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BATS RECOVERING FROM WHITE-NOSE SYNDROME ELEVATE METABOLIC RATE DURING WING HEALING IN SPRING

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ABSTRACT: Host responses to infection with novel pathogens are costly and require trade-offs among physiologic systems. One such pathogen is the fungus *Pseudogymnoascus destructans* (Pd) that causes white-nose syndrome (WNS) and has led to mass mortality of hibernating bats in eastern North America. Although infection with Pd does not always result in death, we hypothesized that bats that survive infection suffer significant consequences that negatively impact the ability of females to reproduce. To understand the physiologic consequences of surviving infection with Pd, we assessed differences in wing damage, mass-specific resting metabolic rate, and reproductive rate between little brown myotis (*Myotis lucifugus*) that survived a winter in captivity after inoculation with Pd (WNS survivors) and comparable, uninfected bats. Survivors of WNS had significantly more damaged wing tissue and displayed elevated mass-specific metabolic rates compared with Pd-uninfected bats after emergence from hibernation. The WNS survivors and Pd-uninfected bats did not significantly differ in their reproductive capacity, at least in captivity. However, our metabolic data demonstrated greater energetic costs during spring in WNS survivors compared with uninfected bats, which may have led to other consequences for postpartum fitness. We suggest that, after surviving the energetic constraints of winter, temperate hibernating bats infected with Pd faced a second energetic bottleneck after emerging from hibernation.

Key words: Chiroptera, energy balance, fitness, little brown myotis, metabolic rate, reproduction, wildlife disease, white-nose syndrome.

INTRODUCTION

When energetic costs are high or available energy is low, such as during periods of food shortage, temperature extremes, or exposure to pathogens, energetic trade-offs in animals can be expected (Ricklefs and Wikelski 2002). Energetic trade-offs affect the energy for competing physiologic demands such as thermoregulation and immune function (Lochmiller and Deerenberg 2000). This may be especially true when novel pathogens exert strong selective pressures. These selective pressures may place additional energetic demands upon the host because of competition for resources between the host and pathogen, costs of repairing tissue damage, and physiologic consequences of mounting an immune response (Svensson et al. 1998).

White-nose syndrome (WNS), a disease of hibernating bats, presents a model for studying energetic trade-offs during times of environmental stress and exposure to infectious disease. First documented in 2006, WNS has devastated populations of hibernating bats in eastern North America, including the little brown myotis (*Myotis lucifugus*) that has been particularly affected (Frick et al. 2010). Bats in temperate environments largely feed on flying insects that do not fly during winter at northern latitudes; these bats survive the winter either by migrating to warmer locations or by using prolonged bouts of torpor (hibernation) and autumnal acquisition of energy stores (Thomas et al. 1990). Seasonal energy availability shapes not only their overwintering behavior and physiology but also their reproductive strategy. These bats display a dissociated pattern of reproduction in which mating occurs largely in late autumn, concurrent with prehibernation fattening (Racey 1982). Subsequently, females store

sperm during hibernation, with ovulation and fertilization occurring in spring (Gustafson 1979; Oxberry 1979; Racey 1979). Although the management of fat reserves over winter is important for both sexes, female bats must emerge from hibernation with sufficient reserves to support ovulation and early pregnancy (Buchanan 1987). Anything that alters energetic demands, such as WNS, would likely have detrimental effects on both winter survival and spring birth rates.

White-nose syndrome is a disease caused by *Pseudogymnoascus destructans* (Pd), a cold-adapted fungal pathogen (Lorch et al. 2011) that invades and damages cutaneous tissue in hibernating bats. Damage to wing tissue is especially disruptive, negatively affecting thermoregulatory and other behaviors, energetic balance, and immune processes (Reeder et al. 2012; Brownlee-Bouboulis and Reeder 2013; Field et al. 2015). The disease significantly reduces an individual's chance of overwinter survival, although mortality rates vary within and across species (Turner et al. 2011; Grieneisen et al. 2015; Johnson et al. 2015). Of these various impacts, differences in energy balance among and within species seem to play the greatest role in determining mortality (Hayman et al. 2016; Moore et al. 2017). In little brown myotis, we have previously shown that infection with Pd causes too-frequent arousals from hibernation (Reeder et al. 2012), which depletes fat reserves (Warnecke et al. 2012). Although mortality is especially high in this species (>90%), some bats survive and are able to heal wing damage within a given year (Dobony et al. 2011). These survivors have persisted in Pd-endemic areas that have experienced WNS upward of 10 yr (Langwig et al. 2015; Lilley et al. 2016). Because Pd infection during hibernation has high costs, with the potential to alter reproduction of female bats and subsequently cause population declines, understanding the energetic and other consequences of surviving a winter with Pd is critical.

Survivorship and reproduction in little brown myotis affected by Pd are likely further impacted by the energetic costs of recovering from infection after emergence from hibernation. Even in uninfected bats, emergence from hibernation in spring involves energetic readjustment and repair to wing tissues (Humphries et al. 2003; Archie 2013; Powers et al. 2013). Damaged wing tissue not only affects flight but also other critical homeostatic functions (Willis et al. 2011; Cryan et al. 2013; Verant et al. 2014). For bats that survive WNS, Pd-induced tissue damage repair must occur. Additional immune responses associated with Pd infection (Meteyer et al. 2012; Field et al. 2015; Lilley et al. 2017) likely increase the energetic cost of the posthibernation period for affected bats and represent an energetic bottleneck.

Our goal was to examine physiologic consequences of surviving Pd infection for both male and female little brown myotis. We hypothesized that bats with overwinter Pd infection would exhibit delayed tissue repair and higher resting metabolic rates relative to uninfected bats due to the energetic costs of these processes. We also hypothesized that females surviving Pd infection would have lower reproductive rates than uninfected bats due to energetic trade-offs that occur between reproduction and other physiologic processes.

MATERIALS AND METHODS

Animal collection, hibernation, and husbandry

We collected 147 Pd-naïve little brown myotis (70 males, 77 females) from hibernacula in Michigan and Illinois on 1 and 2 November 2012, as part of a larger study of WNS survival (Johnson et al. 2014). Bats were transported to Bucknell University, Lewisburg, Pennsylvania; inoculated with Pd (n=118) or a vehicle solution (n=29); and hibernated in captivity for 148 d. All methods were approved by the Institutional Animal Care and Use Committee at Bucknell University (protocol DMR-016). To mitigate the variation inherent in sampling wild animals, bat assignment to treatment group was randomized and balanced by sex and provenance. The only differences in the experience of bats during this study were whether they were inoculated with Pd, at what dose, and at what temperature they hibernated (4 or 10 C). All bats were confirmed to be Pd negative by quantitative PCR at the time of collection; control bats remained uninfected whereas inoculated bats were confirmed Pd

TABLE 1. Scoring system for quantifying wing damage from white light photographs, with a description of the damage present per category. The scale does not necessarily reflect the percentage of wing damage.

Wing damage scale	Description of wing patagium
0	Clean, no visible damage
1	Presence of fewer than five small (<0.5-mm) translucent spots; small, pin-sized dark spots; or small tear(s) (<5 mm) in wing not caused by band injury
2	Pin-sized dark spots visible in larger groups; may have small tear(s) in wing not caused by band injury
3	Pin-sized dark spots and translucent spots visible; may have small tear(s) in wing not caused by band injury
4	Translucent spots visible; may have small tear(s) in wing not caused by band injury
5	Large tear(s) (>5 mm), or more than five small tears, in wing not caused by band injury; may have dark pots, translucent spots, or necrotic skin

positive at the end of the study. In the Johnson et al. (2014) study, 69 of the 147 bats survived the 148 d of hibernation: 69% (20/29) of control bats and 41.5% (49/118) of Pd-infected bats. The Pd-infected bats experienced significantly higher mortality than control bats, especially for bats that received the lowest inoculation dose (500 conidia) and bats that hibernated at 10 C (Johnson et al. 2014).

Posthibernation, the 69 survivors were placed in indoor flight cages (5×3×2 m), with Pd-uninfected bats residing in a separate Pd-free room (which was always entered first daily). Bats were hand-fed mealworms (*Tenebrio molitor*) until they self-fed. Mealworms were gut loaded in a medium containing Purina® Game Bird Maintenance diet (Purina Animal Nutrition LLC, Gray Summit, Missouri, USA), supplemented with apples and water. Each flight cage contained "parakeet"-sized feeding dishes attached to the walls; these dishes were filled each day with new mealworms. Water dishes containing vitamin and mineral supplements (7 mL of Avitron/14 mL of Avimin per 3.8 L of water; Lambert Kay Products, Farebury, Nebraska, USA) were attached to the walls of each cage. Humidifiers maintained 60% relative humidity. Both flight cages were exposed to a simulated natural photoperiod (11 h of dark and 13 h of light, sunset 1945 hours).

Of the 69 survivors, 34 were included in our final analysis of the effects of Pd infection on wing damage and metabolic rate after emergence from hibernation. These bats were selected by excluding pregnant females (n=15) due to potential confounding energetic effects of pregnancy (Kurta et al. 1989) and balancing bats by original experimental conditions (prehibernation Pd inoculation dose of 500, 5,000, 50,000, or 500,000 conidia and subsequent hibernation at either 4 or 10 C; Johnson et al. 2014). This was done to randomize and balance variation in posthibernation wing damage and metabolic rates that might be due to hibernation conditions. We were also constrained in the number of bats that could be tested daily and needed to minimize the overall length in days of the overall testing period at each time. Data on all final 34 study subjects can be found in the Supplementary Table. These subjects included nine Pd-uninfected bats (5 females, 4 males) and 25 infected bats (11 females, 14 males). For the analysis of reproductive success, all females (n=44) from the surviving 69 bats were included.

Wing damage score

White-light photographs were taken of the ventral side of each wing with transillumination from below (Reichard and Kunz 2009) for all bats at 19 and 33 d posthibernation. Waiting until 19 d posthibernation allowed captive bats to adapt to their enclosures and reduced handling stress posthibernation. Each wing was assigned a wing damage score (Table 1) modified from Reichard and Kunz (2009) that was averaged for each bat at each time point.

Mass-specific resting metabolic rate

To assess differences in resting metabolic rate (RMR) over time and between groups, we subjected each of the Pd-uninfected bats and Pd-infected bats described above to two respirometry trials. Beginning on day 22 posthibernation, four or five different bats were tested each day over a period of 2 wk. The timing of the first respirometry trial (late spring) was selected so as to occur within a short period after emergence from hibernation but after a period of acclimatization to our flight cages and feeding regime. Measurements were repeated a second time nearly 2 mo later, beginning at 78 d posthibernation (summer). This

latter time was selected to match a midpoint of the active feeding season, before the start of autumnal fattening. Bats typically consumed their food when lights went off. Metabolic measurements were initiated between 1200 and 1300 hours. This provided about 12 h between last feeding and testing, which ensured that bats were postabsorptive and in the inactive phase of their daily cycle (Willis et al. 2005).

For each trial, a solitary animal was placed into a 50-mL conical tube (Combitips Plus 50 mL, Brinkmann Instruments, Inc., Westbury, New York, USA) lined with plastic mesh to allow the bat to adopt a normal roosting posture. Each animal had its own tube and mesh to avoid cross-contamination. The apparatus (one for each flight cage) was attached to an airtight, open-flow respirometry system (FoxBox, Sable Systems International Inc., Las Vegas, Nevada, USA), to record RMR. We used the rate of oxygen consumption (VO_2) over a 90-min period when bats were at rest as a proxy measure for RMR. This positive pressure system provided a continuous supply of oxygen through the respirometry chamber (Voigt and Cruz-Neto 2009). Incurrent air was dried with calcium sulfate (Drierite, WA Hammond Drierite Co. Ltd., Xenia, Ohio, USA), and carbon dioxide was absorbed with sodium hydroxide (Ascarite, Sigma-Aldrich Co., St. Louis, Missouri, USA). The conical tube, with the bat inside, was placed inside a box to reduce external noise and eliminate light before testing began. Each animal was given 20 min to acclimate to and stop moving within the tube before the 90 min of measurement.

Air through the chamber was pumped at a rate of 215 mL/min (with 2% accuracy) to maintain oxygen gas (O_2) concentration in the excurrent air above 20% (Willis et al. 2005). The constraints of working within a Pd-contaminated flight cage necessitated that oxygen consumption was measured at the temperature of the flight cage (ranging from 21.0 C to 23.0 C) for both Pd-positive and Pd-negative flight cages. This is below the thermoneutral zone for little brown myotis but approximated roost temperatures in the wild during the spring (Studier and O'Farrell 1976). Although bats may choose to use torpor below the thermoneutral zone, bat body temperatures, measured using a thermocouple immediately before and after testing, were at or just above ambient temperature and did not differ significantly across condition or time. We recorded VO_2 every 2 s over the 90-min testing period. Both FoxBox units were calibrated to correct for changes in barometric pressure.

We measured the mass of each bat to the nearest 0.01 g immediately before metabolic trials. At the end of each metabolic trial, bats were returned to their respective flight cages. Metabolic rate, VO_2, was calculated as delta $O_2xFR_i/(1–F_iO_2)$, where Fr_i is the flow rate of 215 mL/min and F_iO_2 is 0.2094. For each bat, RMR was determined as the average rate of those values collected when the rate was at a low point and stable for at least 5 min. The averaged value was then transformed into a mass-specific resting metabolic rate (MSRMR) per hour (average RMR×60 min/initial mass) for each bat.

Reproductive success

Maximum reproductive output for little brown myotis is a single pup per year (Barbour and Davis 1969). Thus, for the analysis of reproductive success by WNS status, the number of pups in each flight cage that survived for at least 6 wk was assessed in relation to the number of adult females in each group (n=14 Pd-uninfected bats and n=30 WNS survivors). Because we avoided handling females and their pups in the first few weeks of life in our flight cages, data on pup mass and growth were not available.

Data analyses

Analyses were performed in IBM SPSS Statistics Version 23 (IBM Analytics, Armonk, New York, USA). Where statistically possible (even when power was low), we attempted to assess the influence of hibernation temperature and initial Pd inoculation dose on our posthibernation measures.

Changes in wing damage over time (from 19 d to 33 d) were assessed using nonparametric Wilcoxon signed ranks tests due to failure to meet the assumptions of parametric statistics. Differences in wing tissue degradation between survivors and uninfected bats at each time point were analyzed using Mann-Whitney U-tests. As nonparametric tests for covariance are limited, the potential relationship between hibernation temperature or Pd inoculation dose and subsequent wing damage were assessed with Mann-Whitney U-test and Kruskal-Wallis test, respectively. Data are presented as median and range.

The MSRMR data met the parametric assumptions of normality and homogeneity of variance, as assessed by Shapiro-Wilk's and Levene's tests. To explore the potential effects of initial Pd inoculation dose and hibernation temperature, and with sex on MSRMR at the two time points, a repeated measures analysis of variance (ANOVA) was conducted using the data for all infected bats (n=25; see Supplementary Material). Both main effects and two-way interactions were explored. Based upon our findings, this was followed by a repeated measures ANOVA to assess time, treatment, and sex effects on MSRMR. Finally, differences in reproductive success (pup alive at 6 wk posthibernation)

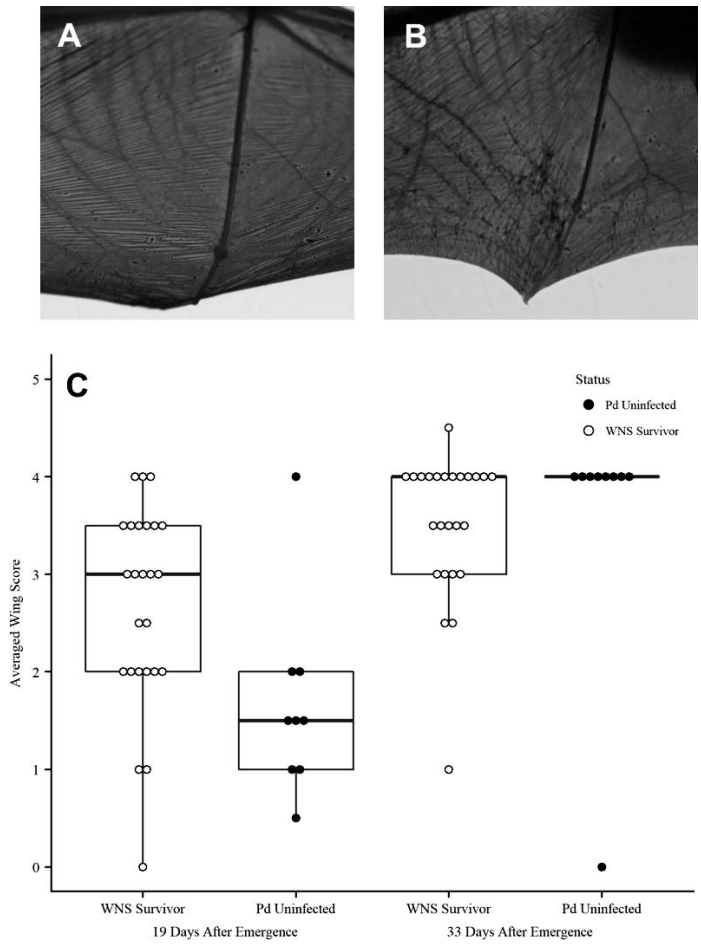

FIGURE 1. Wing damage assessment in white-nose syndrome (WNS) survivors (*Pseudogymnoascus destructans* [Pd]-infected bats) and in Pd-uninfected bats at two time points (19 and 33 d) after emergence from hibernation. Sample wing images of a WNS survivor little brown myotis (*Myotis lucifugus*) wing (A) 19 d after emergence from hibernation (19 April 2013) and (B) 33 d posthibernation (3 May 2013). (C) WNS survivors (n=25) scored significantly higher on wing damage than Pd-unaffected bats (n=9) at day 19 and significantly worsened by day 33. Data are shown as box and whisker plots, showing the median and the second and third quartiles within the box and the minimum and maximum as the whiskers and with individual data points indicated by open (WNS survivor) or closed (Pd-uninfected) circles. A larger, but not significant, rise in wing score over time was seen in Pd-unaffected bats, which were not different from WNS survivors at the second time point. See Table 1 for wing damage score.

between WNS survivors and Pd-uninfected bats were determined using a chi-square test. Data are presented as mean±SD.

RESULTS

Wing damage score

The WNS survivors had significantly higher wing damage scores 19 d posthibernation than Pd-uninfected bats (U=49.50, P=0.012), but this difference was not seen at 33 d post-hibernation (U=81.00, P=0.231; Table 1 and Fig. 1). In both groups, wing damage scores increased from day 19 to day 33, but this difference was only significant for WNS survivors (WNS survivors: 2.68±1.03 at day 19 vs. 3.52±0.74 at day 33, Z=–3.16, P=0.002; Pd-uninfected bats: median 1.5, range 0.5–4.0

at day 19 vs. median 4, range 0–4 at day 33, $Z=-1.61$, $P=0.108$). There were no significant sex differences in wing damage scores, nor did we find any relationships between wing damage score at these postemergence testing dates and hibernation temperature or Pd inoculation dose.

Mass-specific resting metabolic rate

To determine potential inoculation dose effects, MSRMR was first assessed within the Pd-inoculated bats (WNS survivors) only. In this infected group MSRMR was significantly higher in late spring (between 22 d and 35 d posthibernation; 4.55 mL O_2/g per hour±1.66) than more than 2 mo later in the summer (between 78 d and 91 d posthibernation; 3.02 mL O_2/g per hour±1.30; main effect for time: $F_{1,13}=7.857$, $P=0.015$), but there were no main effects for sex, hibernation temperature, or Pd inoculation dose, nor statistically significant interactions between these factors (but the power to detect differences in each case was <0.25). Data for all WNS survivors at both temperatures were then combined and compared to Pd-uninfected bats in a second repeated measures ANOVA. There were significant main effects for WNS status and for time but no main effect of sex or interactions between any factors. Overall, MSRMR was significantly higher in WNS survivors than in Pd-uninfected bats (3.76 mL O_2/g per hour±1.67 vs. 2.67 mL O_2/g per hour±1.29; $F_{1,30}=7.817$, $P=0.009$) and was significantly higher in late spring vs. summer (4.11 mL O_2/g per hour±1.74 vs. 2.88 mL O_2/g per hour±1.30; $F_{1,30}=4.217$, $P=0.038$; Fig. 2).

Reproductive success

Reproductive success in females did not significantly differ between WNS survivors and Pd-uninfected bats (observed vs. expected number of pups; $\chi^2=0.464$, df=1, $n=44$, $P=0.496$). Six (43%) of the 14 uninfected bats and 9 (30%) of the 30 survivors gave birth to a single surviving pup each.

DISCUSSION

Little brown myotis surviving WNS in captivity had significantly more damaged wing tissue than Pd-uninfected bats at 19 d after emergence from hibernation. Two weeks later, median wing score moderately but significantly increased in these WNS survivors, which were then not distinguishable from Pd-uninfected bats. We also found that, overall, MSRMR was significantly higher in WNS survivors than in Pd-uninfected bats and was significantly higher in late spring relative to summer. We found no evidence that hibernation temperature during the previous winter or Pd inoculation dose affected wing tissue damage levels or MSRMR, even though they both affect survivorship (Johnson et al. 2014). Our inability to detect temperature and dose effects is likely due both to low statistical power and to the fact that we designed this study of WNS survivors in such a way as to minimize potential biases in our measures due to these variables through our randomization and balancing across Pd-infected and Pd-uninfected bats. In the wild, hibernating bats select from a variety of temperature microclimates (Boyles et al. 2007), and Pd-infected bats change roost locations and temperature during the winter (Grieneisen 2011; Johnson et al. 2016). They also receive an undetermined and presumably variable loading dose of Pd conidia upon initial exposure. Our WNS survivor study bats had received a variety of doses and hibernated at either 4 or 10 C. Despite the variability in wing damage score and in MSRMR that these factors may have introduced, clear and significant differences between WNS survivors and Pd-uninfected bats were found in both wing tissue damage and MSRMR. Last, both WNS survivors and Pd-uninfected bats were capable of reproduction, at least in captivity. Although a smaller proportion of WNS survivors (30%) compared with Pd-uninfected bats (43%) successfully reared a pup (i.e., pup survived at least 6 wk), this difference was not statistically significant. Both the wing damage and the metabolic rate data suggest that little brown myotis surviving

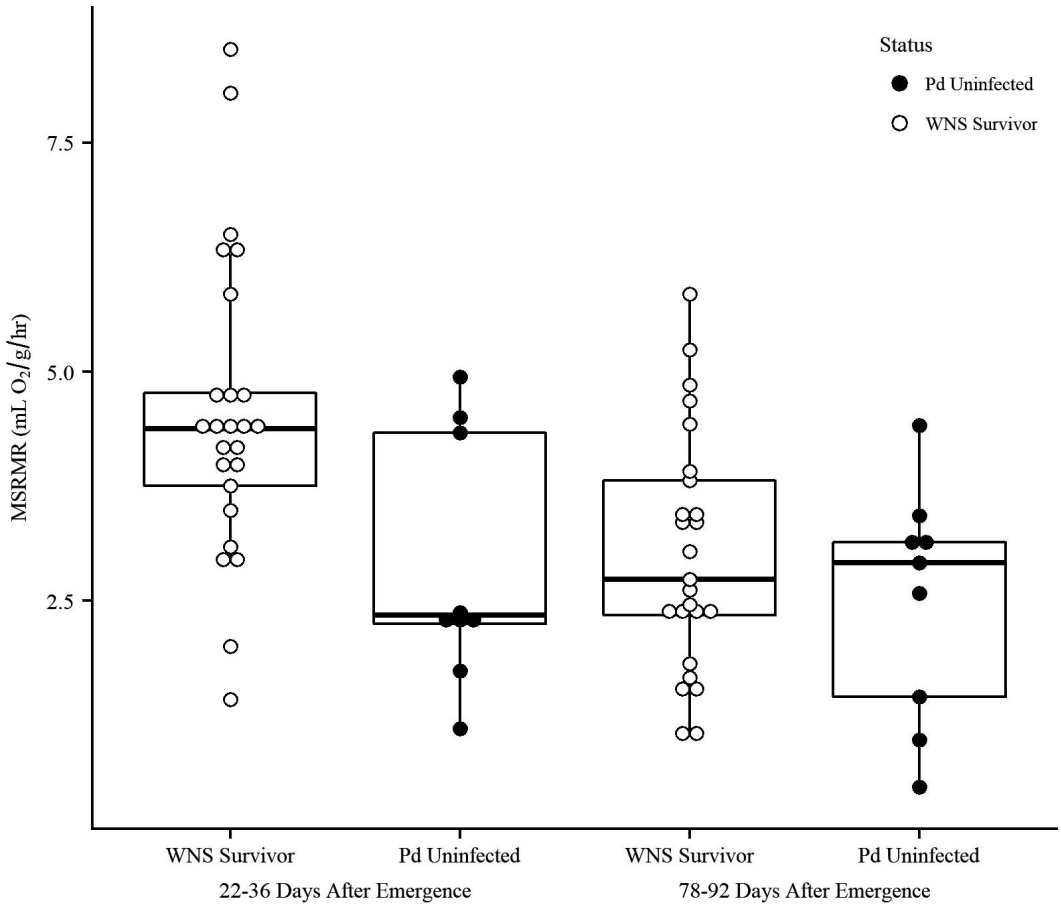

FIGURE 2. Mass-specific metabolic rates (MSRMRs; mL O_2/g per hour) in white-nose syndrome (WNS) survivors (*Pseudogymnoascus destructans* [Pd]-infected bats) and in Pd-uninfected bats at two times (between 22 d and 36 d [late spring] and between 78 d and 92 d [summer]) after emergence from hibernation. Data are shown as box and whisker plots, showing the median and the second and third quartiles within the box and the minimum and maximum as the whiskers and with individual data points indicated by open (WNS survivor) or closed (Pd-uninfected) circles. Overall, MSRMR was significantly higher in WNS survivor little brown myotis (*Myotis lucifugus*; n=25) than in Pd-uninfected bats (n=9) and was significantly higher in late spring vs. summer.

WNS experience additional energetic costs from the disease after hibernating for 148 d after Pd inoculation. However, this prolonged energetic stress may not preclude reproduction.

Damage to wing membranes has been noted in both infected and uninfected bat populations (Reichard and Kunz 2009; Weaver et al. 2009; Powers et al. 2013). In a systematic study of wing condition of bat carcasses recovered before the appearance of WNS, Powers et al. (2013) found that moderate-to-severe wing damage is not uncommon, and in fact, increased between early spring and summer. Our increase in wing damage scores from day 19 to day 33 in our Pd-uninfected bats (albeit insignificant, likely due to sample size), mirrors Powers et al. (2013) from studies of bats collected in the field, at least in some years. The increase in wing damage across the early spring into summer suggests that the physiologic processes underlying what we view as wing damage and its subsequent repair are normal phenomena. Furthermore, because wing tissue plays critical roles, yet is delicate (Cryan et al. 2010), it is likely under low levels of continual repair.

Meteyer et al. (2011) noted elevated peak wing damage at day 27 posthibernation in naturally infected WNS survivors held in captivity, which they suggested may be due to a prolific inflammatory response to Pd infection. Our data, showing a significant increase in wing damage from day 19 to day 33 posthibernation in WNS survivors and greater wing damage at 19 d posthibernation compared with Pd-uninfected bats (Fig. 1), are in line with those of Meteyer et al. (2011) who studied naturally infected bats brought into captivity at emergence from hibernation. Our data also provide the appropriate comparison to Pd-uninfected bats missing from previous studies and suggest that the processes underlying wing damage repair are upregulated earlier and to a greater extent in WNS survivors relative to Pd-uninfected bats, which probably has physiologic consequences for these surviving bats.

These physiologic consequences are likely reflected in our findings of elevated MSRMR. As predicted, WNS survivors had significantly higher MSRMRs than Pd-uninfected bats posthibernation. Increased MSRMR may be related to cutaneous gas exchange rates across damaged wing membranes (Herreid et al. 1968; Cryan et al. 2010). Alternatively, elevated resting metabolic rates may be related to immune activation and the energetic costs related to wound healing (Archie 2013). In a transcriptomic study of Pd-infected and -uninfected wing tissue by using RNA sequencing, we demonstrated altered gene expression in pathways involved in inflammation, wound healing, and metabolism in infected bats during hibernation (Field et al. 2015). These findings suggest that inflammation may contribute to changes in torpor behavior during hibernation or lead to damage upon emergence in the spring, either of which may lead to mortality of bats affected by WNS. We have also found that little brown myotis with WNS upregulate regional antifungal immune responses (Lilley et al. 2017), which may prime bats with WNS to mount an energetically costly inflammatory response to Pd upon emergence from hibernation (Meteyer et al. 2012).

The consideration of immune processes and their timing may explain why our WNS survivors had significantly higher MSRMRs than Pd-uninfected bats. Our first metabolic testing period (late spring, 22–36 d posthibernation) occurred during the same period in which Meteyer et al. (2012) described peak pathology, including inflammation. By our second metabolic testing period (summer, 78–92 d posthibernation), MSRMR in WNS survivors had significantly decreased.

Although WNS survivors in captivity have damaged wings and increased MSRMRs, reproduction was not hindered. Contrary to our prediction, we found no difference between the percentage of WNS survivor and Pd-uninfected adult females that gave birth to pups (30% vs. 43%, respectively). Although an energetic trade-off between immune processes and reproduction is frequently assumed, Burton et al. (2011) found that relationships between RMR and fitness are equivocal and highly context specific. Furthermore, our findings may not reflect natural conditions as the animals in this study were fed ad libitum once active and clearly were not subject to the challenges faced by bats in the wild.

Our study demonstrates that surviving hibernation with Pd infection has significant consequences, resulting in altered physiology (as indexed by increased wing tissue damage and elevated MSRMR) in spring, at least in bats sampled from a WNS-naïve population and tested under captive conditions. Although females in the wild and those in our study are capable of reproduction, our metabolic data suggest greater energetic costs during spring in WNS survivors. This may lead to other postpartum fitness consequences, such as longer gestation (which varies in response to available energy; Racey 1973) and thus later parturition, predisposing young of the year to gain less fat prehibernation and thus reducing their chance of survival (Thomas et al. 1990; Humphries et al. 2003).

The energetic challenges that WNS survivors face after emerging from hibernation represent a significant energetic bottleneck. As bats are forced to allocate resources to

combat Pd-inflicted damage that remains posthibernation, trade-offs with other systems likely occur. Studies that track reproductive rates in the wild and potential postpartum consequences for Pd-surviving female bats and their offspring are needed for this and other affected bat species (Reeder et al. 2016). In contrast to bats from our Pd-naïve sampled population, Lilley et al. (2016) showed that Pd-infected WNS survivors in remnant bat populations (those persisting in Pd-endemic areas) display normal thermoregulatory behavior rather than the altered thermoregulation that typically accompanies Pd infection (Reeder et al. 2012; Warnecke et al. 2012). Thus, it may be the case that surviving bats display physiologic adaptations that allow them to persist and reproduce in the presence of endemic Pd. Whether bats in these remnant populations, which have likely undergone significant selection, suffer consequences posthibernation, including fitness effects, remains to be studied.

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SUPPLEMENTARY MATERIAL

Supplementary material for this article is online at http://dx.doi.org/10.7589/2017-08-195.

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