1 **Modelling invasive pathogen load from non-destructive sampling data**

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20 Abstract

21 Where microbes colonizing skin surface may help maintain organism homeostasis,
 22 those that invade living skin layers cause disease. In bats, white-nose syndrome is a
 23 fungal skin infection that affects animals during hibernation and may lead to mortality
 24 in severe cases. Here, we inferred the amount of fungus that had invaded skin tissue
 25 of diseased animals. We used simulations to estimate the unobserved disease severity
 26 in a non-lethal wing punch biopsy and to relate the simulated pathology to the
 27 measured fungal load in paired biopsies. We found that a single white-nose syndrome
 28 skin lesion packed with spores and hyphae of the causative agent, *Pseudogymnoascus*
 29 *destructans*, contains 48.93 pg of the pathogen DNA, which amounts to about 1560 *P.*
 30 *destructans* genomes in one skin lesion. Relating the information to the known UV
 31 fluorescence in Nearctic and Palearctic bats shows that Nearctic bats carry about 1.7
 32 μg of fungal DNA per cm^2 , whereas Palearctic bats have $0.04 \mu\text{g cm}^{-2}$ of *P.*
 33 *destructans* DNA. With the information on the fungal load that had invaded the host
 34 skin, the researchers can now calculate disease severity as a function of invasive
 35 fungal growth using non-destructive UV light transillumination of each bat's wing
 36 membranes. Our results will enable and promote thorough disease severity assessment
 37 in protected bat species without the need for extensive animal and laboratory labor
 38 sacrifices.

39

40 **Keywords:** pathogen load; skin lesion; fungal infection; *Pseudogymnoascus*

41 *destructans*; white-nose syndrome; bat; UV light diagnostics

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44 1. Introduction

45 Association of pathogen load with infections transmission stands at the forefront of
 46 epidemiological dynamics models, where high pathogen load increases chances of
 47 transmission (Wilson et al., 2008). The amount of the pathogen that can infect a new
 48 host represents an infectious dose. In natural condition, the infectious dose needs to be
 49 transmitted through pathogen shedding via aerosol, direct or vector-mediated oral or
 50 bodily fluids exchange. As such, the pathogen is often quantified in sputum, feces,
 51 urine or blood, but such measures do not directly reflect the systemic infection or
 52 disease severity.

53 Clinical data support that the overall pathogen load positively correlates with disease
 54 severity (Franz et al., 2010; van der Poll and Opal, 2008). Technical and ethical
 55 hurdles hinder estimation of the overall load of the pathogen, where the pathogen may
 56 occur in different tissues in differing quantities during disease progression
 57 (Cunnington, 2015). Thorough sampling can then be possible during autopsy with
 58 pathogen quantification in multiple tissue samples, which is sadly late for the patient.
 59 Research in infectious diseases aims to prevent lethal outcomes and non-lethal
 60 methods must be preferred.

61 In case of white-nose syndrome (WNS), a fungal infection caused by
 62 *Pseudogymnoascus destructans* in hibernating bats (Blehert et al., 2009; Lorch et al.,
 63 2011), the disease severity can be estimated from histopathologic examination of
 64 wing membrane tissue (Meteyer et al., 2009; Pikula et al., 2017; Reeder et al., 2012).
 65 Following skin surface colonization, *P. destructans* invades the skin and forms
 66 cupping erosions diagnostic of WNS (Pikula et al., 2017). A cupping erosion is a cup-
 67 shaped skin lesion densely packed with fungal hyphae (Meteyer et al., 2009) and the
 68 fungus in the cupping erosions produces secondary metabolites that further damage

69 skin of the host (Flieger et al., 2016). Early methodology designed to assess disease
70 severity was lethal, as it required excision and rolling an interdigital segment of the
71 wing in preparation for serial cross-sections used in stained microscopy slides.
72 Number and distribution of the skin lesions were then used to score the severity of the
73 disease (Reeder et al., 2012). More recent methodology utilizes punch biopsy
74 sampling guided by characteristic fluorescence of skin lesions in ultraviolet (UV)
75 light, enabling the bat to survive the examination (Pikula et al., 2017).
76 Progress from lethal to destructive sampling facilitated examination of bats with
77 higher conservation status for WNS. To further reduce handling and disturbance of
78 bats for research purposes, herein we estimated the fungal load that invaded host
79 tissues in WNS skin lesions. Our approach combines previously published data on
80 fungal load estimated as the amount of *P. destructans* DNA on the wing punch where
81 the wing was without a WNS lesion and fungal load in a wing punch where UV
82 transillumination revealed characteristic fluorescence indicative of WNS lesions. The
83 relationship between the fungal loads measured from the paired biopsies can be used
84 to estimate how much fungal DNA there is in the bat wing tissue, because *P.*
85 *destructans* fluoresces under UV light after it had formed skin lesions. As the invasive
86 infection progresses, multiple lesions develop and the fungal load in the skin tissue
87 exceeds the fungal load on the wing surface (Martínková et al., 2018). Sampling in
88 the field cannot distinguish between a single microscopic lesion and multiple
89 confluent lesions as both cases manifest as a fluorescing dot when handling a live bat
90 in a cave (Fig. 1). In the laboratory, quantifying pathogen DNA requires enzymatic
91 tissue digestion, meaning that estimating infection intensity with DNA-based methods
92 as well as disease severity with histological microscopy is not possible from the same
93 sample. We therefore simulate possible histopathologic findings in the wing punch

94 digested for pathogen DNA quantification to infer how much *P. destructans* there is
95 in a single WNS skin lesion. We further calculate how the invasive fungal DNA
96 quantity relates to the biology of the infection, and we estimate the number of nuclei
97 in the invading fungal hyphae. We use these results to compare the invasive infection
98 in the Palearctic and Nearctic bats.

99

100 **2. Materials and Methods**

101 We used previously published data on fungal load present in a wing punch biopsy
102 (Fig. 1a in Flieger et al., 2016). The data comes from paired wing punch samples from
103 the same bat, where one punch was taken over a membrane segment with an orange
104 yellow fluorescence spot indicating a lesion (Turner et al., 2014) and the other punch
105 from an area where no UV florescent lesions were apparent (Fig. 1). In total, 41
106 *Myotis myotis* bats with paired wing biopsies were sampled and fungal load as a
107 measure of fungal DNA was quantified with quantitative polymerase chain reaction
108 (Flieger et al., 2016), i.e. sample size $n = 41$.

109 The dependence of the total fungal load on wing membrane that contains a UV
110 fluorescent lesion diagnostic for WNS Pd on the fungal load representing surface
111 colonization on an infected wing membrane $\widetilde{Pd}$ is linear in logarithmic scale (Flieger
112 et al., 2016). The total fungal load in a biopsy with a lesion Pd is compounded from
113 the surface skin colonization $\widetilde{Pd}$ and the invasive fungal growth, where the invasive
114 fungal growth is proportional to the number of skin lesions present in the sample.

115 The dependence of $\log_{10}(Pd)$ on $\log_{10}(\widetilde{Pd})$ can be used to quantify fungal load in a
116 WNS lesion. Once translated to the quadrant IV of the Cartesian system, the intercept
117 of the linear model α_0 represents the fungal load in the single lesion $\widetilde{Pd}^1$. The
118 modified relationship is given as

$$\log_{10}(Pd) = \alpha_0 + \alpha_1(\log_{10}(\widetilde{Pd}) + c), \quad \text{Eq. 1}$$

119 where the constant for translating the data values $c = -\min \log_{10}(\widetilde{Pd})$. The
 120 unknown model parameters α_0 and α_1 can be estimated using a least square method
 121 from the set of observations.
 122 Following Eq. 1, the fungal load in a single WNS lesion is $\widetilde{Pd}^1 = 10^{\alpha_0}$ for $\alpha_1 \approx 1$.
 123 Knowing the fungal load in a single WNS lesion and having enumerated the number
 124 of WNS lesions on a wing membrane from their UV fluorescence n_{UV} , we can
 125 calculate the tissue invasive fungal load $\widetilde{Pd}$ as

$$\widetilde{Pd} = n_{UV} \cdot \widetilde{Pd}^1. \quad \text{Eq. 2}$$

126 In the empirical study (Flieger et al., 2016), the slope of the regression $\alpha_1 = 0.294$,
 127 meaning that difference in fungal load between a biopsy negative and positive for UV
 128 fluorescence is due to additional factors than simple presence of a single WNS lesion
 129 in one biopsy. To investigate why there is a departure from 1, we departed the
 130 covariates confounding total fungal load of the i -th single wing membrane biopsy
 131 containing a UV fluorescent lesion Pd_i . First, the surface of a WNS lesion influences
 132 the area, where the fungus colonizes bat wing surface. Second, a UV fluorescent spot
 133 observed without magnification in the field might represent a more complex
 134 histopathology detectable with microscopy.

136 **2.1. Influence of wing surface area of a WNS lesion**

137 The first confounding aspect we considered influences intercept α_0 . The surface
 138 colonization affects a smaller area in a biopsy with a lesion as the wing surface area of
 139 the lesion cannot be considered to contain surface colonization by *P. destructans*. The
 140 total fungal load in the i -th wing biopsy is

$$Pd_i = \widetilde{Pd}_i^* + \widetilde{Pd}_i, \quad \text{Eq. 3}$$

141 where $\widetilde{Pd}_i$ represents fungal load in the UV fluorescent lesion for the i -th biopsy, and
 142 for this biopsy, $\widetilde{Pd}_i^*$ represents a surface colonization satisfying the condition of a
 143 sampled area A . It holds that

$$A(\widetilde{Pd}_i^*) = A(\widetilde{Pd}_i) \not\subset A(Pd_i), \quad \forall i \in I, \quad \text{Eq. 4}$$

144 where I is a set of sample indices and $I = \{1, \dots, n\}$.

145 In the sampling regime used to derive the original model (Flieger et al., 2016), the
 146 wing membranes were biopsied with standard 4 mm punch needles. The fungal DNA
 147 was thus quantified from the whole wing biopsy with radius R , meaning that the
 148 surface colonization covers both sides of the biopsy. At least one cupping erosion
 149 with radius r was present in the biopsy, and 46 % of *M. myotis* biopsies contained
 150 multiple cupping erosions (Pikula et al., 2017), i.e. the area covered by a surface
 151 colonization in the i -th biopsy is given as

$$A(\widetilde{Pd}_i^*) = 2\pi R_i^2 - \pi r_i^2 - 0.46 \pi r_i^2. \quad \text{Eq. 5}$$

152 In the empirical studies, the diameter of the i -th wing punch biopsy was $2R_i = 4$ mm
 153 (Flieger et al., 2016) and mean cupping erosion was $2r_i = 86 \mu\text{m}$ (Zukal et al., 2016)
 154 for $\forall i \in I$. Solving Eq. 5 numerically shows that $A(Pd_i) - A(\widetilde{Pd}_i) > 0.99 A(Pd_i)$
 155 for $\forall i \in I$. The effect of presence of the cupping erosion area on the estimation of the
 156 fungal load in a WNS lesion is thus negligible.

157

158 **2.2 Influence of unobserved histopathology**

159 The second aspect affects the regression slope α_1 . The UV fluorescent lesion
 160 recognized without magnification during field sampling could represent a confluent
 161 series of cupping erosions (Fig. 1), increasing the relative invasive growth. The fungal
 162 load of wing biopsies with UV fluorescence should be revised according to

$$Pd' = Pd - \lambda u, \quad \text{Eq. 6}$$

163 where Pd' is the revised fungal load in a wing biopsy with UV fluorescence, Pd is the
164 total fungal load estimated from the biopsy, u is number of additional cupping
165 erosions, and λ is a theoretical fungal load in the UV fluorescent lesion where
166 $\lambda \in [0.1, 10^{\alpha_0}]$ for $\alpha_0 = -1.01527$. The value for α_0 was inferred from the least
167 squares regression as per Eq. 1 from the original data, where each biopsy with a UV
168 fluorescence was assumed to contain a single WNS lesion. Note that the published
169 $\alpha_0 = 0.015$ (Flieger et al., 2016) cannot be used directly, because the original data
170 were not translated following their logarithmic transformation. The published data
171 values are located in the quadrant III of the Cartesian system, where the intercept of
172 the regression does not correspond to the fungal load in a single WNS lesion.
173 In our experience, up to four additional cupping erosions can be associated with one
174 biopsy, i.e. $u \in \{0,1,2,3,4\}$ (Fig. 1). Each biopsy in a set of n biopsies is associated
175 with one u forming a set of alternative histopathologic findings U of n elements u .
176 According to the results (Pikula et al., 2017), probability of multiple UV fluorescent
177 lesions in the set of biopsies is $p(u|u > 0) = 0.46$, i.e. $p(u|u = 0) = 0.54$. The
178 probability of observing n_{UV+} additional cupping erosions in biopsies with multiple
179 UV fluorescent lesions is given as $p(n_{UV+}) = 0.5 - 0.1n_{UV+}$, where $n_{UV+} \in N_{UV+}$,
180 and $N_{UV+} = \{1,2,3,4\}$. It means that $p(u|u > 0) = 0.46(0.5 - 0.1u)$ for $u \in N_{UV+}$.
181 Given the uncertainty in theoretical fungal load in a UV fluorescent lesion λ , there is
182 an infinite number of possible revised fungal loads (Eq. 6) for the available empirical
183 data. We approached the problem using simulations to estimate the effect of multiple
184 UV fluorescent lesions in the biopsy on the regression slope α_1 for sampled
185 combinations of λ and u . For that purpose, we created a vector λ with n_λ evenly
186 distributed λ where $\lambda \in [0.1, 10^{\alpha_0}]$, and we included the estimations of the total

187 fungal loads Pd for all n biopsies with a UV fluorescent lesion into a vector $\mathbf{Pd}$.
 188 Further, we generated randomly n_u permutations of the set U for each $\lambda \in \boldsymbol{\lambda}$. The j -th
 189 permutation of U for the k -th λ is given as $\mathbf{u}_{j,k} = \rho_{j,k}(U)$ where $\rho_{j,k}$ is the j -th
 190 mapping for the k -th λ . In total, $n_u \cdot n_\lambda$ random vectors $\mathbf{u}$ are generated, where the
 191 length of the vector $\mathbf{u}_{j,k}$ is n for $\forall j \in \{1, \dots, n_u\}$ and $\forall k \in \{1, \dots, n_\lambda\}$. For the k -th λ and
 192 the j -th permutation, the revised fungal loads in wing biopsies are given as

$$\mathbf{Pd}'_{j,k} = \mathbf{Pd} - \lambda_k \mathbf{u}_{j,k}. \quad \text{Eq. 7}$$

193 We used the obtained revised fungal loads Pd' (Eq. 7) instead of the measured total
 194 fungal loads Pd while estimating the coefficients α_0 and α_1 of the model Eq. 1
 195 (Fig. 2). Using the least squares estimator, $n_u \cdot n_\lambda$ coefficient estimations, $\hat{\alpha}_0$ and $\hat{\alpha}_1$,
 196 were obtained for the $n_u \cdot n_\lambda$ revised datasets. For each $\lambda \in \boldsymbol{\lambda}$, we searched for
 197 coefficient estimations $\hat{\alpha}_0$ and $\hat{\alpha}_1$ that best match the expected dependence, i.e. we
 198 searched for such setting where $\hat{\alpha}_1 \approx 1$. Considering this, we proposed an objective
 199 function

$$J = |1 - \hat{\alpha}_1^{j,k}|, \quad \text{Eq. 8}$$

200 where $\hat{\alpha}_1^{j,k}$ is the estimation of the coefficient α_1 for λ_k and the j -th permutation $\mathbf{u}_{j,k}$.
 201 For $\lambda_k \in \boldsymbol{\lambda}$, the best setting is given as

$$\hat{\alpha}_0^k, \hat{\alpha}_1^k = \arg \min_{j=1, \dots, n_u} |1 - \hat{\alpha}_1^{j,k}|, \quad \text{Eq. 9}$$

202 where $\hat{\alpha}_0^k$ and $\hat{\alpha}_1^k$ are the best estimates of the coefficients α_0 and α_1 for λ_k . Note that
 203 n_u coefficient estimations were obtained for each $\lambda \in \boldsymbol{\lambda}$, and we calculated fungal
 204 load in a WNS lesion as $\widetilde{Pd}_k = 10^{\hat{\alpha}_0^k}$.
 205 The study assumes that the first biopsy from the pair represents a wing segment
 206 without a WNS lesion, in which *P. destructans* colonized wing surface, and the
 207 second biopsy from the pair contains a wing segment with a WNS lesion, where the

208 fungus invaded the tissue. Theoretically, the difference in fungal loads between the
 209 two biopsies should be greater than or equal to the fungal load in the WNS lesion.
 210 Sampled data that do not conform to the condition are likely influenced by sampling
 211 or laboratory artifacts or by as yet unrecognized natural phenomena. To test for the
 212 effect of possible unknown bias, we ran a set of simulations on the original and the
 213 reduced data. In the case of the reduced data, we used $\forall k \in \{1, \dots, n_\lambda\}$ those paired
 214 biopsies where $Pd - \widetilde{Pd} > \lambda_k$.
 215 The equation Eq. 7 allows negative values of revised fungal load in a wing biopsy
 216 with a UV fluorescent lesion for some combinations of λ_k and $\mathbf{u}_{j,k}$, which is
 217 biologically not feasible. In the absence of *P. destructans*, fungal load must be equal
 218 to zero. We considered simulations based on combinations of λ_k and $\mathbf{u}_{j,k}$ leading to
 219 any $Pd' < 0$ as failed. Indexes k for which any element of $\mathbf{Pd}'_{j,k} < 0, \forall \mathbf{u}_{j,k}$ formed a
 220 set of failed simulations D , which is a subset of all simulations. Fungal load in a
 221 single UV fluorescent lesion was estimated from the sampled distribution as

$$\widetilde{Pd}^1 = P(\widetilde{Pd}_{\forall k \notin D}). \quad \text{Eq. 10}$$

222 The simulations were run in R (R Core Team, 2018) with custom scripts,
 223 implementing equations Eq. 1, Eq. 7, Eq. 8, Eq. 9 and Eq. 10. Data visualization used
 224 package *RColorBrewer* (Neuwirth, 2014).
 225 We then used the estimated $\widetilde{Pd}^1$ to approximate the number of pathogen nuclei in a
 226 skin lesion N . Given the genome size of *P. destructans* $G = 30.685 \times 10^6$ bp
 227 (GCA_000184105.1) and the conversion constant between genome mass and its size
 228 $q = 978 \times 10^6$ (Doležal et al., 2003), the number of *P. destructans* nuclei in a single
 229 WNS skin lesion is

$$N = \frac{\widetilde{Pd}^1 \cdot q}{G}. \quad \text{Eq. 11}$$

230 We applied the results of our simulations to previously published data (Pikula et al.,
231 2017) to infer the invasive fungal load in the Nearctic and Palearctic bats using
232 equations Eq. 2 and Eq. 11.

233

234 **Results**

235 We ran 1 million simulations of unobserved histopathology during estimation of
236 fungal load in a WNS lesion. For $n_\lambda = 1000$, we permuted designation of samples
237 with multiple UV fluorescent lesions $n_u = 1000$ times for each λ_k . When the
238 theoretical fungal load in a UV lesion $\lambda_k \in [0.075, 0.097]$ ng, 127 simulations failed
239 to find any feasible combination of samples with multiple cupping erosions despite
240 attempting one thousand permutations for each λ_k (Fig. 3), indicating that the range
241 might represent an upper limit of fungal load in one WNS lesion in *M. myotis*. Results
242 from 873 successful simulations show that simulating unobserved histopathology
243 improves the objective function value $J \in [0.45, 0.80]$ compared to the result from the
244 original data, where $J = 0.79$ (Flieger *et al.* 2016). The probability density with
245 Gaussian kernel of the simulated fungal load in a lesion has the mean equal to 0.0489
246 ng and standard deviation equal to 0.01134 ng (Fig. 3). This means that one WNS
247 skin lesion contains $\widetilde{Pd}^1 = 48.9 \pm 11.34$ pg (mean $\pm$ SD) of *P. destructans* DNA.
248 The simulated fungal load then translates to 1559 ± 362 pathogen nuclei in a WNS
249 lesion.

250 When the data were subset in each simulation to those where $Pd - \widetilde{Pd} > \lambda_k$, the
251 objective function values further decreased to $J \in [0.28, 0.58]$ and $\widetilde{Pd}^1 = 34.97 \pm$
252 7.42 pg of *P. destructans* DNA. In both simulation modes, using all data and using
253 subsets of data, the theoretical λ with the lowest objective function values were about
254 50.1 to 68.6 pg and the respective estimated $\widetilde{Pd}^1 \in [11.0, 17.1]$ pg.

255 Using the data on disease severity published by Pikula et al. (2017), the Palearctic
 256 bats had $n_{UV} = 0.78 \pm 1.44$ WNS lesions per cm^2 of wing membrane area ($n =$
 257 173), which translates to $\bar{P}d = 38.14 \pm 70.42$ pg of *P. destructans* DNA or hyphae
 258 with 1216 ± 2244 nuclei that invaded the unit area of host tissues. In Nearctic bats
 259 with $n_{UV} = 34.73 \pm 26.35$ UV fluorescent lesions per cm^2 of wing ($n = 11$), the
 260 invasive fungal growth contains $\bar{P}d = 1698.30 \pm 1288.52$ pg of *P. destructans*
 261 DNA, meaning fungal hyphae with 54129 ± 41068 nuclei per cm^2 of wing area.

262

263 Discussion

264 The utility in modelling pathogen load from data originating from non-lethal sampling
 265 provides unquestionable advantages and insight into disease dynamics. Non-lethal
 266 sampling promotes more effective sampling that enables to track distribution,
 267 prevalence, spread, infection intensity and disease severity on population level. Our
 268 approach simulated unobserved histological severity in a biopsy sample that was used
 269 to isolate DNA from the bat as well as the total pathogen biomass in the biopsy.
 270 Having established a density distribution of likely fungal loads in one WNS lesion,
 271 the researchers can now use the information to infer total fungal biomass that invaded
 272 the skin of the hibernating bat. We found that one WNS skin lesion contains about 50
 273 pg of *P. destructans* DNA and thus about 1560 genomic copies in the fungal
 274 multinucleic hyphae and spores in the lesion. Translating the value into context of
 275 published data on the number of UV fluorescent spots that are indicative of the WNS
 276 lesions (Pikula et al., 2017), we found that in some Nearctic bats, 1 cm^2 of their wing
 277 membrane might contain more than 1 ng of pathogen DNA or 54000 pathogen nuclei.
 278 The limitation of the present study lies in the fact that values of the objective function
 279 Eq. 8 did not approach 0 (Fig. 3a). The objective function minimized difference

280 between the slope of regression from adjusted data with simulated histopathologic
281 findings and the ideal slope equal to 1, when the regression intercept would signify
282 the fungal load in a single WNS lesion (i.e. Eq. 9). The lack of convergence towards
283 the optimum may be due to complexity of WNS pathology in the biopsy we did not
284 consider. The WNS lesions have variable size (Zukal et al., 2016) and some animals
285 develop full thickness invasion where the fungus replaces host tissues across the
286 cross-section of the wing membrane (Pikula et al., 2017). Additional noise in the data
287 is likely introduced with precision of the biopsy punch. Trained personnel stretch a
288 bat wing on a clean, firm surface transilluminated with UV light and circles the target
289 area with a punch needle. Although the punch needles have constant diameter, the
290 sampled wing may differ depending on animal movement, needle slippage or local
291 stretch of the wing membrane. The apparent solution suggests careful sampling where
292 the paired biopsies would be taken from wing area equidistant from joints and bones
293 and the punch site would be chosen with help from magnification to pinpoint single
294 UV fluorescent spots of similar size. At this moment, such data is not available, and
295 our simulation provides the best data-driven approximation of the invasive fungal
296 load during a WNS infection.

297 We addressed the potential problems in sampling by reducing the dataset to only
298 those observations where the fungal load on a biopsy without a fluorescing WNS
299 lesion was less than fungal load on the paired biopsy by at least the margin of the
300 theoretical fungal load in a single WNS lesion. The change resulted in lower objective
301 function values, but in no simulation was $J \approx 0$. The uncertainty in the estimate of the
302 fungal load in a single WNS lesion remains influenced by the issues mentioned above.
303 Despite the acknowledged bias, we consider our results useful in infectious disease
304 research of protected bat species, in which using non-destructive methods is

305 warranted. Photography of a bat wing transilluminated with UV light enables
 306 enumeration of the WNS lesions in the laboratory and together with estimation of
 307 fungal load on the wing surface using a swab sample, these data can be used to
 308 calculate infection invasiveness (Martínková et al. 2018). For practical utility in
 309 evaluating invasive fungal growth in an infected bat (Eq. 2), we recommend using the
 310 fungal load of 49 pg of *P. destructans* DNA in a single WNS lesion. The higher
 311 estimate will better incorporate the unconsidered histopathology and also account for
 312 presence of confluent WNS lesions that cannot be distinguished on the photograph of
 313 the transilluminated bat wing. Using the value of 49 pg of fungal DNA will thus likely
 314 better reflect the biological reality of the infection.
 315 Our results provide a valuable tool in assessing invasive infection in endangered
 316 hibernating bats on organismal level. Prior to the current study, the disease severity
 317 was inferred from focal histopathology in a wing biopsy. Now, the researchers can
 318 calculate disease severity as a function of infection invasiveness using non-destructive
 319 UV light transillumination (Turner et al., 2014) in conjunction with our results about
 320 the fungal load in a WNS lesion.

321

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328

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330

331 **Contributors:** NM and JP conceptualized the study, NM designed and implemented

332 the simulation and collated the data, PŠ formalized the mathematical apparatus, NM

333 and PŠ wrote the manuscript to which all authors contributed.

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398 Figure captions

399 **Fig. 1. Scheme of the relation between the biological and the mathematical model**
 400 **in estimating fungal load in a skin lesion.** Bat wing membrane (pink) was biopsied
 401 at two places, once at a wing segment without orange-yellow fluorescence under UV
 402 light, and once at a wing segment with a single fluorescing dot. Each biopsy was
 403 enzymatically digested and total fungal DNA was quantified with a quantitative
 404 polymerase chain reaction. The quantity of fungal DNA, the fungal load, in a biopsy
 405 without UV fluorescence represents the fungus that grows on the wing surface (purple
 406 lines, $\widetilde{Pd}$). The fungal load in a biopsy with a UV fluorescent dot represents the
 407 fungus growing on the wing surface, as well as the fungus that invaded the bat wing
 408 and formed cupping erosions diagnostic of white-nose syndrome (purple lines and
 409 ovals, Pd). In the field, a single cupping erosion cannot be distinguished from a series
 410 of confluent cupping erosions. The unobserved histopathology must thus be simulated
 411 to estimate the fungal load in a single cupping erosion ($\widetilde{Pd}^1$).

412

413 **Fig. 2. Illustrative modification of fungal load in a bat wing membrane biopsy**
 414 **with UV fluorescence to include simulation of the histopathologic findings.** Open
 415 circles – measured quantification of *Pseudogymnoascus destructans* DNA in paired
 416 biopsies from one bat with the respective regression between fungal load in a wing
 417 biopsy without UV fluorescence (and thus without a WNS lesion) and fungal load in a
 418 biopsy with UV fluorescence given as a dashed line. Closed circles – adjusted fungal
 419 loads, mimicking putative presence of multiple WNS lesions in one biopsy (Eq. 7).
 420 The regression line is solid for the adjusted fungal loads. Arrows – direction of
 421 change from measured to simulated fungal load per one WNS lesion in the biopsy.
 422 Where no arrows are present, the closed circles overlap the measured data in the open

423 circles, meaning that the simulation did not assign more than one WNS lesion in the
 424 given biopsy. λ – Theoretical fungal load in one WNS lesion as used in the simulation
 425 (ng).

426

427 **Fig. 3. Load of *Pseudogymnoascus destructans* DNA in one UV fluorescent lesion**

428 **diagnostic for white-nose syndrome in a *Myotis myotis* bat. (A)** Successful

429 simulations of permuted number of additional WNS skin lesions in a wing biopsy.

430 Theoretical starting values of fungal load in a single WNS lesion are indicated by the

431 colour scheme. **(B)** Density with the Gaussian kernel of the sampled distribution of

432 fungal load in a single WNS lesion in successful (orange) and failed (black)

433 simulations. Simulations were considered failed if all random assignments of

434 additional WNS lesion in a biopsy resulted in at least one adjusted fungal load below

435 zero.

436

a)

b)