1 **Potential long-distance dispersal of freshwater diatoms adhering to waterfowl**
2 **plumage**
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4 **Faye S. Manning[1,2], P. Jeff Curtis[3], Ian R. Walker[1,2,3], and Jason Pither[1,2,3]**

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6 [1]Okanagan Institute for Biodiversity, Resilience, and Ecosystem Services (BRAES), University

7 of British Columbia, Okanagan Campus, Kelowna, British Columbia, Canada, V1V 1V7.

8 [2]Department of Biology, University of British Columbia, Okanagan Campus, Kelowna, British

9 Columbia, Canada, V1V 1V7.

10 [3]Department of Earth, Environmental, and Geographic Sciences, University of British Columbia,

11 Okanagan Campus, Kelowna, British Columbia, Canada, V1V 1V7.

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14 Corresponding Author:

15 Jason Pither

16 Email address: jason.pither@ubc.ca

17 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. We dipped individual breast feathers from mallards (*Anas platyrhynchos*) in a pure culture of the freshwater diatom *Nitzschia pusilla* Grunow, then, at room temperature (22.7 °C), 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. We then gently placed the feather on sterile growth medium. After two weeks we used spectrofluorometry to detect diatom growth and thus diatom viability.

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).

4. 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. Using published data about (i) mallard flight speeds, (ii) the geographic distribution of surface waters and of *N. pusilla*, and (iii) vapour pressure deficit (VPD; calculated using RH and air temperature) during the months of April and May, our

43   geospatial model predicted high probabilities of potential dispersal, over tens to hundreds of

44   kilometres, among water bodies of the central migration flyway.

45   5. Taken together, the results of our experiment and geospatial models provide novel insights

46   into ectozoochory of freshwater diatoms, specifically that long-distance dispersal of diatoms via

47   adherence to waterfowl feathers is highly plausible, particularly during the near-dawn hours

48   when waterfowl flight activity peaks and VPD is low. Considered alongside previous evidence

49   suggesting successful internal transport by waterfowl, we conclude that for freshwater diatoms

50   ectozoochory is likely commonplace among waterbodies frequented by waterfowl.

51

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

75   captured on Lake Windermere, and successfully cultured several diatom taxa including species

76   of *Melosira* and *Fragilaria*. Using samples from the esophagus and colons of gulls collected in

77   Texas and North Carolina, Sides (1973) successfully cultured a variety of diatom taxa including

78   species of *Fragilaria*, *Navicula*, and several species of *Nitzschia*. However, these authors did not

79   know the length of time the diatoms had been present in the sampled birds.

80   The degree to which diatoms successfully disperse via ectozoochory (external transport)

81   by waterbirds remains an open question. To be successful, the diatom propagules would need to

82   (i) come into contact with the bird, (ii) adhere or attach to the bird, (iii) survive and remain

83   attached during transport, (iv) detach in the new habitat, and (v) successfully colonize and persist

84   in the new habitat (Coughlan *et al.*, 2017). Diatoms have been observed on waterbird plumage

85   (e.g. Schlichting, 1960; Kristiansen, 1996; Figuerola & Green, 2002), but we are unaware of any

86   quantitative data about diatom survival and viability following ectozoochory. A key limiting

87   step is surviving exposure to desiccation (Kristiansen, 1996; Coughlan *et al.*, 2018), which is

88   governed by flight time and the humidity experienced by the propagules during transport.

89   Souffreau *et al.* (2010, 2013) experimentally tested the tolerance of 69 strains (34 species) of

90   diatom to desiccation, and found only five to exhibit some tolerance to desiccation. Combined

91   with their findings of limited tolerance to freezing and heating, the authors concluded that the

92   physiological sensitivities of vegetative diatom cells to harsh conditions are likely to severely

93   limit dispersal capacity. However, desiccation risk could be lessened if sufficiently high

94   humidity is maintained around the diatom cells during transport, as might be the case for cells

95   buried within waterfowl plumage, which has strong insulating properties (Coughlan *et al.* 2015).

96   Considering that waterfowl such as mallards (*Anas platyrhynchos*) fly at 60-70 km•h^{-1} (McDuie

97   *et al.*, 2019), diatoms that survive transport could conceivably cover considerable distances in

98   short periods of time. For reference, we use the phrase "long distance dispersal" to simply refer

99   to dispersal among disjunct waterbodies separated by tens to hundreds of kilometres.

100   Here, we present the results of a novel experiment designed to partially emulate diatom

101   dispersal via adherence to waterfowl feathers. We used breast feathers from mallards, a species

102   that is common throughout temperate regions of the world (Wetlands International, 2021), with

103   numbers in North America estimated near 10 million (U.S. Fish & Wildlife Service, 2019). The

104   mallard is an omnivorous, widely dispersed migratory species that frequents a broad range of

105   aquatic habitats (Wetlands International, 2021), and thus has the capacity to transport diatoms to

106   a broad range of environments. As waterfowl such as mallards forage in productive littoral

107   zones, it is reasonable to expect their plumage – especially breast plumage – comes into contact

108   with large numbers of benthic, epiphytic and epipelic diatoms, which can reach very high

109   densities in favourable conditions (Patrick, 1977; Wehr & Sheath, 2004). Barbed feathers

110   provide relatively large surface area for potential adherence, especially for micro-algae such as

111   diatoms. Our experiment mimicked this encounter process by dragging individual breast

112   feathers through solution droplets containing relatively high densities of the benthic diatom

113   *Nitzschia pusilla* Grunow. We examined how relative humidity (RH) and exposure time

114   individually and interactively affected the viability of the diatoms adhered to feathers. We

115   predicted that viability would decrease with decreasing RH and increasing exposure time. To

116   place our experimental results in real-world contexts, we combined them with data about mallard

117   flight speeds, and geospatial data describing waterbody distribution and vapour pressure deficit

118   (VPD) (based on RH and air temperature) within the states of North Dakota, South Dakota, and

119   Nebraska. These states are situated in the central migration flyway of North America, and of the

120   four major north-south flyways in North America (see map at

121   https://www.fws.gov/birds/management/flyways.php), the central flyway hosts the largest

122   numbers of mallards (U.S. Fish & Wildlife Service, 2019).

123   **Materials & Methods**

124   *Diatom culture*

125   We obtained a pure culture of a strain of *Nitzschia sp.* (CPCC 499) from the Canadian

126   Phycological Culture Centre (Waterloo, Ontario). Among the genera available from the centre,

127   we chose *Nitzschia* because it is a relatively common, primarily benthic genus found throughout

128   inland waters in North America (Potapova & Charles, 2002), and we chose this particular strain

129   because it is readily maintained in culture. According to the centre, the original material for

130   strain CPCC 499 was sourced in 1999 from an effluent pond at St. Mary's River pulp and paper

131   mill in Sault Ste. Marie, Ontario, Canada. Upon completion of our study, this strain was

132   identified to be *Nitzschia pusilla* Grunow (pers. comm. Kathryn Thomas, Stillwater

133   Environmental) using standard taxonomic references (Krammer & Lange-Bertalot, 1988; Cox,

134   1996) and morphological assessments of preserved and live material under 1000x magnification

135   (Supporting Information, Fig. S1).

136   Throughout the duration of our study, we grew and maintained the diatom culture in its

137   exponential growth phase at approximately 20-23° C and 21-24 μmol m^{-2} s^{-1} using serial

138   dilutions in 125mL Erlenmeyer flasks capped with tinfoil.

139   *Feathers*

140   We collected mallard feathers under Environment Canada Scientific Permit No. BC-18-

141   0005 and adhered to the *Migratory Birds Convention Act*, (Government of Canada, 1994). In

142   response to a request communicated by a local wildlife biologist / waterfowl hunter, waterfowl

143   hunters in the Okanagan Valley region of southern British Columbia, Canada, donated mallard

144   skin patches from the breast/abdominal section (i.e., the section typically immersed when the

145   duck is in water) with the feathers attached. We provided hunters a video depicting the skinning

146   method alongside written instructions for reference (video available at https://osf.io/ujnw2/), to

147   standardize the collections as much as possible. Each bird yielded a single feather patch. Once

148   removed, the patch was placed in a Ziploc® bag, labelled with the sex, collection date, site, and

149   hunting context (over water or field) and stored in a freezer at -18°C as soon as possible. We

150   obtained a total of twelve suitable mallard feather patches (six male and six female). By the time

151   the experiment began, the patches had been stored in the freezer for at least 38 weeks. Any

152   diatoms that may have been on the feathers would have been killed by the prolonged freezing

153   (cf. Souffreau *et al.*, 2010, 2013). This was confirmed by the results of control trials (see below).

154   Using latex gloves we plucked fifteen feathers haphazardly from different parts of each feather

155   patch and trimmed at the shaft to remove the downy portion of the feather to isolate the part of

156   the feather that is normally exposed. Feathers from this pile were then randomly selected for

157   each experimental run.

158   *Experimental apparatus*

159   We constructed four apparatuses in total, and a single experimental apparatus consisted

160   of the following: one plastic filter holder that served as the makeshift airflow chamber

161   (henceforth "chamber") within which the feather was placed; three lengths of inline tubing; one

162   250mL Erlenmeyer flask filled with a glycerol and water solution; a WhirlPak® bag, and a

163   hygrometer (Supporting Information, Fig. S2). The first length of inline tubing connected the

164   benchtop air valve to the Erlenmeyer flask, which in turn was connected to the top of the

165   chamber with the second length of tubing. The third and final length of tubing was used to direct

166   outflow air from the bottom of the chamber into the WhirlPak® bag, in which the HOBO 12-bit

167   smart sensor (model S-THB-M002; Onset Computer Corporation, Bourne, Massachusetts, USA)

168   simultaneously measured RH and temperature. The bag was twist-tied shut around the tubing.

169   We connected the sensor to a Decagon Em50® Series Data Collection System Data Logger

170   (METER Group Inc., Pullman, Washington, USA), and took RH and temperature readings every

171   minute. We assume that the RH and temperature measured in the WhirlPak® bag reliably

172   estimated the RH and temperature in the chamber proper. We also monitored the RH and

173   temperature of the room using another identical sensor, attached to the same datalogger.

174   The RH within the chamber was manipulated using glycerol-water solutions.

175   Specifically, increasing the glycerol:water ratio in the flask reduces the equilibrium relative

176   humidity in the air within the sealed chamber, as the glycerol directly affects the water activity of

177   the solution (Forney & Brandl, 1992). The Erlenmeyer flask contained one of four ratios of

178   glycerol:water (approximately 100:0, 90:10, 60:30, or 0:100), which were adjusted as needed to

179   maintain one of four target RH levels within the main chamber: near 0%, 35%, 70%, and near

180   100%, respectively. The mean RH that we achieved for each of the four target RH levels

181   (calculated using 32 samples per group; see below) was 8.1% (± one standard error: 0.35), 35.9

182   (± 0.54), 71.1 (± 0.35), and 88.4 (± 0.44), and the corresponding mean temperatures were 22.7 (±

183   0.04), 22.8 (± 0.04), 22.9 (± 0.04), and 22.7 (± 0.04) °C (Supporting Information, Fig. S3). For

184   our statistical analyses (below) we used the average RH value calculated using the 1-minute

185   interval readings taken during the given experimental run.

186   The air valves were turned on 30 minutes before the start of each experimental run. We

187   did not have the means to directly measure the rate of airflow inside the chamber, but we strove

188   to ensure airflow rate was consistent across experimental runs: the airflow valve was opened just

189   enough so air could be felt moving through the system and would ruffle the feather slightly. We

190   were not attempting to mimic the rapid airflow that outermost feathers might experience during

191   flight. Rather, we envision this small rate of airflow simulating the conditions that might be

192   experienced by subsurface plumage (*sensu* Coughlan *et al.* 2015).

193   *Experimental procedure*

194   The procedure for a single experimental run consisted of the following steps: a 1mL

195   droplet of culture was pipetted into the centre of a petri dish (60mm diameter x 15mm depth).

196   Using forceps, each side of a single feather was dragged through the droplet, ensuring that the

197   entire surface of the feather made contact. We verified in preliminary trials that this procedure

198   resulted in diatoms readily adhering to feather barbs, as seen in Figure 1. The feather was then

199   placed in the chamber with the base of the feather shaft compressed in the seal, to hold the

200   feather in place. The chamber containing the feather was maintained near the desired RH for the

201   desired time. The feather was then transferred to a new petri dish containing 7mL of sterile

202   growth medium (enough to cover the bottom of the dish). The feather was gently placed on the

203   liquid medium, but not submerged or pressed downward. The dish was maintained under the

204   same growing conditions as the pure culture for fourteen days (determined during preliminary

205   trials as sufficient time for growth).   The medium was then assessed for evidence of diatom

206   growth using spectrofluorometry (see below). Pre-experiment trials in which inoculated feathers

207   were directly transferred to the growth medium confirmed that this procedure resulted in

208   successful establishment of diatoms.

209   During the procedure the RH occasionally varied beyond desired ranges immediately

210   following sample changeovers. We ignored fluctuations to a higher humidity immediately

211   following sample changeovers, regardless of how long the higher humidity persisted, as the

212   sensor was likely detecting the water evaporating off the feather as it exited the system. When

213   the RH varied beyond the desired range at any other point during the procedure, the sample was

214   discarded, and the procedure started over.

215   We used eight replicates (feathers) for each combination of target RH (four levels) and

216   exposure time (four times), for a total of N = 128. We conducted the experiment over two four-

217   day intervals, separated by one day. We completed one replicate from each unique treatment

218   (sixteen unique combinations of target RH level and exposure time) each day, and we

219   randomized the order of treatments within each day. We also randomly assigned the target

220   humidity levels to the four chambers. At the end of each experimental day, we replaced the

221   volume drawn from the culture with an equal volume of sterile media. This ultimately diluted

222   the culture over the course of the experiment, but concentrations remained very high (> 1.7 x 10^5

223   cells•mL^{-1}) and were thus suitable for ensuring feather inoculation (Supporting Information, Fig.

224   S4).

225   We conducted all assessments of diatom growth using an RF-1501 Shimadzu

226   spectrofluorophotometer (Shimadzu Corp., Japan), which yields a fluorometer intensity reading

227   as an indicator of cell concentration. We set the excitation and emittance wavelengths for the *in*

228   *vivo* fluorometry to 530nm and 680nm, respectively, to target chlorophyll a and fucoxanthin

229   (Vincent, 1983; Watras & Baker, 1988; Beutler *et al.*, 2002), with gap length set to 10nm (5nm

230   per side). Fucoxanthin is unique to brown algae including diatoms (Beutler *et al.*, 2002), and

231   thus our estimates of cell density are unlikely to be affected by any other microbial organisms, if

232   present. We equated evidence of diatom growth with the survival of viable cells. To ensure the

233   reliability of the measurement, we first established the limit of detection (LOD) for our

234   spectrofluorometer intensity readings, as described below.

Experimental controls and Limit of Detection

236   On each of the eight days, we ran one control replicate in which a feather was dipped in

237   sterile growth medium instead of diatom culture, placed in the chamber as described above, then

238   removed after two seconds (thus it did not undergo RH and exposure time treatments), and

239   subsequently placed in a petri dish with growth medium. The spectrofluorometer intensity

240   readings obtained after fourteen days from these eight controls served to establish our LOD. We

241   additionally ran eight controls for which we used a $2cm^2$ square of filter paper instead of feathers

242   as the experimental unit. The spectrofluorometer intensity readings for the feather and paper

243   controls were statistically indistinguishable (Supporting Information, Fig. S5). We are thus

244   confident that the feathers used in our experiment were not pre-contaminated with organisms

245   producing fucoxanthin or chlorophyll. We equated the LOD with the mean intensity reading

246   from the feather controls plus three times the standard deviation of the readings (Shrivastava &

247   Gupta, 2011; Choo *et al.*, 2018).

248   *Statistical analyses*

249   For our experiment, our response variable of interest was binary: viable or inviable. We

250   equated evidence of diatom growth (i.e. intensity readings > LOD) after fourteen days with

251   evidence of viability. We analyzed this binary response variable in relation to RH (%) and

252   exposure time (minutes), and their interaction, using a generalized linear model (GLM) with a

253   binomial link (i.e., logistic regression). We coded RH and time as continuous variables. Coding

254   them as ordinal factors yielded qualitatively similar results. We computed the McFadden

255   pseudo-R^2 value (McFadden, 1974), which, for logistic regression, is analogous to the coefficient

256   of determination for general linear models.

257   *Extrapolating our experimental results to real landscapes*

258   We conducted our experiment at a near-constant air temperature of 22.8ºC, which

259   simplified the interpretation of our RH treatments. In the real world, air temperature varies

260   spatially and temporally, and VPD is a more meaningful measure of desiccation potential

261   because it provides an absolute measure of the atmospheric moisture state independent of

262   temperature (Anderson, 1936). VPD is the difference between the actual moisture content of the

263   air and the saturation point of the air at a given temperature, and it varies as a function of RH and

264   air temperature. Desiccation potential increases with increasing VPD (Anderson, 1936). For

265   example, our experimental treatment of 35% RH at 22.8ºC yields a VPD value of 1.81kPA, but

266   at an air temperature of 10ºC, the same RH yields a VPD value of 0.80kPa. Thus, despite RH

267   being the same, desiccation would occur much faster at 22.8ºC compared to 10ºC. To facilitate

268   extrapolation of our results to the real world, we therefore re-analyzed our experiment data using

269   VPD as a predictor in lieu of RH within the GLM. Specifically, we used the default arguments

270   of the `rH.to.VPD` function within the `bigleaf` R package (Knauer *et al.*, 2018) to estimate

271   VPD in the experimental chambers, using the measured RH and temperature. The average VPD

272   values corresponding to the four RH treatments (from low to high RH), were 2.53 ($\pm$ 0.01), 1.78

273   ($\pm$ 0.02), 0.80 ($\pm$ 0.01), and 0.32 ($\pm$ 0.01) (Supporting Information, Fig. S3c). The results of this

274   analysis are provided in Table S1 and Fig. S6 in the Supporting Information.

275   We extrapolated our results to North Dakota, South Dakota, and Nebraska, and gathered

276   *in situ* VPD data for the period between April 1 and May 31, which corresponds with high

277   frequencies of mallard occurrences in the region (eBird, 2020), and also diatom growth. These

278   three states are located along the central flyway for waterfowl migration, and host the Prairie

279   Potholes region, a crucial breeding ground for North American waterfowl including mallards

280   (U.S. Fish & Wildlife Service, 2019). We could not extend our geospatial analyses north into

281   Canada due to a lack of data. As we did not manipulate temperature in our experiment, our

282   geographical extrapolations should be interpreted with caution.

283   We first used our GLM in combination with the average flight speed of 69 km•h^{-1} for

284   mallards (the middle value between average breeding time flight speeds and migration flight

285   speeds; McDuie *et al.*, 2019) to estimate the probability of remaining viable across a range of

286   distances (with corresponding flight times) and VPD values typical of the study region and time

287   periods (0.25 to 1.25kPa; Supporting Information, Fig. S7). We present these predictions

288   alongside (i) data about within-day dispersal distances of mallards, based on banding

289   observations (Viana *et al.*, 2013a, 2013b), and (ii) distances between each surface water body (>

290   0.1 km^2 area) in the study region and its nearest neighbour water body, calculated using the

291   Global Lakes and Wetlands Database (GLWD), downloaded from the World Wildlife Fund.

292   Finally, we generated a map of probability of potential dispersal among the region's water

293   bodies, using *N. pusilla* Grunow as the focal diatom taxon. To do this, we used our GLM in

294   combination with (i) the average flight speed of mallards (as above), (ii) spatially interpolated

295   VPD for the region, calculated using RH and air temperature data from the ASOS Network

296   online database, and based on the average measurements for three times of day: near dawn

297   (04:00 and 07:00), mid-day (12:00 to 15:00), and dusk (20:00 to 23:00) during the first week of

298   May, (iii) estimated flight times between surface water bodies (using distances from the GLWD

299   data), and (iv) data about the distribution of *Nitzschia* taxa, including *N. pusilla*, acquired from

300   the 2007 and 2012 National Lakes Assessment. All data sources are detailed in the Data

301   Availability Statement. We included the dawn and dusk VPD values because the daily flight

302   activity of ducks typically peaks just before dawn and just after dusk (Bengtsson *et al.*, 2014;

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303   Kleyheeg *et al.*, 2017). We included mid-day VPD values for comparison. We used lakes

304   hosting *N. pusilla* Grunow as the focal "source" lakes for diatoms, and generated a raster layer of

305   probability of potential dispersal based on estimated flight times (based on distances and flight

306   speed) from these source lakes, combined with the spatially-interpolated VPD values. Then for

307   each waterbody hosting diatoms of the *Nitzschia* genus, we extracted the probability of potential

308   dispersal values from the resulting raster layer. This procedure (i) equates probability of

309   potential dispersal with the probability of diatoms remaining viable after the given exposure time

310   and VPD (based on spatially interpolated VPD), and (ii) makes the simplifying assumption that

311   waterbodies hosting the genus *Nitzschia* are suitable for colonization by *N. pusilla* Grunow (see

312   Discussion). Additional details of these methods and of our results are provided in the fully

313   annotated R Markdown script available online (https://osf.io/ujnw2/).

314   *Ensuring computational reproducibility*

315   All our analyses were performed using R version 3.6.3 (R Core Team, 2019) within the

316   RStudio IDE (RStudio Team, 2019). To ensure computational reproducibility we used the `renv`

317   package (Ushey, 2020) that manages R package versions, and we generated an annotated R

318   Markdown script that, along with all data, are freely available for download from the Open

319   Science Framework (https://osf.io/ujnw2). We used numerous R packages within our analyses,

320   and these are detailed in the Supporting Information. We encourage readers to contact the

321   corresponding author (JP) if any part of our study is unclear and / or irreproducible.

322   **Results**

323   The logistic regression from our experiment yielded a McFadden pseudo-R^2 value of

324   0.51, indicating a significant amount of the deviance was accounted for by the full model (Table

325   1). Exposure time and RH interacted significantly to affect diatom viability: at high RH, the

326   probability of being viable was moderate to high across all exposure times, including the

327   maximum four-hour period (Fig. 2). For instance, at an average RH value of 88% and an

328   exposure time of four hours, the predicted probability of being viable was 0.45 (95% confidence

329   interval: 0.18 to 0.75), and for a one-hour exposure time it was 0.78 (95% confidence interval:

330   0.61 to 0.89) (Fig. 2). At lower RH, the probability of being viable declined more rapidly with

331   increasing exposure time, and at the lowest RH values (8 and 36%), viability was predicted to be

332   possible (between 0 and 0.42 probability) over only the shortest duration of exposure (10 min)

333   (Fig. 2).

334   Using VPD in lieu of RH in a GLM yielded qualitatively identical outcomes (Table S1),

335   including a significant interaction between VPD and exposure time (Table S1; Fig. S6). Based on

336   this GLM, Figure 3A depicts the predicted probability of remaining viable across a range of

337   travel distances (using an estimated average mallard flight speed of 69 km•h^{-1}) and VPD values

338   that are typical of those observed in the study region during April and May (Fig. S7). Figure 3B

339   shows the frequency distribution of distance travelled by mallards within a single day, and

340   Figure 3C shows the frequency distribution of distance to nearest neighbouring water body

341   among lakes and wetlands (those > 0.1 km^2 area) in the study region. Collectively, these figures

342   suggest strong potential for long-distance diatom dispersal by mallard vectors, especially over

343   tens of kilometres, and given the highly favourable VPD conditions (< 0.5kPa) that are typical of

344   dawn hours during April and May (Fig. S7).

345   The U.S. National Lakes Assessment survey revealed that diatoms of the genus *Nitzschia*

346   were observed in 85 waterbodies in the study region in the years 2007 and 2012, with five of

347   these locations hosting *N. pusilla* Grunow in 2007 (species-level identifications were only

348   available for the 2007 surveys) (Fig. 4). Using these five waterbodies as potential sources of

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349   mallard-borne *N. pusilla* diatoms, Figure 4 shows how the probability of potential dispersal

350   varies geographically in the study region, based on (i) distance from the source lakes, (ii) average

351   mallard flight speed (69 km•h^{-1}), and (iii) VPD interpolated throughout the study region (Fig.

352   S8), using data from the first week of May for illustration, and for three different times of day

353   (dawn, mid-day, and dusk). The maps show considerable potential for dispersal throughout the

354   study region, particularly during dawn hours (04:00 – 07:00) when VPD is ubiquitously low

355   (rarely reaching 0.25kPa; Fig. S8) owing to the cool temperatures and high RH that are typical of

356   spring mornings in the region (Fig. S7). Using data extracted from these prediction maps, Figure

357   5 shows the probability of potential dispersal from the five source lakes into each of the other

358   eighty waterbodies in the region that host *Nitzschia* diatoms. It shows the considerable influence

359   that time of day has on the probability of potential dispersal, owing to pronounced variation in

360   VPD that occurs through the day (Fig. S8). During dawn hours, predicted probabilities are

361   generally greater than 0.75, whereas during mid-day hours, most are less than 0.25, though some

362   lakes exhibit higher probabilities owing to their proximity to source lakes (Fig. 4). At dusk, the

363   geographical pattern is more varied, and the probabilities of potential dispersal are more evenly

364   distributed between zero and 0.8 (Fig. 5).

365   **Discussion**

366   Consistent with our predictions, we found that the viability of diatoms adhered to feathers

367   declined significantly with increasing exposure time and decreasing RH. However, these

368   treatments interacted significantly: exposure time had diminished negative effects under high RH

369   conditions. For example, the predicted probability of remaining viable after four hours of

370   exposure was near zero at 8 and 36% RH, whereas at 88% RH plausible values for the

371   probability of remaining viable ranged from 0.18 to as high as 0.75. Such interactions between

372 RH and exposure duration have previously been observed in desiccation experiments involving

373 aquatic macrophytes (e.g. Coughlan *et al.*, 2018), but to our knowledge, ours are the first such

374 findings pertaining to diatoms. Souffreau *et al.* (2010, 2013) examined desiccation tolerance

375 among an impressively long list of diatom taxa (69 strains), but RH was not manipulated

376 directly, and nor was exposure time examined simultaneously. Rather, solutions of diatom

377 culture within well plates were air-dried for five or ten minutes in separate experiments. A

378 factorial experimental design is key for detecting treatment interactions, and in our case revealed

379 the important interaction between RH and exposure time.

380   Our experiment was also unique in examining desiccation tolerance of diatoms adhered

381 to feathers. Although a number of studies have examined the potential for internal transport of

382 diatoms by waterfowl (reviewed in Green *et al.*, 2016), we are unaware of any that have

383 experimentally evaluated the potential for ectozoochory of diatoms via adherence to feathers.

384 For diatoms to successfully disperse via waterbird-mediated ectozoochory, propagules would

385 need to (i) come into contact with the bird, (ii) adhere or attach to the bird, (iii) survive and

386 remain attached during transport, (iv) detach in the new habitat, and (v) successfully colonize

387 and persist in the new habitat (Coughlan *et al.*, 2017).   Below we discuss our findings with

388 reference to each of these five points.

389 *Contact and adherence of diatoms to waterfowl*

390   As waterfowl such as mallards forage in productive littoral zones, their breast plumage

391 likely comes into contact with numerous benthic, epiphytic, and epipelic diatoms. In our

392 experiment we aimed to emulate this process by dragging individual breast feathers through

393 solution droplets containing relatively high densities of diatom (see Supporting Information

394 Figure S4). We cannot be sure whether using an entire patch of breast feathers as the

395   experimental unit would have affected the adherence process, but it certainly would have

396   provided more surface area of feather barbs for the diatoms to adhere to. While designing the

397   experiment we tested the procedure of dragging a single feather through a droplet of diatom

398   solution, and each time large numbers of diatoms readily adhered to the feather barbs (as seen in

399   Figure 1). We speculate, therefore, that in productive littoral zones, large numbers of diatom

400   may adhere to mallard breast plumage. If correct, dispersal success is unlikely to be limited by

401   the number of diatoms that successfully adhere to mallard feathers, especially when one

402   considers the vast numbers of diatoms and mallards involved – almost 10 million mallards in

403   North America alone, (U.S. Fish & Wildlife Service, 2019).

404   The apparent ease with which diatoms were observed to adhere to feathers could be due

405   in part to properties of diatom frustules. First, diatom frustules contain polysaccharides

406   (Gélabert *et al.*, 2004; Le Costaouëc *et al.*, 2017) that are typically attracted to hydrophobic

407   substances, potentially including the so-called "preening oil" secreted by waterbirds from the

408   uropygial gland (Bakken *et al.*, 2006), and that has been found to contain chemical derivatives

409   found in waxes (wax esters) (Stenhagen & Odham, 1971). Second, the polysaccharides in

410   diatom frustules can reduce the surface tension of water (Ozkan & Berberoglu, 2013), which

411   could further facilitate contact between diatoms and feathers. Third, *Nitzschia pusilla* Grunow is

412   a comparatively small diatom, with reference material suggesting lengths between 8 and 33 μm,

413   and widths of 2.5 to 5 μm (Krammer & Lange-Bertalot, 1988; Cox, 1996). We speculate that the

414   small size of *N. pusilla* Grunow may facilitate adherence to feather barbs. Specifically, we

415   propose that its small size enables it to better "fit" within the barb structure (see Fig. 1), and

416   promotes interaction over a greater surface area (for a given volume) between the

417   polysaccharides on the diatom frustules and the hydrophobic substance in waterfowl plumage

418   (see above). Future experiments could test these ideas by using diatoms of contrasting body size,

419   and by using feathers that have and have not been washed of its hydrophobic substance. More

420   generally, body size has long been linked to dispersal capacity among microorganisms (Finlay,

421   2002): smaller body size is associated with larger population size, which will promote dispersal

422   capacity, and with respect to microbes, smaller organisms are more efficiently dispersed.

423   *Survival and adherence during transport*

424   We did not evaluate the extent to which diatoms adhered to feathers might dislodge

425   during flight, and nor did we attempt to mimic the potentially strong wind shear that might be

426   experienced by diatoms adhered to the outermost feathers on a bird. Rather, in our experiment,

427   we exposed the feathers to a light breeze that may be more representative of conditions

428   experienced by diatoms adhering to subsurface plumage, though this remains speculative. Our

429   experimental design could readily be modified to test the effects of wind speed and angle on

430   propagule dislodgement and viability, using a wind-tunnel design, for example.

431   For any aquatic plants successfully adhering to feathers for the duration of flight,

432   desiccation is thought to be the key limiting process (Green *et al.*, 2016; Coughlan *et al.*, 2017).

433   In the only other studies we are aware of that examined diatom tolerance to desiccation,

434   Souffreau *et al.* (2010, 2013) showed that diatoms are broadly intolerant of this form of stress.

435   Our findings are generally consistent with this: we directly manipulated relative humidity at

436   room temperature and found a dramatic decrease in viability with decreasing relative humidity

437   (Figure 2) and corresponding vapour pressure deficits (Fig. S6). However, we did not examine

438   the physiological status of the diatoms in our experiment, so cannot be certain that inviability

439   was due to desiccation per se. Importantly, we found that under levels of vapour pressure deficit

440   that are representative of the favourable springtime, near-dawn conditions in the central flyway

20

441   of North America (e.g. < 0.25kPa; Supporting Information Fig. S7 and Fig. S8), diatoms

442   remained viable after prolonged periods adhered to feathers, including 4 hours at a vapour

443   pressure deficit of 0.33kPa. It should be noted that our experiment examined a single

444   temperature regime (between 22 and 23ºC; Fig. S3), and we do not know how diatom viability

445   might respond to the much cooler air temperatures typical of springtime dawns in the region. On

446   one hand, we suspect that air temperatures between 0 and 10ºC are more favourable to diatom

447   viability, as cooler temperatures yield lower vapour pressure deficit (all else being equal), and

448   diatoms are well adapted to cold aquatic environments (Wehr & Sheath, 2004). On the other

449   hand, waterfowl plumage includes microclimates that are buffered to some extent from ambient

450   conditions (Coughlan *et al.*, 2015), and may include warm pockets proximate to the skin. The

451   plumage can also maintain a humid microclimate (Coughlan *et al.*, 2015). Thus, the conditions

452   experienced by the diatoms in our experiment may have been more harsh than would be

453   experienced in transit within duck plumage.

454   It is important to note that the strain of diatom that we used in our experiment was chosen

455   specifically because it is easily maintained in culture. It may therefore be hardier and better

456   adapted to surviving ectozoochory than most diatom taxa. For instance, among the 69 strains (34

457   species) of diatom examined by Souffreau *et al.* (2010, 2013), only 5 exhibited some tolerance to

458   their desiccation treatment, including two strains of *Pinnularia borealis*, one strain of *Hantzschia*

459   *amphioxys*, and two strains of *Navicula radiosa*. It would be beneficial to explore the tolerances

460   of more taxa using our experimental design.

461   Flight duration is another factor that will influence the number of diatoms that remain

462   attached and survive transport. Our study explored this in three ways. In our experiment we

463   directly manipulated exposure time and found that increasing it from 10 minutes to 4 hours

464 decreased viability, but only moderately so under high relative humidity (Fig. 2) and

465 correspondingly low vapour pressure deficit (Fig. S6). Then, assuming an average flight speed

466 of 69 km•h^{-1} (McDuie *et al.*, 2019), we used the statistical model from our experimental results

467 to make spatially implicit predictions of the probability of remaining viable across a range of

468 vapour pressure deficits and flight distances. For example, with values of vapour pressure deficit

469 ≤ 0.5kPa, which are representative of springtime dawn and dusk conditions (Fig. S7) in the

470 central flyway, our model predicts that diatoms would remain viable over 120 km with a

471 probability of almost 0.5, and this increases to 0.7 for a distance of 40 km (Fig. 3A). In many

472 parts of the North American range of mallards these distances more than span the distances

473 among neighbouring water bodies (e.g. Fig. 3C). Nevertheless, these predictions make a number

474 of key assumptions that should be borne in mind. Most importantly, we assume that diatoms can

475 successfully remain adhered to feathers for the duration of the flight, despite the strong airflow

476 that would impact outermost feathers with flight speeds of 69 km•h^{-1}. Below the plumage

477 surface, it is possible that feathers are protected from strong airflow due to the insulating

478 properties of the plumage and its microstructure (Coughlan *et al.*, 2015). In our experiment we

479 exposed the feathers to a light breeze rather than a strong wind. We assume that this could

480 represent the conditions experienced by diatoms adhered to subsurface feathers, but this requires

481 testing.

482   The same assumptions apply to our spatially-explicit predictions of the probability of

483 potential dispersal (Fig. 4), which indicated strong potential for dispersal among the region's

484 waterbodies, particularly during dawn and dusk hours when ducks, including mallards, tend to

485 fly between daytime roosting sites and nighttime foraging sites (Bengtsson *et al.*, 2014;

486 Kleyheeg *et al.*, 2017). Indeed, based on air temperature and relative humidity data gathered

487   from ninety-eight ASOS stations distributed throughout the study region, we found that vapour

488   pressure deficit was quite low ($\leq$ 0.5kPa) and thus favourable in April and May during the hours

489   around dawn (Figs. S7, S8). During mid-day hours (noon to 3pm), higher temperatures combine

490   with lower relative humidity to yield much higher vapour pressure deficits on average (Figs. S7,

491   S8), so feather-borne dispersal during these times are predicted to be much less likely (Figs. 4,

492   5).

493   *Detachment in the new habitat*

494   Diatoms that withstand transport could be dislodged from feathers during landing, by

495   preening, and/or could remain adhered to feathers that themselves become dislodged (Coughlan

496   *et al.*, 2017). Our experiment mimicked the latter scenario in which feathers detach from the

497   bird (due to molting, for example), and rest on the water surface. During early spring and late

498   summer molting seasons, the surfaces of ponds and lakes hosting large numbers of waterfowl are

499   often littered with detached feathers. Although plausible, we do not know how effective this

500   scenario is at enabling diatoms to actually colonize the new habitat. Future experiments could

501   compare alternative dislodgment scenarios, including dragging the feather through sterile media.

502   *Successful colonization*

503   The final step of successful colonization and establishing a local population is clearly

504   dependent on the suitability of local conditions (e.g., water chemistry) for the given diatom

505   taxon. In our experiment we aimed to ensure that diatoms surviving the treatments would not be

506   limited by subsequent growth conditions, and therefore provided algal growth medium as the

507   receiving habitat. The diatom strain we used was sourced from an effluent pond at St. Mary's

508   River pulp and paper mill in Sault Ste. Marie, Ontario, Canada, and was identified using standard

509   morphological techniques to be *Nitzschia pusilla* Grunow. According to algaebase.org (accessed

510   March 10, 2020), this freshwater taxon has been recorded at locations throughout North America

511   and Europe, and in the 2007 National Lakes Assessment it was observed in 23 lakes in 13

512   different states. It occurred in five lakes within our study region (Fig. 4A). Thus, it is not an

513   especially common taxon. However, there is considerable uncertainty surrounding the taxonomy

514   of the genus *Nitzschia* (Rimet *et al.*, 2011), so the available data about the distribution of *N.*

515   *pusilla* should be interpreted with caution. For instance, members of the genus appear to occupy

516   an extremely diverse range of abiotic conditions (Potapova & Charles, 2002), and this can be

517   indicative of a taxonomic group in need of revision. In the case of *N. pusilla*, there is insufficient

518   data upon which to define "suitable habitat". Based on our geospatial predictions, there is

519   considerable potential for dispersal from the five waterbodies that host *N. pusilla* (Fig. 4), but

520   given the small number occurrence records, perhaps "suitable habitat" is comparatively rare in

521   the region.

522   It is also important to note that the available survey and geospatial data (e.g. the lakes

523   and wetlands data used for Figures 3 and 4) may underestimate the distribution and abundance of

524   potential diatom habitat, because they do not include very small and ephemeral waterbodies.

525   Given that the mallards frequents a broad range of aquatic habitats (Wetlands International,

526   2021), it has the capacity to transport diatoms to a broad range of environments. Future research

527   should modify our experimental design to explore multiple species of diatom simultaneously,

528   using receiving solutions with contrasting conditions.

529   *Conclusion*

530   We have provided novel evidence consistent with the idea that adherence to waterfowl

531   feathers is a potentially effective mode of ectozoochory for freshwater diatoms. More generally,

532   our study adds to a growing body of evidence that waterfowl are potentially effective long-

533   distance dispersal vectors for aquatic organisms via both endozoochory and ectozoochory

534   (Figuerola & Green, 2002; Viana *et al.*, 2013c; Tesson *et al.*, 2015; Green *et al.*, 2016; Coughlan

535   *et al.*, 2017; Lovas-Kiss *et al.*, 2018). Considering (i) the vast numbers of waterfowl that migrate

536   annually and visit numerous waterbodies en route, and (ii) the high densities of diatom that many

537   aquatic habitats host, it is possible that the number of diatoms that successfully disperse adhered

538   to waterfowl feathers is extremely large. Nevertheless, the efficacy of this mode of dispersal is

539   likely to vary among diatom taxa, and future research should seek to quantify this variation

540   experimentally.

541

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557   **Data Availability Statement**

558   All data and scripts are freely available for download from the OSF (https://osf.io/ujnw2), and

559   are citable as: Pither and Manning (2020). Data about within-day dispersal distances of mallards

560   are available on Dryad (https://datadryad.org/resource/doi:10.5061/dryad.619gd). The Global

561   Lakes and Wetlands Database (GLWD) is available from the World Wildlife Fund

562   (https://www.worldwildlife.org/pages/global-lakes-and-wetlands-database). The ASOS Network

563   online database is freely accessible

564   (https://mesonet.agron.iastate.edu/request/download.phtml?network=IA_ASOS). Data about the

565   distribution of *Nitzschia* taxa, including *N. pusilla*, are freely accessible within the 2007 and

566   2012 National Lakes Assessment online repository (https://www.epa.gov/national-aquatic-

567   resource-surveys/data-national-aquatic-resource-surveys).

568

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740 **Tables**

741

742 **Table 1.** Logistic regression (generalized linear model with binomial link) of the effects of

743 relative humidity (RH) (%), exposure time (TIME) (minutes), and their interaction (RH × TIME) on

744 the probability of diatoms remaining viable. Shown are the coefficient estimates, lower and

745 upper 95% confidence limits (CL), Z-values, and associated probability values (P-value). The

746 null and residual deviance was 146.1 and 71.5 respectively, on 127 and 124 degrees of freedom

747 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	9.251×10^{-4}	1.427×10^{-4}	20.044×10^{-4}	2.000	0.046

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750 **Figures**
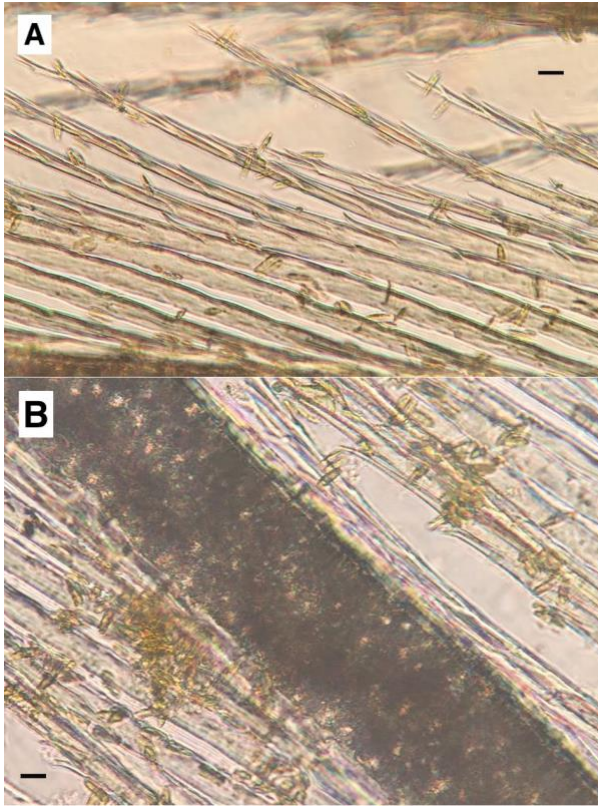
751

752 **Figure 1**. Two views (panels A and B) of *Nitzschia pusilla* Grunow diatoms embedded within a

753 mallard breast feather. The black scale bar in each panel is approximately 10 μm. For reference,

754 individual diatoms examined during identification work were, on average, 13.7 μm long (see

755 Figure S1).

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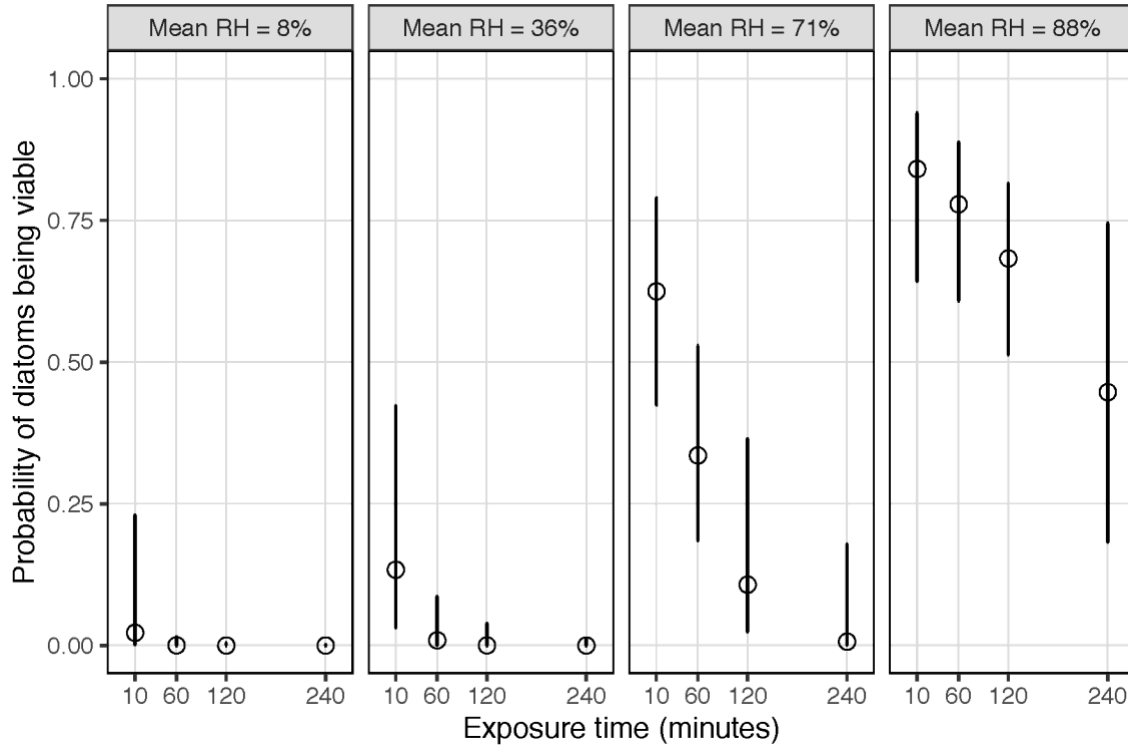
Figure 2. Predicted probability (circles) of *Nitzschia pusilla* Grunow diatoms being viable as a function of relative humidity and exposure time. Bars indicate 95% confidence intervals. Panels display results grouped by target relative humidity (from lowest to highest, left to right), and panel labels 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).

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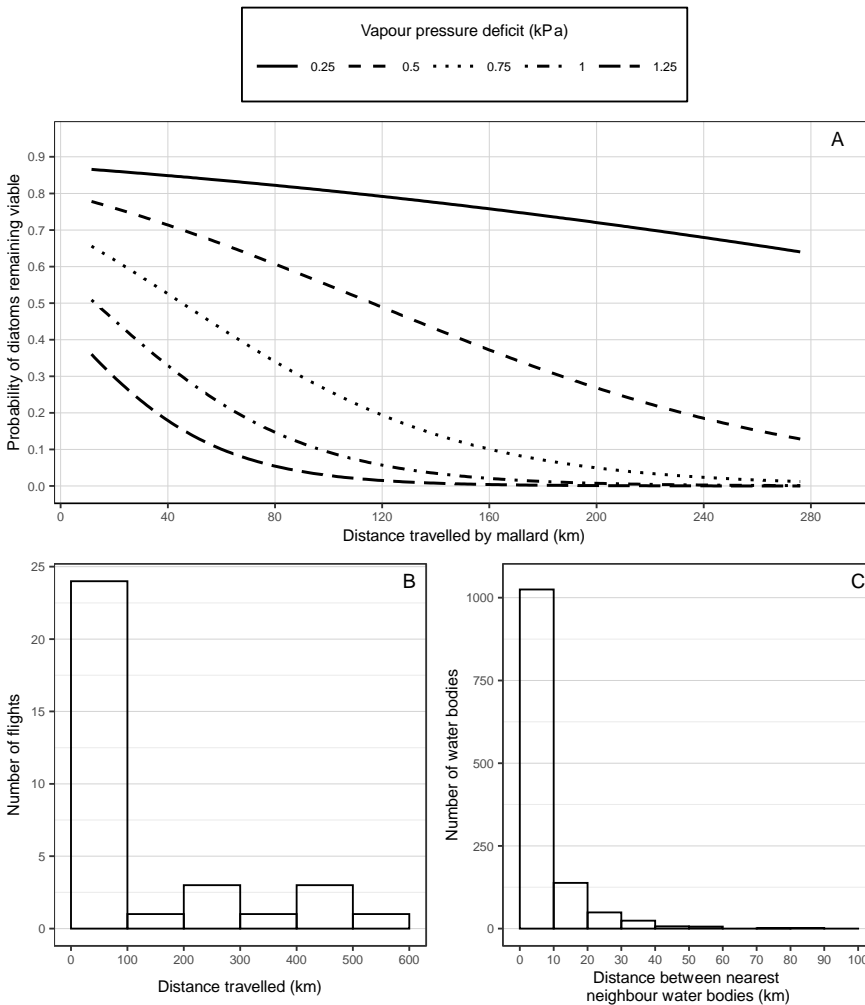
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771 **Figure 3.** Panel (A): Predicted probability of *Nitzschia pusilla* Grunow diatoms remaining

772 viable as a function of vapour pressure deficit and travel distance. Predictions are based on our

773 VPD-based GLM, and assume average flight speed of 69 km•h^{-1}. (B) Histogram of the distance

774 travelled within a single day by each of 33 individual, banded mallards in North America (Viana

775 *et al.*, 2013a, 2013b). (C) Histogram of distances between surface water bodies and their nearest

776 neighbours within North Dakota, South Dakota, and Nebraska (N = 1252 water bodies).

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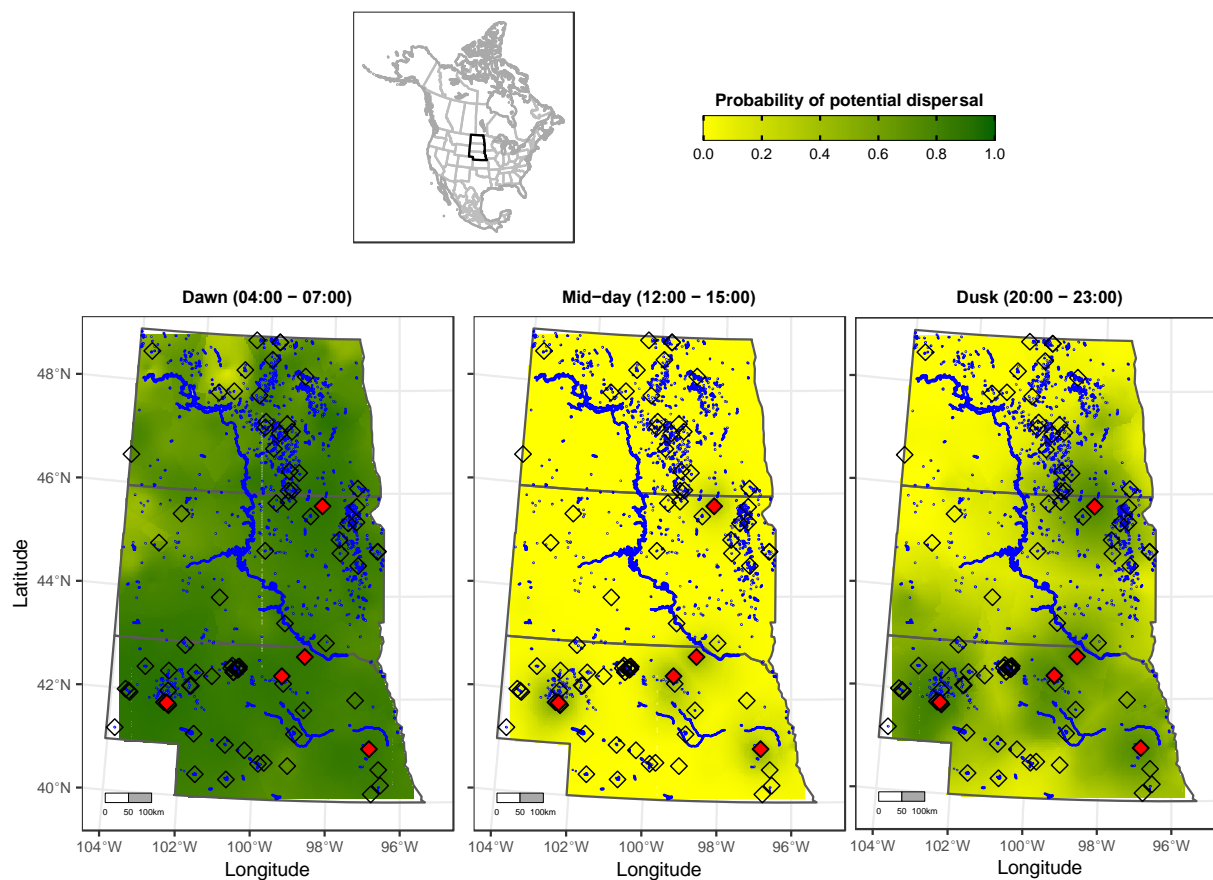
Figure 4. Geographical predictions of the probability of potential diatom dispersal

(omnidirectional) from five source lakes hosting *Nitzschia pusilla* Grunow (red diamonds).

Predictions are derived from the VPD-based GLM from our experiment, using estimates of

mallard flight duration (based on average flight speed of 69 km•h^{-1} and geographic distances)

and spatially interpolated vapour pressure deficit 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). See

Materials and Methods for details. All surface waterbodies are indicated in blue. Hollow black

diamonds denote the 80 waterbodies that in 2007 or 2012 hosted diatoms of the genus *Nitzschia*

(aside from the five hosting *N. pusilla*). The map projection is North American Equidistant

788   Conic. The map at the top shows the study region outlined in black within North America

789   (North American Lambert Conformal Conic projection).

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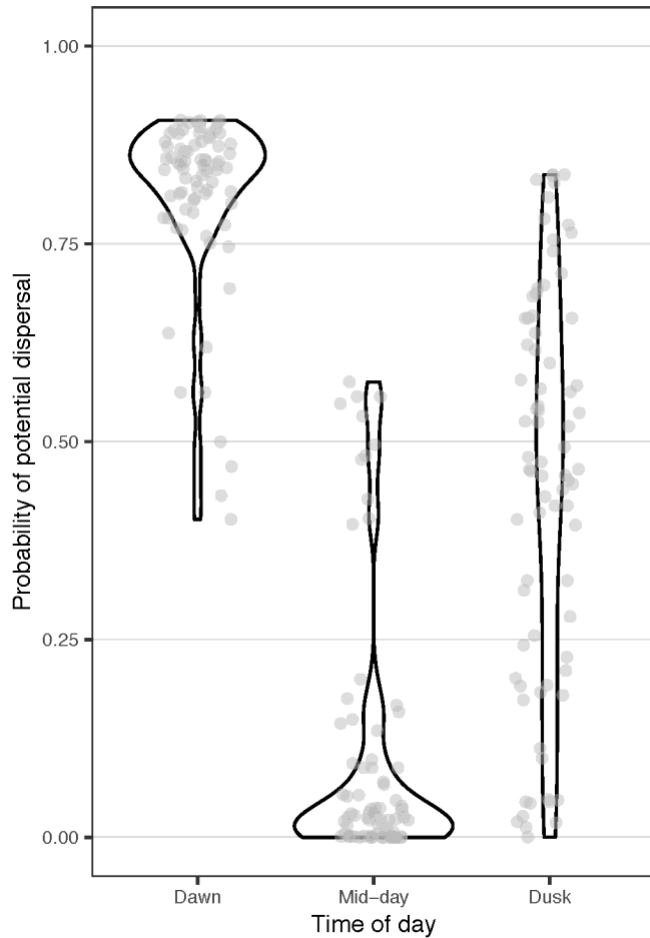
Figure 5. For waterbodies in the study region (Fig. 4) known to have hosted members of the

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.

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