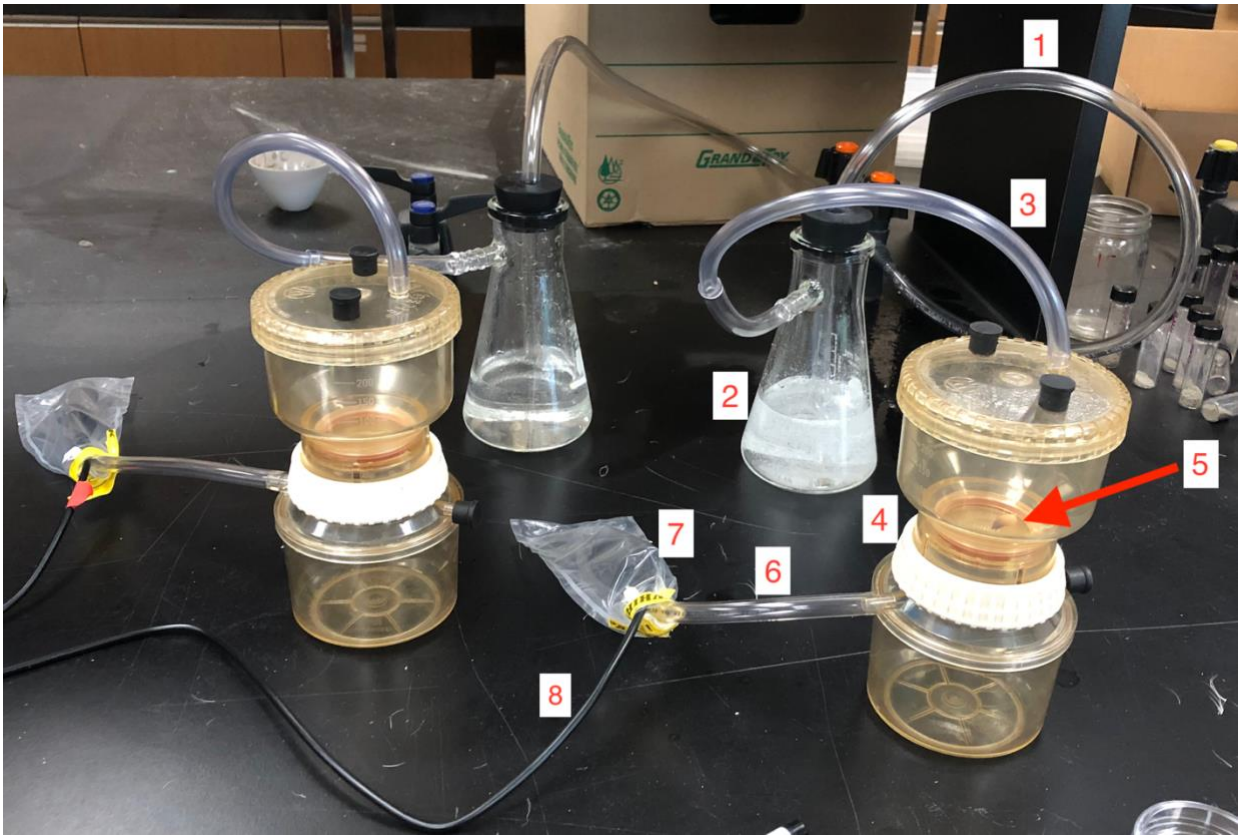



**Figure S2:** Two experimental apparatuses. In the right-hand apparatus, the red numbers correspond to: (1) tubing attached to benchtop air valve, connecting to (2) a flask with a glycerol solution that moderates relative humidity. This flask is connected by (3) tubing to (4) a makeshift airflow chamber, in which (5) the feather is secured by clasping the shaft in the seal. This chamber is then connected by (6) tubing that takes outflow air from the chamber to (7) the Whirlpack® bag containing the hygrometer. (8) The black cable exiting the Whirlpack® bag is the hygrometer cable, which connects to a datalogger (not shown).

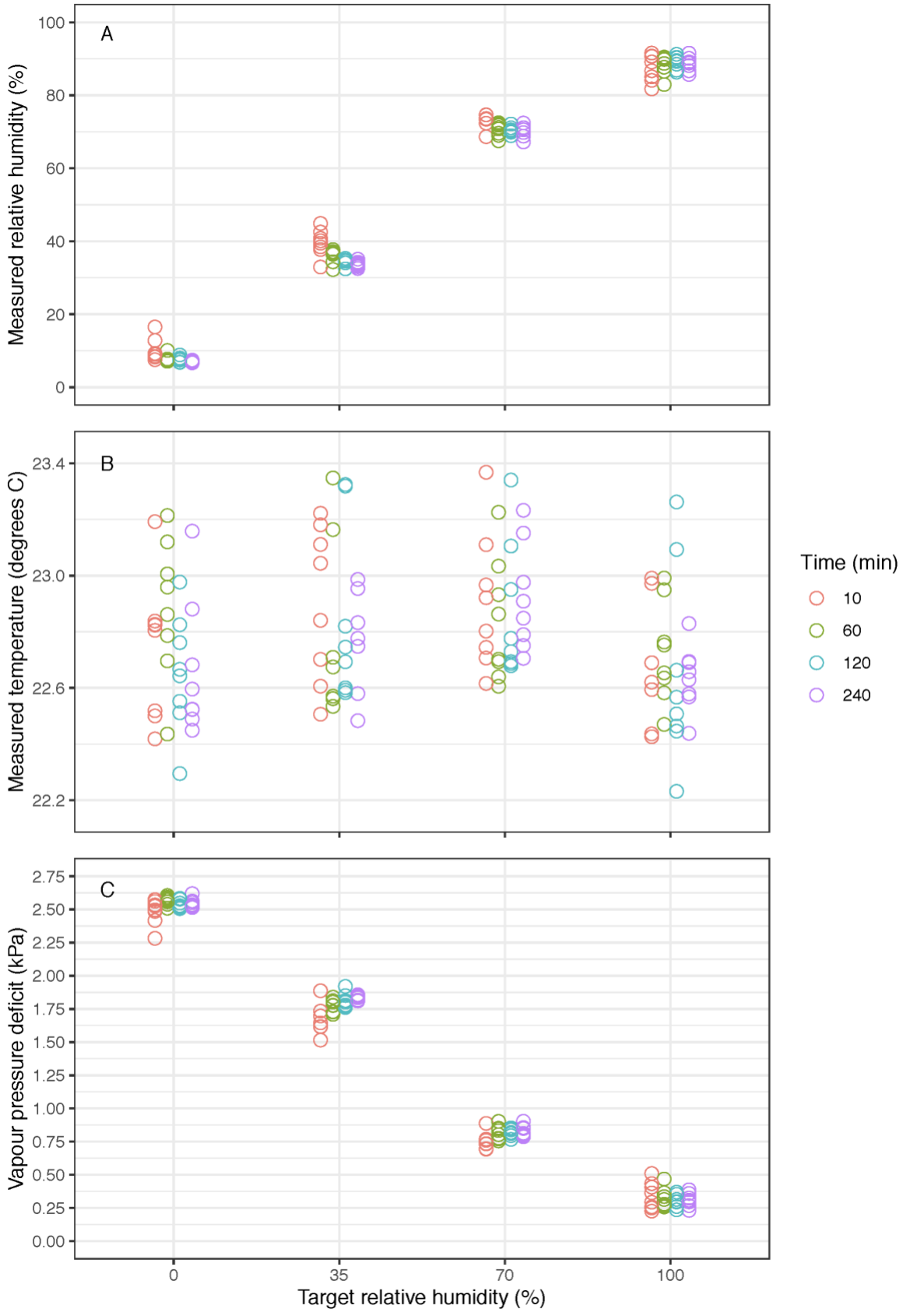

A
B
C
Measured relative humidity (%)
Measured temperature (degrees C)
Vapour pressure deficit (kPa)
Target relative humidity (%)
0
35
70
100
Time (min)
10
60
120
240

**Figure S3:** The time-averaged (A) relative humidity, (B) temperature measured in each experimental replicate plotted against the target relative humidity levels (with the 0 and 100 targets representing the "near-0" and "near-100" target values), and grouped by duration treatment. Panel (C) shows the corresponding calculated vapour pressure deficit values. There were n = 8 replicates per combination of target relative humidity and duration. Temperature values in panel B are jittered in the vertical direction to avoid overlapping points.

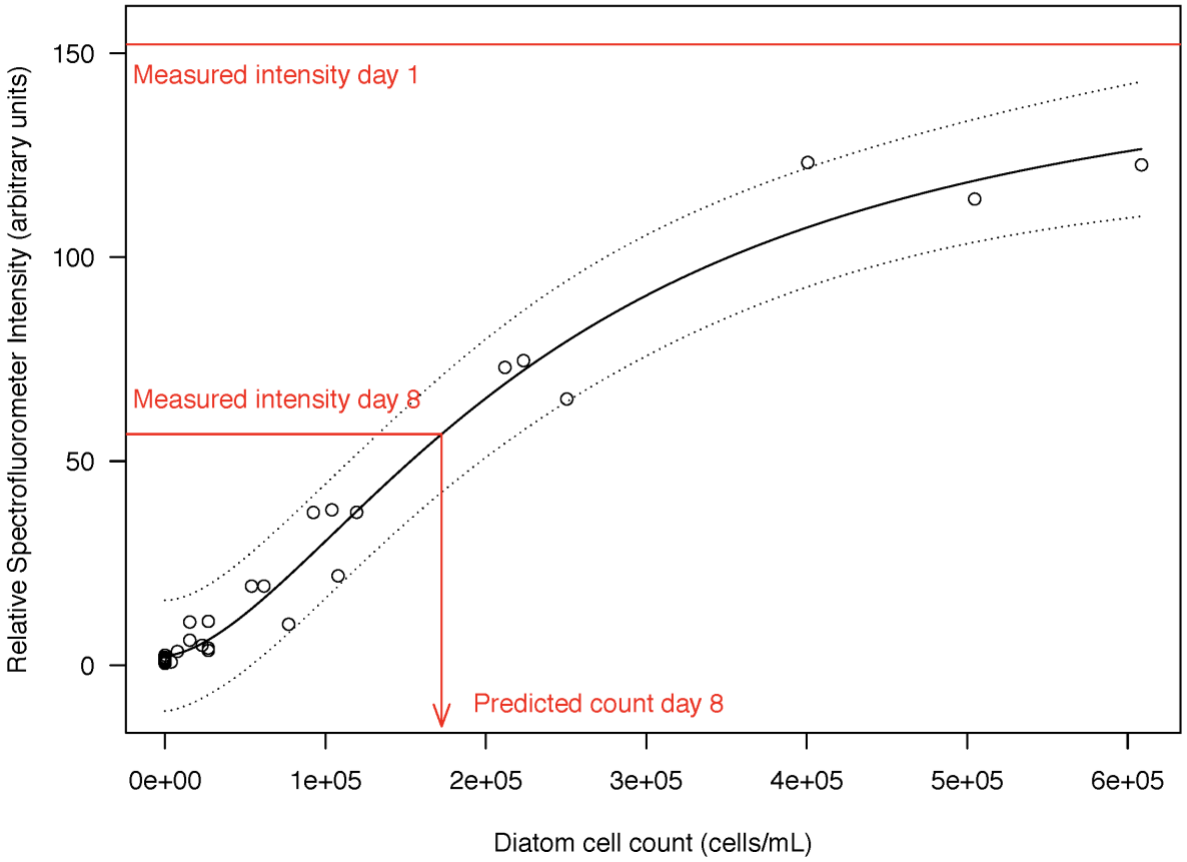



**Figure S4**: Calibration curve for estimating diatom cell density in the cell culture. The solid black line depicts the best-fit 4-parameter non-linear model, and the dotted lines depict the 95% confidence bands. The cell density of the culture on the final day of the experiment (day 8) was estimated to be 1.72 x 10^5^ cells•$mL^{-1}$. See the section "Estimating Cell Density" in the R Markdown script for methodological details.

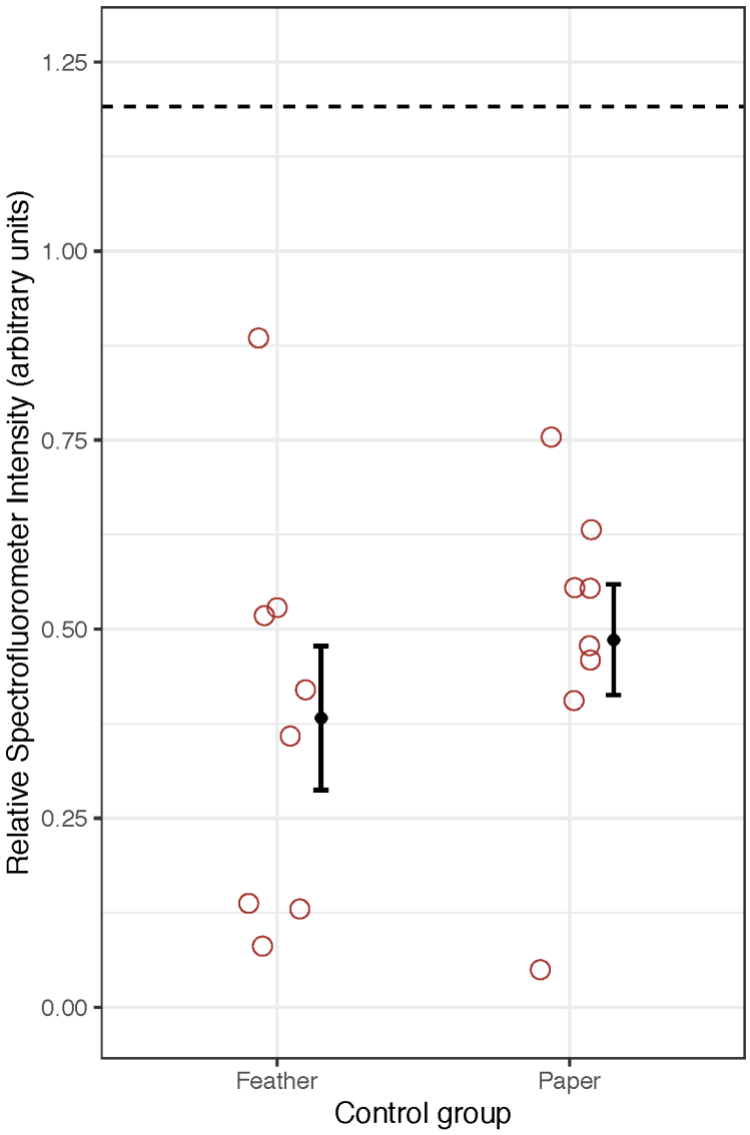



**Figure S5**: Spectrofluorometer readings for the feather and paper control groups (n = 8 per group). Solid black dots represent groups means, and bars extend to +/- one standard error. The horizontal dashed line depicts the limit of detection (LOD), calculated as the mean intensity from the Feather control group + 3 standard deviations.

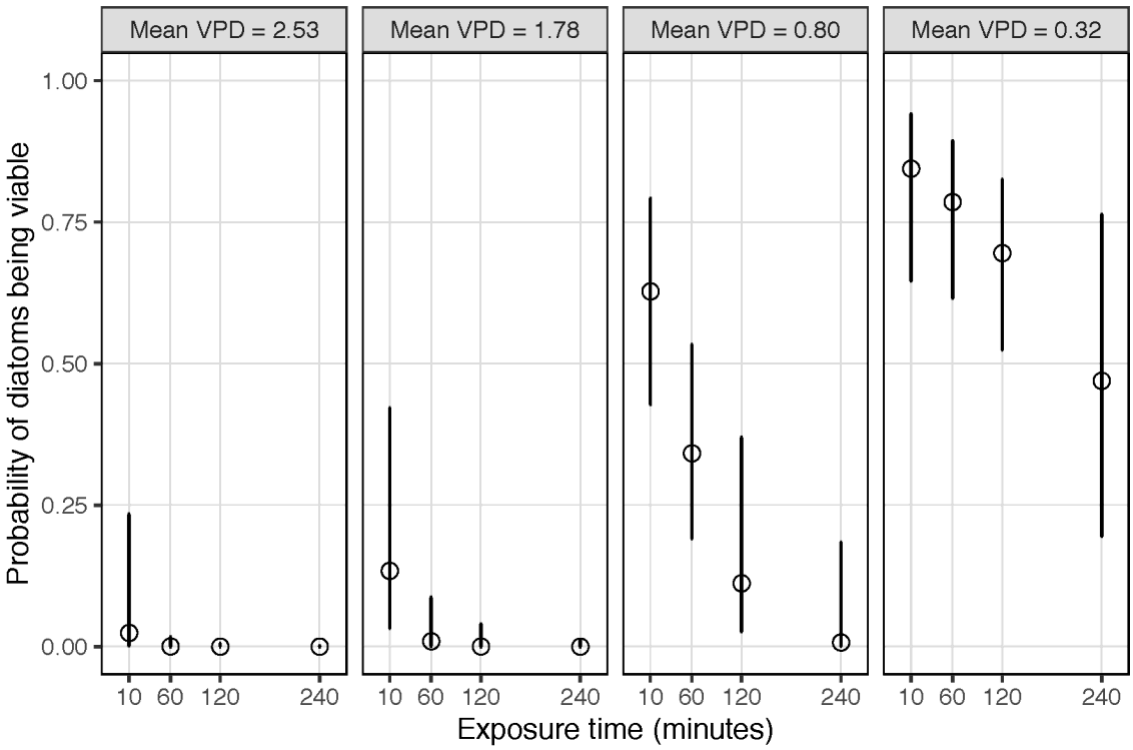
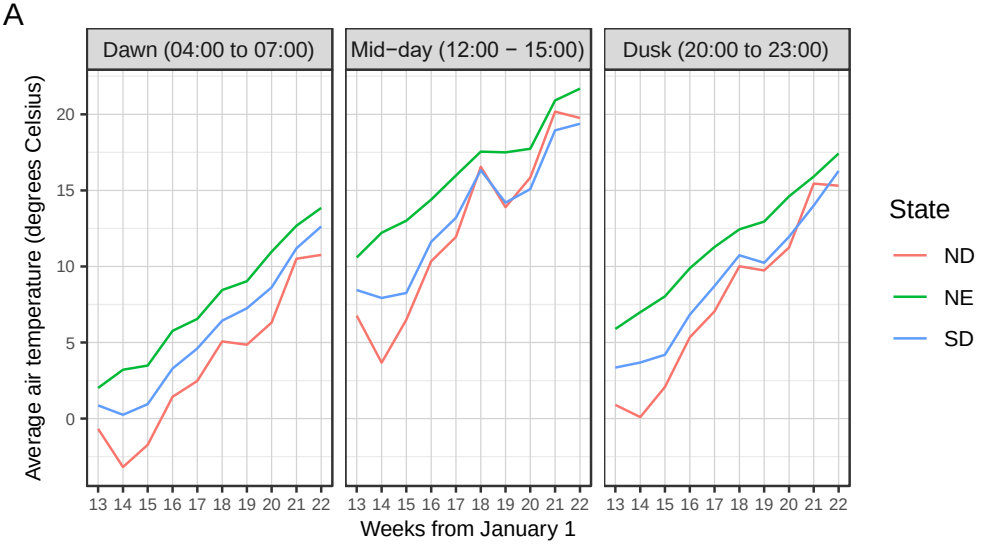
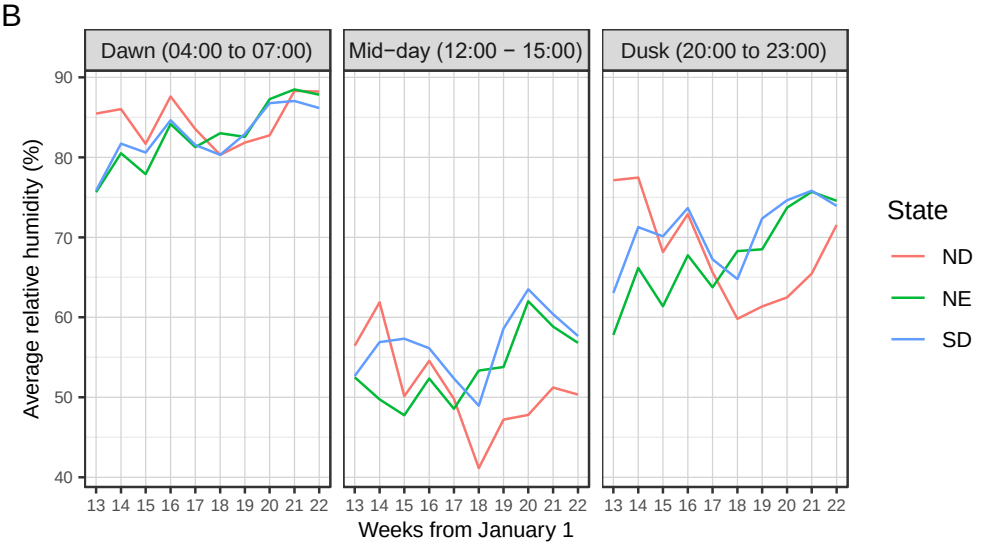



**Figure S6.** Predicted probability (circles) of *Nitzschia pusilla* Grunow diatoms being viable as a function of vapour pressure deficit and exposure time. Bars indicate 95% confidence intervals. Panels display results grouped by vapour pressure deficit (from highest to lowest, left to right), and panel labels show the mean vapour pressure deficit (calculated using measured relative humidity and temperature) across replicates of the corresponding group. All experimental trials occurred at temperatures between 22.7 and 22.9 °C. Predictions are based on a GLM with a binomial link, and use marginal responses, the default approach in the R package ggeffects (Lüdecke, 2018).

A


Dawn (04:00 to 07:00)
Mid−day (12:00 − 15:00)
Dusk (20:00 to 23:00)
Average air temperature (degrees Celsius)
Weeks from January 1
State
ND
NE
SD

B


Dawn (04:00 to 07:00)
Mid−day (12:00 − 15:00)
Dusk (20:00 to 23:00)
Average relative humidity (%)
Weeks from January 1
State
ND
NE
SD

C

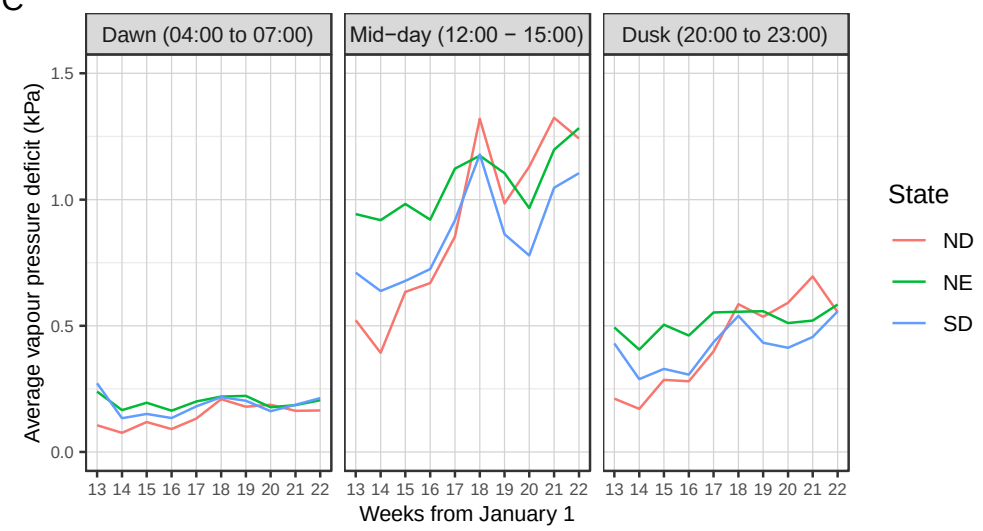

Dawn (04:00 to 07:00)
Mid−day (12:00 − 15:00)
Dusk (20:00 to 23:00)
Average vapour pressure deficit (kPa)
Weeks from January 1
State
ND
NE
SD

**Figure S7**: Average values of (A) air temperature, (B) relative humidity, and (C) vapour pressure deficit for the three states in the study region, during the months of April and May, averaged over the six years spanning 2015 to 2020. Averages were calculated using data from 35, 21, and 40 ASOS station locations across North Dakota (ND), South Dakota (SD), and Nebraska (NE), respectively, and from three distinct time periods daily (panels left to right). Week 13 overlaps with the first week of April, and week 18 overlaps with the first week of May, which is used for generating geospatial predictions (Fig. 4).

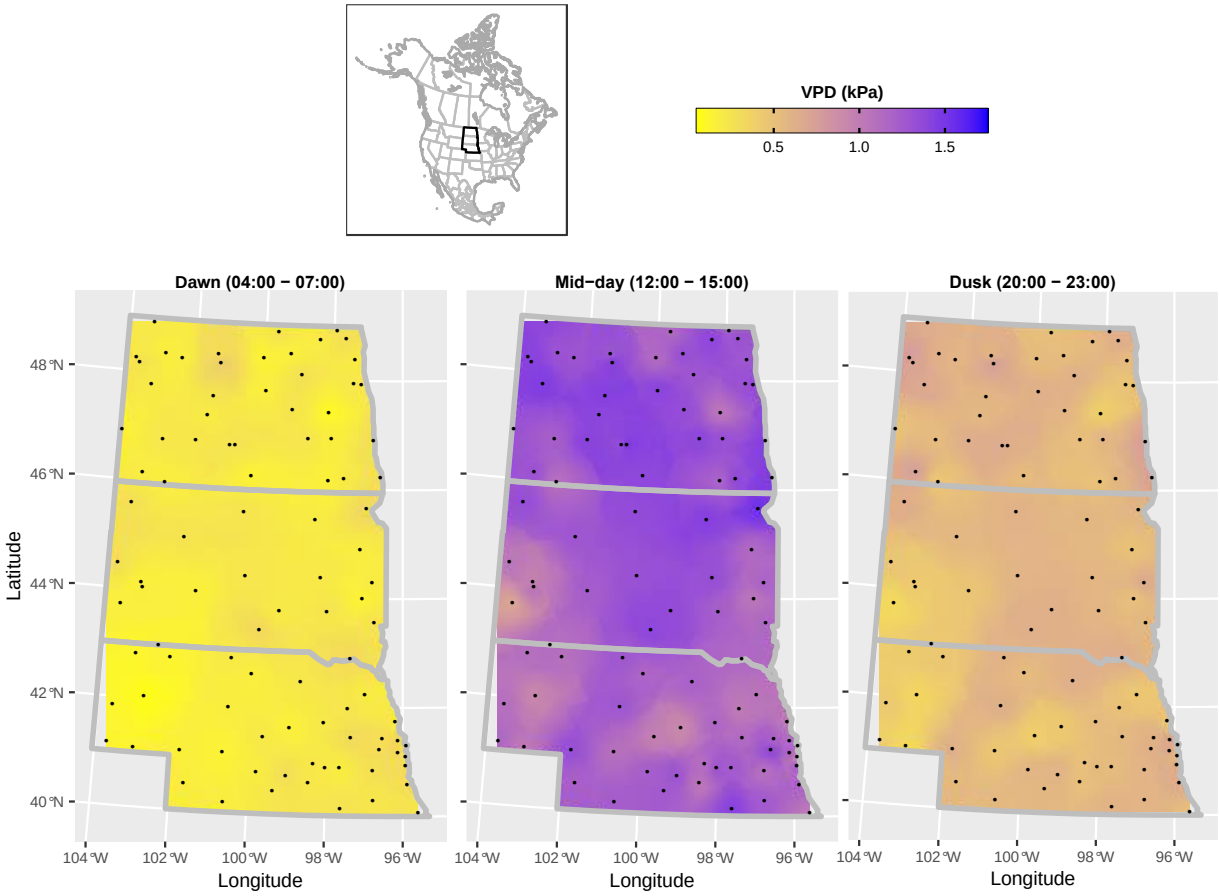



**Figure S8.** Inverse-distance weighted interpolations of vapour pressure deficit (VPD) based on relative humidity and air temperature readings from ninety-eight ASOS stations (black dots) for the first week of May, averaged over the years 2015 through 2020, and for three different times of day (dawn, mid-day, and dusk). In the three map panels the projection is North American Equidistant Conic. The map at the top shows the study region outlined in black within North America (North American Lambert Conformal Conic projection).

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