K.J. Vanderwolf, D. Malloch, and D.F. McAlpine – Detecting viable *Pseudogymnoascus destructans* (Ascomycota: Pseudeurotiaceae) from walls of bat hibernacula: effect of culture media. *Journal of Cave and Karst Studies*, v. 78, no. 3, p. 158–162. DOI: 10.4311/2015MB0138

DETECTING VIABLE *PSEUDOGYMNOASCUS DESTRUCTANS* (ASCOMYCOTA: PSEUDEUROTIACEAE) FROM WALLS OF BAT HIBERNACULA: EFFECT OF CULTURE MEDIA

Karen Jane Vanderwolf[1,2]*, David Malloch[1], and Donald F. McAlpine[1]

Abstract: *Pseudogymnoascus destructans* (*Pd*) causes the fungal disease white-nose syndrome (WNS), which has led to high mortality in some hibernating bat species in eastern North America. The ability to detect viable *Pd* in hibernacula is important for understanding the role the environment plays as a reservoir for infectious *Pd*. Previous studies have generally used the high-sugar medium Sabouraud-dextrose (SAB) and have had low yields of viable *Pd* from environmental samples of *Pd*-positive hibernacula. While culture-independent methods (i.e., molecular genetics) have previously shown much better success in detecting *Pd*, these methods cannot determine viability. In 2012 and 2015, we swabbed walls in four hibernacula with WNS-positive bats in New Brunswick, Canada, and cultured the samples using dextrose-peptone-yeast extract agar (DPYA), SAB, and Malt extract (MEA) media. Samples cultured on DPYA produced viable *Pd* 43.7 to 50.0 % more frequently than SAB, with a maximum overall return for DPYA among sites of 62.5 % *Pd*-positive samples over both years. During the initial outbreak of WNS in our study region, *Pd*-positive swabs were produced from 40.0 to 83.3 % of samples on DPYA, whereas SAB produced a maximum of 40.0 %. At one site we detected *Pd* from 83.3% of swabs cultured on DPYA and 0 % on SAB. MEA produced no viable *Pd*. Our figures for *Pd* detection are as high as or higher than previously published culture-independent methods, while also confirming the viability of the *Pd* present. We found that the yield of viable *Pd* from hibernacula walls decreased from 2012 to 2015 as the hibernating bat population decreased due to WNS mortality, but patterns varied amongst hibernacula, and overall, were not statistically different. It is possible that environmental growth of *Pd* contributes to its persistence within hibernacula. We suggest that future studies on the environmental persistence of viable *Pd* discontinue the use of high-sugar media that lack inhibitory fungal growth ingredients, such as SAB and MEA, as they favor fast-growing fungal species that overgrow and mask slower-growing fungi such as *Pd*.

Introduction

White-nose syndrome (WNS) is a fungal disease of hibernating bats that has rapidly spread through the eastern United States and Canada, killing more than 6.7 million bats since it was first reported in 2006 in Albany, New York (USFWS, 2012a). WNS is caused by the fungus *Pseudogymnoascus destructans* (Blehert & Gargas) Minnis & D.L. Lindner, thought to be an invasive species from Europe (Lorch et al., 2011). The ability to detect *Pd* within a hibernaculum is an important part of disease surveillance. Detection often relies on the observation of visible fungal growth and behavioral changes in hibernating bats, but *Pd* can arrive before such changes are noticeable (Janicki et al., 2015). *Pd* surveillance is often conducted with culture-independent methods of molecular genetics, but such approaches cannot discriminate between viable and nonviable fungus. Viable cultures of *Pd* are required by researchers for physiological testing and other studies. Additionally, the ability to detect viable *Pd* in hibernacula is important for understanding the role the environment plays as a reservoir for infectious *Pd*. DNA from various organisms is able to persist long term in the environment, as shown by its recovery from paleontological remains, but the limit of persistence of non-viable *Pd* DNA in bat hibernacula is unknown. Viable *Pd* is known to persist in hibernacula soil in the absence of bats (Lorch et al., 2013), and it may be able to propagate as a saprobe (Raudabaugh and Miller, 2013). Reynolds et al. (2015) successfully grew *Pd* on a variety of sterilized sediments from caves in the laboratory. However, the ability of *Pd* to grow in cave sediments in the presence of native microorganisms has not yet been demonstrated. The relative importance of spores shed from bats in maintaining an environmental reservoir of *Pd* compared to possible environmental growth is unknown. Langwig et al. (2015) found

* Corresponding author: kjvanderw@gmail.com
[1] New Brunswick Museum, 277 Douglas Avenue, Saint John, NB, Canada E2K 1E7
[2] Canadian Wildlife Federation, 350 Promenade Michael Cowpland Drive, Kanata, ON Canada K2M 2W1

higher concentrations of *Pd* DNA on cave walls close to roosting bats compared to locations more than 2 m away, suggesting that bats shed *Pd* spores into their immediate environment. The long-term persistence of these shed spores is unknown. Modelling has shown that *Pd* persists in hibernacula for only three years beyond the loss of bats in the absence of environmental growth, while it persists for decades when environmental growth is possible (Reynolds et al., 2015).

Previous studies have produced low yields of viable *Pd* from *Pd*-positive hibernacula, with only 13 to 33 % positive samples using culture-dependent methods (Lorch et al., 2013; Martinkova et al., 2010; Zhang et al., 2014), as compared to 44 to 91 % with culture-independent methods (Zhang et al., 2014; Langwig et al., 2015). Lorch et al. (2013) found that the probability of detecting *Pd* in a *Pd*-positive hibernaculum was 56 % for each sample using real-time PCR, compared to 14% using culture-dependent methods.

WNS was first observed in New Brunswick, Canada, in 2011 (McAlpine et al., 2011) and had spread to all known hibernacula in the province by 2013 (Vanderwolf et al. 2015). The study reported here had two objectives: to compare the yield of viable *Pd* from hibernacula walls using a variety of culture-media types and to determine if the yield of viable *Pd* from hibernacula walls decreased over time as the hibernating bat population declined due to WNS-associated mortality.

Methods

Swabs were collected from hibernacula where bats (*Myotis lucifugus*, *Perimyotis subflavus* and *M. septentrionalis* with visible *Pd* growth were observed in southern New Brunswick, Canada. Data on physical characteristics of study sites, including location and temperatures, can be found in Vanderwolf et al. (2012). The mean winter (Nov. 1–Apr. 30) temperature in the dark zone of New Brunswick hibernacula is 5.1 °C ± 1.1SD (Vanderwolf et al., 2012). We followed the protocol of the United States Fish and Wildlife Service (2012b) for minimizing the spread of WNS during all visits to caves. Walls were swabbed in three hibernacula, Dorchester Mine, Berryton Cave, and Glebe Mine, both in April 2012 and April 2015. An additional site, White Cave, was sampled in April 2015 because hibernating bats were still roosting there, unlike the other sites. The number of hibernating bats present during swabbing is listed in Table 1, as well as the date of first detection of *Pd* in each site. *Pd* was initially detected at sites by assessing live hibernating bats for the presence of characteristic *Pd* fungal growth. However, lack of visible *Pd* growth does not equate to its absence (Janicki et al. 2015), so visual assessments of *Pd* presence on bats may underestimate arrival date.

Swabs were collected about 2 m above the cave floor deep in hibernacula in areas where bats were known to routinely roost, but always more than 1 m from the nearest roosting bat. Samples were collected from the same areas within hibernacula in 2012 and 2015. At each hibernaculum areas of approximately 20 by 20 cm on the wall were swabbed during each visit, with one applicator used for each area. Swabs were taken with a sterile, dry, cotton-tipped applicator and immediately streaked on the culture medium surface in a petri plate. Diluting streaks were completed within hibernacula within 1 h of the initial streak, after which plates were sealed in situ with Parafilm. A new applicator was used for each swab. Plates were shuffled in the field to ensure media types were not inoculated consecutively. In 2012, dextrose-peptone-yeast extract agar (DPYA; Papavizas and Davey, 1959) and Sabouraud-dextrose agar (SAB) were used, with five plates of each media type for Dorchester Mine and Glebe Mine and six of each for Berryton Cave. In 2015, DPYA, SAB, malt extract (MEA), and modified DPYA were used, with five plates of each media type for each of the four hibernacula. The antibiotics chlortetracycline (30mg/L) and streptomycin (30mg/L) were added to all media. Modified DPYA differed from DPYA in that the agar was autoclaved separately from the other ingredients. Tanaka et al. (2014) found that autoclaving ingredients separately increased the number of bacterial species that could be cultured. MEA consisted of 20 g Malt extract, 1 g peptone, 20 g dextrose, and 20 g agar per liter. DPYA consisted of 5 g dextrose, 1 g peptone, 2 g yeast extract, 1 g NH_4NO_3, 1 g K_2HPO_4, 0.5 g $MgSO_4 \cdot 7H_2O$, 0.01 g $FeCl_3 \cdot 6H_2O$, 5 g oxgall, 1 g sodium propionate, and 20 g agar per liter (Papavizas and Davey, 1959).

Plates were incubated inverted in the dark at 7 °C, a temperature that approximates that found in our study sites (Vanderwolf et al., 2012), and monitored over four months, in the manner of Vanderwolf et al. (2013). Initial morphological identifications of *Pd* (using Gargas et al., 2009) were confirmed by sequencing as part of other studies (2012 samples: Khankhet et al., 2014; 2012 and 2015 samples: pers. comm. J. Foster and K. Drees, University of New Hampshire). Permanent dried cultures are housed in the New Brunswick Museum (NBM# F-05262–05282, 05339, 05345, 05404, 05457, 05486).

Since the data were not normally distributed, a Friedman test was used to test the effect of media type and site on the number of *Pd*-positive swabs in 2015. After testing for normality, a general linear model was used to test the effect of media type, site, and year on the number of *Pd*-positive swabs using data from media types and sites sampled in both 2012 and 2015. All tests were performed using Minitab.

Results

Pd was cultured from 40.6 % of wall swabs ($n = 32$) in 2012, and 31.3 % of swabs ($n = 80$) in 2015 (Table 2). When restricting 2015 data to the same hibernacula and media types used in 2012, 26.7 % of swabs ($n = 30$) yielded viable *Pd*, with Dorchester Mine and Berryton Cave decreasing to 10 % and 20 % positive samples from 2012 to 2015, and Glebe Mine increasing to 50 %. These trends were not significantly different between years ($F_{1,11} = 1.26$, $p = 0.299$) or sites ($F_{2,11}$

Table 1. The number of hibernating bats (*Myotis lucifugus*, *M. septentrionalis*, and *Perimyotis subflavus*) present during sampling. Sampling was not conducted in White Cave in 2012. The first detection of *Pd* was determined by the observation of visible fungal growth on hibernating bats.

Site Name	2012	2015	First *Pd* Detection
Berryton Cave	5	0	March 2011
Dorchester Mine	1	1	December 2011
Glebe Mine	174	0	March 2012
White Cave	115	5	December 2011

$= 0.15$, $p = 0.863$), but overall yield was significantly higher on DPYA compared to SAB ($F_{1,11} = 8.51$, $p = 0.022$). Samples cultured on DPYA produced viable *Pd* 43.7 to 50.0 % more frequently than SAB, with a maximum overall return for DPYA among sites of 62.5 % *Pd*-positive samples over both years. During the initial outbreak of WNS in our study region, *Pd*-positive swabs were produced from 40.0 to 83.3 % of samples on DPYA, whereas SAB produced a maximum of 40.0 %. The date of first *Pd* detection in each site did not appear to affect *Pd* yield. However, the date of *Pd* arrival in Dorchester Mine is uncertain, since we observed visible fungal growth on hibernating bats ($n = 140$ bats) on our initial visit to the site in December 2011, while the other sites had been monitored since 2009.

In 2015, *Pd* yield was not significantly different between sites ($S_{3,15} = 4.78$, $p = 0.189$, adjusted for ties), but was significantly different among media types ($S_{3,15} = 9.65$, $p = 0.022$; adjusted for ties). *Pd* yield was higher on DPYA (60 %) compared to SAB (10 %; Table 2). The modification to DPYA (autoclaving the agar separately from the other ingredients) did not increase *Pd* yield compared to unmodified DPYA (11 vs. 12 positive swabs in 2015). Malt medium tended to be quickly overgrown with *Mucor* spp. and *Mortierella* spp., and no *Pd* was obtained.

DISCUSSION

Pd is known to persist in hibernacula soil/sediments in the absence of bats, but healthy bats only occasionally come into contact with the ground (Lorch et al., 2013; Rysgaard, 1942), and *Pd* is not thought to be transmitted aerially (Lorch et al., 2011). Our study shows that viable *Pd* persists on walls of hibernacula that bats regularly come into contact with years after bat populations have declined at these sites or disappeared entirely. Hibernacula walls may act as a source of infection when bats return to hibernate or during swarming activity (Langwig et al., 2015). The number of *Pd*-positive samples did decrease in two hibernacula (Berryton Cave and Dorchester Mine) after the hibernating-bat population declined or disappeared, but increased at the third site (Glebe Mine), although these trends were not significant and low sample sizes complicate interpretation. In Glebe Mine, no more than one bat has been seen since winter 2013 during annual surveys, although the site previously supported hundreds of hibernating bats (Vanderwolf et al., 2012). No more than three bats each have been observed in Berryton Cave and Dorchester Mine during annual winter surveys 2012-2015, although the sites previously had thousands and hundreds of hibernating bats, respectively (Vanderwolf et al., 2012). The reasons for the increase in *Pd* yield in Glebe Mine are unknown. It is interesting to note that the *Pd* yield in Glebe Mine was higher than that from White Cave, where low numbers of bats with visible *Pd*-growth still persist and presumably continue to shed *Pd* spores into the environment. It is possible that environmental growth explains the patterns. Reynolds et al. (2015) found that the greatest growth of *Pd* in the laboratory occurred on cave sediments with high levels of organic matter. The levels of organic matter in our study sites have not been quantified, although our mine sites have old wooden support pillars with mushroom growth that are absent in the caves, and Glebe Mine and White Cave have accumulations of porcupine (*Erethizon dorsatum*) dung. The ability of *Pd* to grow on non-bat substrates in the field and the mechanism for spore dispersal within hibernacula in the absence of bats requires further study.

Verant (2016) found that the prevalence of *Pd* on hibernacula walls increased over time after the first detection of WNS in hibernating bats at sites, although at a slower and lower rate than in sediment samples. Verant (2016) did not detect *Pd* on hibernacula walls until one year after WNS was first detected on bats at the sites. Prevalence was initially low

Table 2. Number and percentage of *Pd*-positive wall swabs taken in four New Brunswick hibernacula April 2012 and 2015 on different media types with the total number of swabs in brackets. DPYA = dextrose-peptone-yeast extract agar. SAB = Sabouraud-dextrose agar. MEA= Malt extract agar. ND = no data.

	2012			2015				
Site Name	DPYA	SAB	Overall Positive	DPYA	SAB	Mod DPYA	MEA	Overall Positive
Berryton Cave	5(6) 83.3%	0(6) 0%	41.7% (12)	2(5) 40%	0(5) 0%	1(5) 20%	0(5) 0%	15.0% (20)
Dorchester Mine	3(5) 60%	2(5) 40%	50.0% (10)	1(5) 20%	0(5) 0%	4(5) 80%	0(5) 0%	25.0% (20)
Glebe Mine	2(5) 40%	1(5) 20%	30.0% (10)	4(5) 80%	1(5) 20%	5(5) 100%	0(5) 0%	50.0% (20)
White Cave	ND	ND	ND	5(5) 100%	1(5) 20%	1(5) 20%	0(5) 0%	35.0% (20)
Overall positive	62.5% (16)	18.8% (16)	40.6% (32)	60.0% (20)	10.0% (20)	55.0% (20)	0% (20)	31.3% (80)

(8 % *Pd*-positive samples from walls and 30 % from sediment using culture-independent methods), but increased to 17 to 75 % from walls and up to 90 % from sediment two years after the first detection of WNS in hibernating bats at each site (Verant 2016). We did not find a lag in *Pd*-detection from hibernacula walls, as prevalence of *Pd* in Dorchester Mine and Glebe Mine was 30.0 % and 41.7 % one month and four months after the first detection of WNS in bats at the sites, respectively. We also did not find such large increases in *Pd*-prevalence more than two years after the first detection of WNS in bats at our study sites. These inconsistencies may reflect differences in the detection of *Pd* DNA using culture-independent approaches compared to culture-dependent methods, which reveal only viable spores, or possibly site differences.

Lorch et al. (2013) and Zhang et al. (2014) found that the probability of detecting *Pd* was higher using molecular methods compared to culture techniques. Viable *Pd* was cultured from only 13.8 % ($n = 195$) of soil samples in *Pd*-positive hibernacula using SAB (Lorch et al., 2013). Zhang et al. (2014) obtained viable *Pd* from 18 % of sediment samples from *Pd*-positive hibernacula in New York using SAB and Rose Bengal medium. In Estonia, 100 % of wall swabs ($n = 4$) on SAB yielded *Pd*, but these swabs were taken within a few centimeters of where one bat with visible white fungal growth had been roosting a few days earlier (Puechmaille et al., 2011). Langwig et al. (2015) found that the percentage of *Pd*-positive samples from cave walls using culture-independent techniques varied with the distance from roosting bats. Swabs taken just under roosting bats were 91 % positive, 66 % were positive 10–20 cm away from bats, and 44 % were positive more than 2 m from bats (Langwig et al., 2015). We swabbed cave walls more than 1 m from roosting bats, if any were present, and obtained results with DPYA that generally match or exceed those obtained with culture-independent methods, while also allowing us to confirm the viability of *Pd*. Our yield of *Pd* from wall swabs using SAB is similar to Lorch et al. (2013) and Zhang et al. (2014).

Discrepancy in *Pd* yield between SAB and DPYA media was less marked when we directly swabbed bats (Vanderwolf et al., 2015), presumably because bats have greater numbers of spores than cave walls. However, Martinkova et al. (2010) cultured *Pd* on SAB from only 33.3 % ($n = 48$) of swabs taken directly from European bats with visible white fungal growth. This may reflect decreased growth of *Pd* on European bats compared to infected bats in North America, or possibly the observed growth on some bats was of a different fungal species than *Pd* (such as *Trichophyton* spp., Lorch et al., 2015).

As pointed out by Cooke (1968), culture media high in soluble sugars, such as MEA and SAB, tend to accumulate extracellular biproducts, leading to premature staling of the medium. We recommend against the use of these media in studies on the environmental persistence of viable *Pd*. We also recommend the use of fungal-growth-inhibiting ingredients, such as sodium propionate and oxgall, as additives to any medium used for this purpose. High-glucose media such as MEA and SAB favor fast-growing species, such as *Mortierella* spp. and *Mucor* spp., which overgrow and mask slower growing fungal species such as *Pd*. Other media types, such as those that mimic the more complex nutrient contents of bats, may produce even higher yields of viable *Pd* from environmental samples collected in hibernacula.

Acknowledgements

Access to hibernacula located on private lands was generously provided by David Roberts, Joan Chown, and Tony Gilchrist. Scientific permits for handling bats and/or entering sites was provided by the New Brunswick Department of Natural Resources Species-at-Risk Program and the New Brunswick Protected Natural Areas Program. Research funding was provided by the Canadian Wildlife Federation, Crabtree Foundation, New Brunswick Environmental Trust Fund, New Brunswick Wildlife Trust Fund, New Brunswick Department of Natural Resources, and Parks Canada.

References

Cooke, W.B., 1968, Carbon/nitrogen relationships of fungus culture media: Mycopathologia et Mycologia Applicata, v. 34, p. 305–316. doi:10.1007/BF02051856.

Gargas, A., Trest, M.T., Christensen, M., Volk, T.J., and Blehert, D.S., 2009, *Geomyces destructans* sp. nov. associated with bat white-nose syndrome: Mycotaxon, v. 108, p. 147–154.

Janicki, A.F., Frick, W.F., Kilpatrick, A.M., Parise, K.L., Foster, J.T., McCracken, G.F., 2015, Efficacy of visual surveys for White-Nose Syndrome at bat hibernacula: PLOS ONE, v. 10, no. 7, e0133390, 12p. doi:10.1371/journal.pone.0133390.

Khankhet, J., Vanderwolf, K.J., McAlpine, D.F., McBurney, S., Overy, D.P., Slavic, D., Xu, Jianping, 2014, Clonal expansion of the *Pseudogymnoascus destructans* genotype in North America is accompanied by significant variation in phenotypic expression: PLOS ONE, v. 9, no. 8, e104684, 9p. doi:10.1371/journal.pone.0104684.

Langwig, K.E., Hoyt, J.R., Parise, K.L., Kath, J., Kirk, D., Frick, W.F., Foster, J.T., Kilpatrick, A.M., 2015, Invasion dynamics of white-nose syndrome fungus, Midwestern United States, 2012-2014: Emerging Infectious Diseases, v. 21, no. 6, p. 1023–1026. doi:10.3201/eid2106.150123.

Lorch, J.M., Meteyer, C.U., Behr, M.J., Boyles, J.G., Cryan, P.M., Hicks, A.C., Ballmann, A.E., Coleman, J.T.H., Redell, D.N., Reeder, D.M., and Blehert, D.S., 2011, Experimental infection of bats with *Geomyces destructans* causes white-nose syndrome:Nature, v. 480, p. 376–378. doi:10.1038/nature10590.

Lorch, J.M., Muller, L.K., Russell, R.E., O'Connor, M., Lindner, D.L., and Blehert, D.S., 2013, Distribution and environmental persistence of the causative agent of white-nose syndrome, *Geomyces destructans*, in bat hibernacula of the Eastern United States: Applied and Environmental Microbiology, v. 79, p. 1293–1301. doi:10.1128/AEM.02939-12.

Lorch, J.M., Minnis, A.M., Meteyer, C.U., Redell, J.A., White, J.P., Kaarakka, H.M., Muller, L.K., Lindner, D.L., Verant, M.L., Shearn-Bochsler, V., and Blehert, D.S., 2015, The fungus *Trichophyton redellii* sp. nov. causes skin infections that resemble white-nose syndrome of hibernating bats: Journal of Wildlife Diseases, v. 51, no. 1, p. 36–47. doi:10.7589/2014-05-134.

Martinková, N., Bačkor, P., Bartonička, T., Blažkova, P., Červeny, J., Falteisek, L., Gaisler, J., Hanzal, V., Horáček, D., Hubálek, Z., Jahelková, H., Kolařik, M., Korytár, L., Kubátova, A., Lehotská, B., Lehotský, R., Lučan, R.K., Májek, O., Matějů, J., Řehák, Z., Šafář, J., Tájek, P., Tkadlec, E., Uhrin, M., Wagner, J., Weinfurtová, D., Zima, J., Zukal, J., and Horáček, I., 2010, Increasing incidence of *Geomyces destructans* fungus in bats from the Czech Republic and Slovakia: PLOS ONE, v. 5, no. 11, e13853, 7p.. doi:10.1371/journal.pone.0013853.

McAlpine, D.F., Vanderwolf, K.J., Forbes, G.J., and Malloch, D., 2011, Consumption of bats (*Myotis* spp.) by raccoons (*Procyon lotor*) during an outbreak of white-nose syndrome in New Brunswick: implications for bat mortality estimates: Canadian Field-Naturalist, v. 125, p. 257–260.

Papavizas, G.C., and Davey, C.B., 1959, Evaluation of various media and antimicrobial agents for isolation of soil fungi: Soil Science, v. 88, p. 112–117.

Puechmaille, S.J., Wibbelt, G., Korn, V., Fuller, H., Forget, F., Mühldorder, K., Kurth, A., Bogdanowicz, W., Borel, C., Bosch, T., Cherezy, T., Drebet, M., Görföl, T., Haarsma, A.-J., Herhaus, F., Hallart, G., Hammer, M., Jungmann, C., Le Bris, Y., Lutsar, L., Masing, M., Mulkens, B., Passior, K., Starrach, M., Wojtaszewski, A., Zöphel, U., and Teeling, E.C., 2011, Pan-European distribution of white-nose syndrome fungus (*Geomyces destructans*) not associated with mass mortality: PLOS ONE, v. 6, no. 4, e19167, 11 p.. doi:10.1371/journal.pone.0019167.

Raudabaugh, D.B., and Miller, A.N., 2013, Nutritional capability of and substrate suitability for *Pseudogymnoascus destructans*, the causal agent of bat white-nose syndrome: PLOS ONE, v.8, no. 10, e78300, 9 p. doi:10.1371/journal.pone.0078300.

Reynolds, H.T., Ingersoll, T., and Barton, H.A., 2015, Modeling the environmental growth of *Pseudogymnoascus destructans* and its impact on the white-nose syndrome epidemic: Journal of Wildlife Diseases, v. 51, no. 2, p. 318–331. doi:10.7589/2014-06-157.

Rysgaard, G.N., 1942, A study of the cave bats of Minnesota with especial reference to the large brown bat, *Eptesicus fuscus fuscus* (Beauvois): American Midland Naturalist, v. 28, no. 1, p. 245–267. doi:10.2307/2420702.

Tanaka, T., Kawasaki, K., Daimon, S., Kitagawa, W., Yamamoto, K., Tamaki, H., Tanaka, M., Nakatsu, C.H., and Kamagata, Y., 2014, A hidden pitfall in the preparation of agar media undermines microorganism cultivability: Applied and Environmental Microbiology, v. 80, no. 24, p. 7659–7666. doi:10.1128/AEM.02741-14.

USFWS, 2012a, United States Fish and Wildlife Service, North American bat death toll exceeds 5.5 million from white-nose syndrome, posted January 17, 2012. https://www.whitenosesyndrome.org/sites/default/files/files/wns_mortality_2012_nr_final_0.pdf. [accessed January 21, 2012?]

USFWS, 2012b, United States Fish and Wildlife Service, Revised decontamination protocol: June 25, 2012. http://www.whitenosesyndrome.org/resource/revised-decontamination-protocol-june-25-2012. [accessed January 5, 2015?]

Vanderwolf, K.J., McAlpine, D.F., Forbes, G.J., and Malloch, D., 2012, Bat populations and cave microclimate prior to and at the outbreak of white-nose syndrome in New Brunswick: Canadian Field Naturalist, v. 126, p. 125–134.

Vanderwolf, K.J., McAlpine, D.F., Malloch, D., and Forbes, G.J., 2013, Ectomycota associated with hibernating bats in eastern Canada caves prior to the emergence of white-nose syndrome: Northeastern Naturalist, v. 20, no. 1, p. 115–130. doi: 0.1656/045.020.0109.

Vanderwolf, K.J., Malloch, D., and McAlpine D.F., 2015, Fungi associated with over-wintering tricolored bats, *Perimyotis subflavus*, in a white-nose syndrome region of Eastern Canada: Journal of Cave and Karst Studies, v. 77, no. 3, p. 145–151. doi:10.4311/2015MB0122.

Verant, M.L., 2016, Defining the epidemiology and host-pathogen interactions of bat white-nose syndrome to inform surveillance and management strategies [Ph.D. thesis]: University of Wisconsin-Madison.

Zhang, Tao., Victor, T.R., Rajkumar, S.S., Li, Xiaojiang, Okoniewski, J.C., Hicks, A.C., Davis, A.D., Broussard, K., LaDeau, S.L., Chaturvedi, S., and Chaturvedi, V., 2014, Mycobiome of the bat white nose syndrome affected caves and mines reveals diversity of fungi and local adaptation by the fungal pathogen *Pseudogymnoascus (Geomyces) destructans*. PLOS ONE, v. 9, no. 9, e108714, 12 p. doi:10.1371/journal.pone.0108714.