Title: A viral mimic increases body temperature but does not affect mass or inflammation in a wild frugivorous bat

Author list: Alexis M. Heckley[1], Valeriia Bohodist[2,3,4], Camilo Calderon-Acevedo[5], Alona Prylutska[2,6], Maryna Yerofeieva[2], Kateryna Zemliana[2], Rachel A. Page[5], Ralph Simon[7,8], Daniel J. Becker[1].

Author affiliations: [1]School of Biological Sciences, University of Oklahoma, Norman, OK, USA; [2]Ukrainian Bat Rehabilitation Center, Ukrainian Independent Ecology Institute, Kharkiv, Ukraine; [3]Finnish Museum of Natural History, University of Helsinki, Helsinki, Finland; [4]Educational and Research Bat Biology Laboratory, H.S. Skovoroda Kharkiv National Pedagogical University, Alchevskyh st., 29, Kharkiv, Ukraine; [5]Center for Animal Behavior, Smithsonian Tropical Research Institute, Gamboa, Panama; [6]Falz-Fein Biosphere Reserve "Askania Nova", Ukraine; [7]Behavioral Ecology and Conservation Lab, Nuremberg Zoo, Nuremberg, Germany. [8]Department of Artificial Intelligence in Biomedical Engineering, Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Germany;

ORCIDs: Heckley: 0000-0002-5644-3535; Bohodist: 0009-0004-3068-6127; Calderón: 0000-0002-1468-3565; Prylutska: 0000-0002-0875-0573; Yerofeieva: NA; Zemliana: 0009-0004-7259-7149; Page: 0000-0001-7072-0669; Simon: 0000-0002-6083-0394; Becker: 0000-0003-4315-8628.

Corresponding author e-mail address: alexis.heckley-1@ou.edu

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Abstract:

The acute phase response is a component of innate immunity that helps fight infections. Understanding variation in this response is particularly critical in bats, which can be asymptomatic hosts of pathogens that cause disease in other animals. Although bats are most famously tolerant of viruses, research on the bat acute phase response has focused predominantly on bacterial antigens. To improve understanding of bat viral responses, we challenged wild Seba's short-tailed bats (*Carollia perspicillata*) with a viral mimic (polyinosinic:polycytidylic acid; poly:IC). We injected nine bats subcutaneously with 2 mg/kg of poly:IC (n = 3), 5 mg/kg of poly:IC (n = 3), or phosphate buffered saline (n =3). Over the next 24 hours, we measured body temperature hourly and collected body mass and blood smears for leukocyte counts every four hours. Regardless of dose, poly:IC-challenged bats had higher body temperatures compared to control bats but did not exhibit leukocytosis or reduced body mass. These findings improve understanding of how wild bats physiologically respond to viral challenges. Moreover, in showing that as little as 2 mg/kg of polyI:C can induce a febrile response, our study provides a framework to facilitate future investigations into causes and consequences of wild bat viral responses.

Keywords: Chiroptera, antiviral, poly(I:C), ecoimmunology, innate immunity, sickness

Introduction

The acute phase response is the first line of defence against pathogens (Cray et al. 2009). Mounting this immune response is energetically costly and manifests as a suite of behavioural and physiological symptoms that include but are not limited to lethargy, fever, and mass loss (Kelley et al. 2003; Adelman et al. 2009). Because these "sickness" effects can trade-off with other ecologically important processes, such as foraging, mating, or territorial defence, these responses can be deleterious to hosts (Lochmiller and Deerenberg, 2000). Yet despite its potential costs, sickness has also been hypothesized to be adaptive (Lopes et al. 2021). The behavioural and physiological changes that characterize sickness permit energy allocation towards fighting infections and thus can be ultimately advantageous to hosts. As such, characterizing the acute phase response within and among species can provide critical insight into variable infection outcomes including morbidity and mortality.

Bats (order: Chiroptera) are a particularly interesting host group for investigating variation in the acute phase response, because they can harbour pathogens that cause clinical disease in other animals, including humans, yet often while presenting as asymptomatic (Baker et al. 2013; Banerjee et al. 2020; Irving et al. 2021). For instance, whereas Marburg virus (MARV) causes severe hemorrhagic fever in humans, Egyptian fruit bats (*Rousettus aegyptiacus)* inoculated with MARV do not show signs of clinical disease (Amman et al. 2015; Jones et al. 2015). A notable absence of disease also occurs for other bat species inoculated with other viruses, including Jamaican fruit bats (*Artibeus jamaicensis*) with Middle East respiratory syndrome coronavirus (MERS-CoV; Munster et al. 2016) and *Pteropus* species with both Hendra virus (HeV) and Nipah (NiV) virus (Halpin et al. 2011). Similarly, when experimentally challenged with antigens that mimic bacterial infections (in the absence of an actual pathogen),

bats do not always show characteristic features of the acute phase response, such as fever and leukocytosis (e.g., Stockmaier et al. 2015; Melhado et al. 2020); however, these and other sickness responses do sometimes occur (e.g., reduced food intake, altered foraging and social behaviour, increased resting metabolic rates and body mass losses; Schneeberger et al. 2013a; Stockmaier et al. 2015; Otálora-Ardila et al. 2016; Otálora-Ardila et al. 2017; Cabrera-Martinez et al. 2018; Guerrero- Chacón et al. 2018; Stockmaier et al. 2018; Cabrera-Martinez et al. 2019; Melhado et al. 2020; Moreno et al. 2021; Viola et al. 2022; Viola et al. 2024). Understanding the causes and consequences of variation in the acute phase response across bats is foundational for research on the ecology and evolution of infections and could facilitate development of novel therapies to treat infections in humans and other wildlife (Banerjee et al. 2020; Becker et al. 2025).

Experimental immune challenges are desirable for studying the acute phase response in wildlife, because they isolate the effects of the immune response without possible confounding effects of an actual pathogen (Boughton et al. 2011). Moreover, in contrast to correlative observational work, these manipulations provide causal insight into how immune responses affect downstream processes (e.g., physiological responses). In bats specifically, where experimental infections can be prohibitively challenging for logistical and regulatory reasons, immune challenge in the wild can serve as a more tractable system for studying infection *in vivo* (Gilman et al. 2026). However, most experimental research in bats and other animals has focused on immunological responses to simulated bacterial infections using lipopolysaccharide (LPS), a Gram-negative bacterial cell wall component (Adelman and Martin, 2009). This work has advanced understanding of the mechanisms underlying variable host responses to bacterial infections, revealing substantial inter- and intra-specific differences in the acute phase response

63 driven by organismal features (e.g., life history variation; Previtali et al. 2012) and environmental
64 conditions (e.g., ambient temperature; Viola et al. 2024). LPS challenges have also shed light on
65 consequences of the acute phase response, such as how sickness behaviour can alter pathogen
66 transmission dynamics (e.g., Lopes et al. 2016). Given the eco-evolutionary, biomedical, and
67 conservation importance of many bat-harboured viruses (e.g., MARV, HeV, NiV, lyssaviruses), a
68 need exists for more focused work on how viral challenges (rather than bacterial challenges)
69 affect the acute phase response in bats.

70 Polyinosinic-polycytidylic acid (poly:IC) is a double-stranded RNA virus analogue that
71 initiates immune responses similar to those trigged by viral infections (Caskey et al. 2011). In
72 bats, *in vitro* poly:IC challenges have revealed diverse effects on gene expression and protein
73 abundance (e.g., Cowled et al. 2012; Li et al. 2015; Banerjee et al. 2017; Irving et al. 2020;
74 Schneor et al. 2023). To provide some examples, bat cell lines transfected with poly:IC
75 upregulate interferon signalling and interferon-stimulated gene transcription, both of which work
76 to control viral infections (e.g., Cowled et al. 2012; Mok et al. 2015). Moreover, poly:IC-
77 transfected black flying fox (*Pteropus alecto*) cell lines upregulate glycolysis-associated proteins,
78 suggesting increased energy metabolism, and downregulate ribosomal-associated proteins,
79 possibly to limit viral replication (Mok et al. 2015). As a final example, macrophages of greater
80 mouse-eared bats (*Myotis myotis*) challenged with poly:IC show both proinflammatory and anti-
81 inflammatory responses, which are hypothesized to simultaneously control infections while
82 minimizing damage to host cells (Kacprzyk et al. 2017).

83 To our knowledge, only two studies have experimentally challenged wild bats *in vivo*
84 with poly:IC. With *M. myotis*, a Eurasian vespertilionid species, Seltmann et al. (2022) found no
85 evidence of fever, leukocytosis, or body mass changes following poly:IC treatment, although

challenged bats displayed an overall increase in the neutrophil-to-lymphocyte ratio. Some of these findings contrast with work on the great fruit-eating bat (*Artibeus lituratus*), a phyllostomid distributed across the Greater Antilles, Central America, and northern South America, where poly:IC caused bats to lose body mass and develop fever in addition to increasing resting metabolic rate (Triana-Llanos et al. 2019). Much like responses to LPS, these poly:IC studies suggest a high extent of diversity in virus-associated acute phase responses. Yet given the scarcity of this work, additional studies in other species are critically needed to improve understanding of viral-associated acute phase responses in wild bats (Becker et al. 2025).

Here, we investigated how simulated viral challenges with poly:IC affect the acute phase response in Seba's short-tailed bat (*Carollia perspicillata*), a small-to-medium sized phyllostomid bat species that is likewise distributed across Central and South America (Cloutier and Thomas, 1992). We specifically injected wild-caught *C. perspicillata* in Panama with polyI:C and recorded changes to body temperature, body mass, and total leukocyte counts over a 24-hour period. Our results show that *C. perspicillata* develop fever, but do not exhibit body mass losses or changes to leukocyte counts, following poly:IC challenge. These results improve understanding of how these bats respond to viral antigens while also providing a framework for future researchers conducting experimental immune challenges in wild bats.

Methods

Bat capture and immune challenge

On February 12th, 2026, we captured nine adult male *C. perspicillata* using mist nets outside of a large roost in Gamboa, Parque Nacional Soberanía, Panama. We focused only on males because

most captured females during this time were pregnant. Upon capture, we transferred bats to a large outdoor flight cage at the Smithsonian Tropical Research Institute, where they were housed together within a smaller tent (60 cm x 40 cm x 40 cm). We injected each bat with a thermo-sensitive pit tag (Biotherm 13 PIT tag, Biomark) for individual identification and body temperature monitoring. Over the next several nights of acclimation, bats were provided water and banana *ad libitum*.

The experiment began slightly less than three days after capture (~ 68 hours). Starting at 7 pm, we removed bats from their tent and recorded mass with a Pesola scale (to the nearest 0.1 gram) and body temperature by scanning the thermo-sensitive PIT tags. Using 26- or 27-gauge sterile needles, we drew a small amount of blood (< 10 μL) by lancing the propatagial vein. We collected blood with heparinized capillary tubes and transferred a drop to a clean glass slide to make a thin blood smear. We next injected each bat subcutaneously with one of either a low-dose of poly:IC (2 mg/kg; n = 3 bats), a high-dose of poly:IC (5 mg/kg; n = 3 bats), or phosphate-buffered saline (PBS; using the equivalent volume as a 5 mg/kg poly:IC dose; n = 3 bats). While injecting, we alternated through treatments rather than injecting all three bats within one treatment group first.

Following injections, we immediately placed bats into small tents (n = 9 tents; each bat held individually), still within the outdoor flight cage. Within each tent, we provided 60 g of banana. Six of the tents were 60 cm x 40 cm x 40 cm, and the other three tents were 30 cm x 30 cm x 30 cm. Every hour following the injections (for 24 hours), we recorded body temperature by scanning each bat with a portable PIT tag reader (GPR Plus, Biomark). We aimed to be as minimally disruptive as possible, by holding the PIT tag reader to the side of the tent to minimize disturbance. Every four hours following the challenges, we removed bats from their tents, re-

measured mass, and collected blood to prepare another thin blood smear. The total volume of blood collected over this 24-hour period represented substantially less than 1% body mass (Sikes, 2016). After the trial was complete, bats were released at their capture location.

Hematological analysis

We used blood smears to estimate total white blood cell counts (Maceda-Veiga et al. 2015). Specifically, we used a light microscope to quantify the mean number of leukocytes from 10 fields of view at 400X magnification (Schneeberger et al. 2013b). One observer (AMH) performed all white blood cell counts.

Statistical analysis

We conducted all analyses in R v 4.3.1 (R Core Team, 2023). To test how immune challenges affect *C. perspicillata* body temperature (°C), body mass (g), and total white blood cell counts, we fit three generalized additive mixed models (GAMMs)—one for each physiological measurement—using the *mgcv* package (Wood, 2011). Rather than using the raw physiological measurements, we instead calculated the change from "baseline" (i.e., the measurement taken immediately prior to injections, at hour zero) for each sampling event. In each model, we included treatment (PBS, low-dose poly:IC, high-dose poly:IC) as an ordered factor as a fixed effect, along with smoothed effects of time and treatment–time interactions using thin plate regression splines (Wood, 2003). We also included bat identity as a random intercept to account for repeated measurements from the same individuals. All GAMMs were fit using restricted maximum likelihood and used Gaussian errors.

Results

We found that poly:IC treatment significantly increased *C. perspicillata* body temperature, and that temperature increased linearly with dose (i.e., PBS < low dose < high dose; Figure 1; Table 1). Within the low-dose group, temperature changes differed non-linearly over time (EDF = 2.50), whereas we did not detect a time effect for the high-dose group (Figure 1; Table 1). This GAMM explained 57% of the deviance and 55% of the variance in temperature (i.e., $R^2 = 0.55$).

Although our preliminary sample size precludes formal statistical investigation into pairwise hourly differences, body temperature was immediately elevated in the high-dose poly:IC group, while it initially decreased in the PBS and low-dose poly:IC groups (mean temperature change from pre-injection baseline at hour one = high-dose poly:IC: 0.23°C; low-dose poly:IC: -1.87°C; PBS: -1.93°C). The high-dose and low-dose poly:IC groups differed by an average of >1°C only for the first three hours of the trial (3/24 hours); however, by hour four and throughout the remainder of the trial, the mean temperature difference between these groups was <1°C. By contrast, the high-dose poly:IC and PBS bats differed by an average of >1°C for most of the trial (17/24 hours), including in hours 21–23.

Treatment did not affect body mass (Figure 2) or total leukocyte counts (Figure 3) independently or in interaction with time (Table 1). These GAMMs explained 77% and 28% of the deviance and 72% and 23% of the variance, for mass and leukocyte counts, respectively.

Discussion

Characterizing the acute phase response across species and contexts can help explain variation in infection outcomes (e.g., morbidity and mortality). These investigations are particularly interesting and epidemiologically relevant with bats, as these flying mammals often harbor pathogens—particularly viruses—that are dangerous to other animals, while seemingly remaining asymptomatic (Baker et al. 2013; Banerjee et al. 2020; Irving et al. 2021). To improve understanding how bats respond to viral challenges, we experimentally challenged wild phyllostomid bats with a viral mimic (i.e., poly:IC). Regardless of the dose administered (i.e., 2 mg/kg vs 5 mg/kg), immune-challenged bats did not differ from control bats in body mass or total leukocyte counts over a 24-hour period. Instead, the primary indicator of an immunological response was fever; immune-challenged bats had higher body temperatures compared to control bats. Collectively, our preliminary findings improve understanding how wild bats respond to viral infections and will help facilitate future work on viral challenges in wild bats.

In our study, regardless of the poly:IC dose administered, *C. perspicillata* exhibited increased body temperature. Between the high-dose and control bats, this temperature difference was >1°C for 71% of the trial, revealing a substantial energetic cost to *C. perspicillata*. Indeed, foundational physiological work suggests a temperature increase of 1°C is associated with a metabolic rate increase of at least ~10% (Kluger 1991), and, in bats specifically, LPS challenges that increase body temperature by 1.5°C are associated with a 40% increase in resting metabolic rate (Guerrero- Chacón et al. 2018). Following poly:IC treatment, *A. lituratus*, another bat species from the same family as *C. perspicillata* (Phyllostomidae), also develops fever (Triana-Llanos et al. 2019), whereas the more distantly related *M. myotis* does not—even at much higher doses (i.e., 25 mg/kg compared to our highest dose of 5 mg/kg; Seltmann et al. 2022). These results suggest that more closely related or more ecologically similar species could show similar

197 febrile responses to viral challenges. However, methodological differences also point to the
198 possibility that these discrepancies could be artefacts rather than shared ecology or evolutionary
199 history. For instance, in addition to different doses, prior work on *A. lituratus* reported body
200 temperature six hours after poly:IC treatment, whereas that on *M. myotis* reported body
201 temperature 24 hours later (Triana-Llanos et al. 2019; Seltmann et al. 2022). As revealed in our
202 study, febrile responses to poly:IC can vary in a time- and dose-dependent manner; as such, these
203 sampling windows (six vs 24 hours) might be comparatively incompatible. This last point
204 emphasizes the value of fine-scale temporal sampling for characterizing febrile responses to
205 poly:IC and stresses that future comparative work with wild bats necessitates standardizing
206 protocols to the fullest extent possible, while acknowledging that different species might require
207 different doses to elicit similar responses and that these responses might differ under varying
208 conditions or timescales (Kluger 1991).

209 We did not observe any body mass or leukocyte count differences between poly:IC- and
210 PBS-injected bats. Given our small sample size, it is possible that we simply did not have the
211 statistical power to detect these effects. On the other hand, these findings could be biologically
212 meaningful, and they are consistent with previous work on *M. myotis*, which also do not lose
213 body mass or exhibit leukocytosis following poly:IC treatment (Seltmann et al. 2022). In *A.*
214 *lituratus,* although leukocytosis was not measured, bats injected with poly:IC lost ~1 g of body
215 mass more than control bats after six hours, although this effect had seemingly dissipated by 24
216 hours post-treatment (Triana-Llanos et al. 2019). Variable body mass responses to viral
217 challenges differ from many bacterial challenge studies, which are largely more consistent, at
218 least for Neotropical bat species. Indeed, Neotropical bats, including but not limited to *C.*
219 *perspicillata,* typically lose mass when challenged with LPS (e.g., Schneeberger et al. 2013a;

Stockmaier et al. 2015; Otálora-Ardila et al. 2016; Otálora- Ardila et al. 2017; Cabrera-Martinez et al. 2018; Cabrera-Martinez et al. 2019; Melhado et al. 2020; Viola et al. 2022; Viola et al. 2024). Body mass loss following an immune challenge can be due to decreased appetite or increased energy metabolism, both of which facilitate allocating resources towards fighting infection (Lochmiller and Deerenberg 2000). The mechanisms for why bats do not always lose mass following viral challenges remain unclear. Future comparative work could aim to determine the extent to which this effect is a feature of the bacterial versus viral response and by how much this variation can be explained by ecological (e.g., diet), evolutionary, or methodological (e.g., time in captivity, challenge dose) differences between individuals, species, and studies.

Despite the lack of mass or leukocyte effects associated with poly:IC treatment, the febrile response in our study provides the practically useful information that as little as 2 mg/kg of poly:IC is sufficient to elicit an acute phase response in *C. perspicillata*. Similar results were observed with 5 mg/kg of poly:IC, with the timing of body temperature changes being the apparent difference between our two doses. Specifically, body temperature of the high-dose group (i.e., 5 mg/kg) was seemingly elevated relative to the control group immediately, whereas temperature of the low-dose group (i.e., 2 mg/kg) increased more gradually, converging with the high-dose group approximately four hours after treatment. Although these hourly pairwise comparisons were not validated statistically, and must therefore be interpreted cautiously, we highlight them to help inform the methodological decisions of researchers studying viral-associate responses in wild bats. In short, depending on the goals of a study and the timing of desired physiological effects, either dose could be suitable to administer to wild bats.

Our work therefore reveals that while *C. perspicillata* do not show all the classically expected components of the acute phase response to viral challenge, a febrile response does

develop following subcutaneous injection with poly:IC. Discussing our findings in the context of other bat immunology research emphasizes that study-level and species-level variation in the acute phase response is the norm. More widespread challenge trials with viral mimics are needed in wild bats to untangle the sources and extent of this variation and its ecological and evolutionary implications. Our study provides practically useful information on the dosage and timing of the acute phase response initiated by poly:IC in one of the most studied Neotropical bat species. Our findings are therefore useful to guide this future work for researchers interested in making comparisons within or across species and for designing studies investigating how the acute phase response affects, or is affected by, other behavioural or physiological processes. More broadly, bats harbour many viruses that cause disease in other animals while presenting as asymptomatic, yet most past work with bats has focused on their responses to bacterial antigens. In monitoring fine-scale physiological changes following challenge with a viral mimic, our study begins to fill an important gap in understanding how wild bats respond to viral infections.

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Tables and figures

Table 1. Effects of treatment (as an ordered factor) on change from pre-injection baseline for each physiological measurement. Effects are presented as model coefficients or estimated degrees of freedom (EDF) and test statistics. All GAMMs include a random intercept of bat identity.

Response	Term	β	t	EDF	F	p
Body	Intercept	-0.90	-7.73			<0.001
temperature	Treatment (linear)	0.86	4.27			<0.001
	Treatment (quadratic)	-0.25	-1.25			0.213
	s(time point)			1.39	11.39	<0.001
	s(time point) : low-dose poly:IC			2.50	2.85	<0.001
	s(time point) : high-dose poly:IC			0.40	0.15	0.12
Body mass	Intercept	1.76	7.45			<0.001
	Treatment (linear)	-0.20	-0.49			0.62
	Treatment (quadratic)	0.70	1.71			0.09
	s(time point)			4.03	19.05	<0.001
	s(time point) : low-dose poly:IC			0.00	0.00	0.24
	s(time point) : high-dose poly:IC			0.00	0.00	0.77
Leukocyte count	Intercept	0.46	3.39			<0.01
	Treatment (linear)	-0.36	-1.54			0.13
	Treatment (quadratic)	-0.24	-1.00			0.32
	s(time point)			0.92	2.60	<0.001

s(time point) : low-dose poly:IC	0.00	0.00	0.35
s(time point) : high-dose poly:IC	0.14	0.04	0.19

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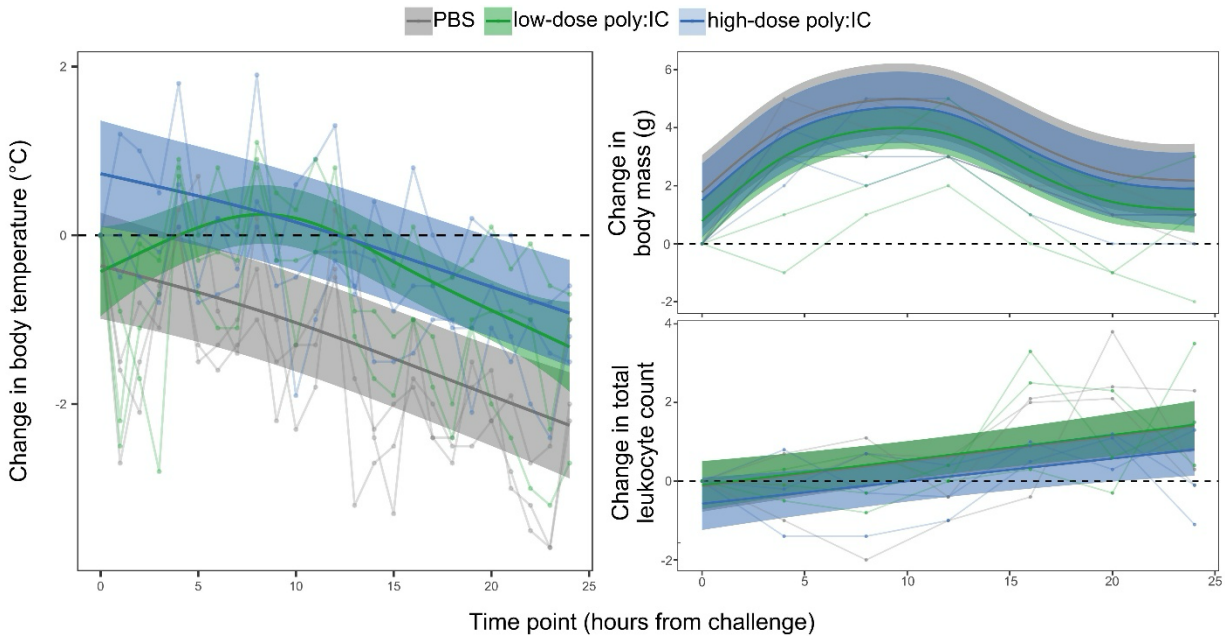

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405 Figure 1. Effect of time on change in body temperature, body mass, and total leukocyte count
406 from baseline. The trendlines with 95% confidence intervals were estimated from the GAMM,
407 and the data points reflect raw data. The data, trend lines, and ribbons are colored by treatment,
408 and the horizontal dashed line reflects baseline (i.e., measurement at hour 0).