The Wilson Journal of Ornithology 133(3):000–000, 2021 DOI: 10.1676/20-00123

Sample point variation in gastrointestinal bacteria of migrating Northern Saw-whet Owls (*Aegolius acadicus*) of eastern North America

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ABSTRACT—We characterize bacteria of the gastrointestinal tract of 28 Northern Saw-whet Owls (*Aegolius acadicus*) netted on the Mohonk Preserve near New Paltz, New York, during autumn of 2017. We used selective culture media to determine the presence and assess the point infection level of *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella* spp., and *Staphylococcus aureus* in the buccal, cloaca, feces, and pellets of Northern Saw-whet Owls. We tested for associations between bacteria type, sample point location, and infection levels and then assessed the influence of bacterial infection on the health of owls using multivariate analysis of mass/wing chord body condition index estimates and heterophil to lymphocyte ratios. All Northern Saw-whet Owls tested positive for at least one type of bacteria. We found an inverse relationship between the number of bacteria types detected per individual and the percentage of Northern Saw-whet Owls affected, with 46.43%, 32.14%, and 3.57% of individuals showing presence of 2, 3, and 4 types of bacteria, respectively. Multivariate analysis revealed a significantly heightened presence of *S. aureus* in the buccal cavity, and Spearman's rho analysis revealed positive correlations between site colonization levels and bacterial species levels. We found no significant relationship between bacterial load and mass/wing chord body condition indexes or heterophil to lymphocyte ratios. We did, however, find a positive relationship between heterophil to lymphocyte ratios and *Leucocytozoon* infections. Providing baseline information of common microflora can help future research recognize potentially harmful gastrointestinal pathogens. Therein, as the first study to characterize bacteria of the Northern Saw-whet Owl, this research adds to the information portfolio of the species and may benefit future research efforts. *Received 1 November 2020. Accepted 1 October 2021.*

Key words: avian, b lymphocyte, body condition index, buccal, heterophil, *Leucocytozoon*, microbiome.

Variación en las bacterias gastrointestinales en un punto de muestreo de mochuelos *Aegolius acadicus* migratorios del este de Norteamérica

RESUMEN (Spanish)—Caracterizamos las bacterias del tracto gastrointestinal de 28 mochuelos *Aegolius acadicus* redeados en la reserva Mohonk Preserve cerca de New Paltz, New York, durante el otoño de 2017. Usamos medios de cultivo selectivos para determinar la presencia y determinar el nivel de infección de *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella* spp. y *Staphylococcus aureus* en la cavidad bucal, cloaca, heces y egagrópilas de estos mochuelos. Sometimos a prueba asociaciones entre tipos de bacteria, localización del punto de muestreo y niveles de infección, y posteriormente determinamos la influencia de infecciones bacterianas en la salud de los mochuelos usando análisis multivariados de estimaciones de índices de condición corporal de masa/cuerda alar y proporción de heterófilos a linfocitos. Todos los mochuelos dieron positivo a al menos un tipo de bacteria. Encontramos una relación inversa entre el número de tipos de bacteria detectados por individuos y el porcentaje de mochuelos afectados, con 46.43, 32.14 y 3.57% de los individuos mostrando la presencia de 2, 3 y 4 tipos de bacteria, respectivamente. Los análisis multivariados revelaron una presencia significativamente elevada de *S. aureus* en la cavidad bucal y los análisis rho de Spearman revelaron correlaciones positivas entre niveles de colonización de sitios y niveles de especies de bacterias. No encontramos relación significativa entre carga bacteriana y los índices de condición corporal masa/cuerda alar o en las proporciones de heterófilos a linfocitos e infecciones de *Leucocytozoon*. Proporcionar información de línea base de microbiota común puede ayudar futuras investigaciones de patógenos gastrointestinales. Por ello, como primer estudio que caracteriza las bacterias de este mochuelo, esta investigación agrega al portafolio de información de esta especie y podría beneficiar futuros esfuerzos de investigación.

Palabras clave: aviar, bucal, heterófilo, índice de condición corporal, *Leucocytozoon*, linfocitos b, microbioma.

Intestinal microbiota may affect the hosts' nutrition physiology, body growth, protection to pathogen infection, immunity, and other physiological mechanisms (Kohl 2012, Montiel-Castro et al. 2013, Vela et al. 2015). In mammals, the microbiota, derived from the mother through the birthing process and from the environment, can impact cognitive functions such as learning and memory (Montiel-Castro et al. 2013). Birds, however, hatch from near-sterile eggs and must acquire their microbiome almost completely from the environment (Kohl 2012). It is hypothesized that the pressures of flight have selected for a unique peristalsis–retroperistalsis pattern in digestion to reduce the weight of the gastrointestinal (GI) tract in birds (Orosz 2015). Body weight has also been minimized by the loss of teeth and limited jaw musculature. Therein, birds do not chew their food and rely on the grinding action of the gizzard and on chemical digestion (Johnston 2014), the latter of which results in a dramatic difference in the biochemical environment of different parts of the GI tract (Klasing 1999). It

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is hypothesized that carnivorous birds acquire the bulk of their intestinal biota from the prey they eat. While seed eating birds are found to host mostly gram-positive bacteria, birds of prey also host gram-negative bacilli in their normal bacterial flora (Houston and Cooper 1975, Bangert et al. 1988). This disconnect between host and microbiota in birds may explain why our understanding of the role of GI microbes in avian hosts is lagging behind that of mammals, with about one-tenth as many publications on avian gut microbiota as there are on mammals (Grond et al. 2018).

The Northern Saw-whet Owl (*Aegolius acadicus*, hereinafter saw-whet owl) is a common raptorial species that breeds in boreal and temperate forests of North America and winters in temperate forests (Cannings 1993, Rasmussen et al. 2020). Feeding mostly on small rodents, such as red-backed vole (*Myodes* spp.) and deer mice (*Peromyscus maniculatus*) (Swengel and Swengel 1992), it may be reasonable to assume the intestinal flora of saw-whet owls is similar to that of other carnivorous birds. Recent studies, however, reveal that intestinal flora may vary significantly between species, and even individuals, dependent on the level of cohabitation of microflora with pathogens such as helminths (Reynolds et al. 2015) and haemosporidia. Unlike other birds of prey, owls lack a true crop to store food and initiate the absorption of amino acids before their meal is passed to the glandular stomach (proventriculus).

Owls also differ from other raptors by the fact that they have 2 comparatively large ceca. This is important because the peristalsis–retroperistalsis action of the rectum in birds transports urates to the ceca where the nitrogen of the urine is used by cecal bacteria to produce amino acids and protein. The newly produced proteins are then digested and absorbed through the cecal epithelium. Thus, from the get-go digestion in owls is set apart from other raptors, and birds in general, by the lack of a crop and their comparatively large ceca. Non-pescatarian birds that eat meat also have shorter intestines than grain- or fish-eating birds, which may partially explain the larger ceca in owls. Indigestible materials such as fur, bones, teeth, and feathers are compressed by the gizzard to form a pellet that is regurgitated. Compared to raptors such as hawks or falcons, owls produce pellets with more bones and complete skeletons because their stomachs are less acidic (Duke et al. 1975, Cummings et al. 1976). These differences in anatomy suggest that the gastrointestinal microflora of the saw-whet owl could be unique to the species.

In this study, we used both general and selective media to characterize the presence and absence of 4 common bacterial species, i.e., *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella* spp., and *Staphylococcus aureus*, in migrating saw-whet owls. These 4 bacterial species are easily cultured in the laboratory, and much is known about their life cycles, metabolism, and pathogenicity. Furthermore, these bacteria are known to colonize the GI tract of other birds of prey (Houston and Cooper 1975, Cooper 1980, Bangert et al. 1988, Vela et al. 2015).

Escherichia coli is a facultatively anaerobic proteobacterium that can use either oxygen or nitrate as its terminal electron acceptor, can utilize amino acids as an energy source when oxygen is not available, and can ferment lactose (Russell and Jarvis 2001). Its ability to respire both aerobically and anaerobically allows it to colonize anaerobic intestines. For the most part, *E. coli* is a harmless symbiont in the avian GI tract unless it crosses the epithelial lining and enters the blood, where it can produce colisepticemia in the heart, liver, and spleen (Jawetz et al. 1974, Russell and Jarvis 2001, Barnes et al. 2003). Colisepticemia results in high morbidity and high mortality and is of particular concern to the poultry industry (Barnes et al. 2003). *Pseudomonas aeruginosa* is a common opportunistic avian pathogen that affects the upper respiratory tract, causing rhinitis, sinusitis, and laryngitis (Benskin et al. 2009). Salmonellae are gram-negative facultative anaerobes that are known to cause gastroenteritis (Gianella 1996). *S. aureus* is relatively common in domestic poultry and is known to cause arthritis, tendonitis, septicemia, and bumblefoot (Cooper 1980, Benskin et al. 2009). *Staphylococcus* spp. are opportune pathogens, often infecting individuals with impaired immunity (Benskin et al. 2009, Zigo et al. 2017). In humans, the primary reservoir for staphylococci is the vestibulum nasi, or nostril of the nose. It is hypothesized that staphylococcal cells adhere to human epithelial cell surface receptors through physicochemical mechanisms, possibly mediated by hydrophobic interactions (Kluytmans et al. 1997).

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Here, we conducted a field survey of GI bacteria in migrating saw-whet owls. We expected to observe patterns of bacterial colonization since different GI sample sites have different biochemistry creating different environments (Klasing 1999, Vela et al. 2015). We also assumed results would most likely indicate chronic levels of bacteria and would not be indicative of clinical infection since the individuals sampled were healthy enough to migrate. However, in an attempt to unveil subtle effects of infection, we assessed the relationship between bacterial presence and disease markers since presence of pathogenic bacteria might dampen immune response, or commensal bacteria might provide protection against pathogens (Kohl 2012, Montiel-Castro et al. 2013, Young and Proudfoot 2014). Additional variables included in this study are body condition (the log-adjusted body mass/natural wing chord length index; Whalen and Watts 2002) as a proxy for general health, heterophil to lymphocyte ratios (H:L) as a marker of inflammatory response, and exoerythrocytic *Leucocytozoon* co-infections (Mabbott 2018). We chose these variables for 2 reasons, the first being convenience and the second was the opportunity to test the utility of different metrics. The body condition index (BCI) is a simple measure that can easily be collected in the field without the use of laboratory equipment, and it was proven sensitive enough to reveal the effect of population density on the health of migrating saw-whet owls: the BCI went down as owl density increased (Whalen and Watts 2002). Assessing the effect of infection on heterophil to lymphocyte ratios is more invasive and time consuming than BCI analysis as it requires collecting and analyzing blood samples (Wilcoxen et al. 2015). However, H:L ratios may provide a more immediate response to stress than the BCI, which requires considerable loss of body fat before analysis of BCI is affected (Schamber et al. 2009, Labocha and Hayes 2012, Young and Proudfoot 2014). Heterophils, the predominant granulocyte in birds and reptiles, are highly phagocytic and present in large quantities in the first 6–12 h of the inflammatory response; lymphocytes tend to appear later in the inflammatory response (Harmon 1998). In addition, establishing baseline data on H:L ratios of infected and noninfected owls may ultimately allow researchers to assess the organism's ability to resist microbial infections (Millet et al. 2007). Fortunately, our GI study was conducted concurrent to a long-term study on haemosporidia prevalence in saw-whet owls that required us to estimate H:L ratios to assay the effect of infection (GAP, unpubl. data). Therefore, we capitalized on the opportunity to add this metric, and the haemosporidia variable, to the analysis by incorporating a small portion of data from a concurrent parasite study into our analysis. And, to our knowledge, the relationship between exoerythrocytic parasites and intestinal flora has not been explored.

Methods

Field sampling

We used the audiolure mist-netting technique (Erdman and Brinker 1997) to capture 122 saw-whet owls at Mohonk Mountain Preserve near New Paltz, New York, from 1 October to 28 November 2017. We tagged each saw-whet owl with a USGS aluminum leg band for future identification, weighed, estimated the natural wing chord length using a "wing and tail ruler" (Avinet Inc., Portland, Maine, USA), and collected ~200 µL of whole blood from the metatarsal vein. We used ~5 µL of whole blood to create a thin blood smear on a microscopy slide to assess prevalence of haemosporidia. We labeled each slide with the corresponding bird's band number, then air dried and immediately fixed each dry blood smear with 100% methanol (Bennett 1970). We stored the remaining blood (~195 µL) in 2 mL cryotubes with 1 mL of preservation buffer (Longmire et al. 1988) for a long-term genetics study on haemosporidia in saw-whet owls (GAP, unpubl. data).

In a concurrent audition study, a subset of saw-whet owls ($n = 20$) was transported to animal facilities at Vassar College in Poughkeepsie, New York (de Koning et al. 2020). Each saw-whet owl was housed individually in commercial pet carriers (61 × 41 × 37 cm; PetCo, San Diego, California, USA) lined with white food-grade butcher paper. The owls were provided with a perch and commercially obtained frozen adult mice (The Gourmet Rodent, Newberry, Florida, USA) ad libitum. The pet carriers provide ample room for the saw-whet owls to move about and fully stretch their wings. We used sterile polyester swabs (Puritan Medical Products, Guilford, Maine, USA) with a 2 mm tip to swab the buccal cavity

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and cloaca of all owls held in captivity; any feces or pellets released during their tenure were also swabbed for cultivable bacteria. All saw-whet owls were released at the site of capture within 48 h. In addition to the 20 saw-whet owls sampled while being held for audition studies, we swabbed the buccal cavity and cloaca of 8 saw-whet owls at the banding station and released the owls on site.

Pellets pass through the buccal cavity when regurgitated. This action may cause bacteria that reside in the buccal cavity to be picked up by the pellet during regurgitation. To reduce the chance of cross contamination between the buccal cavity and pellet samples, we used sterile stainless steel lab probes to tease pellets apart and expose the core pellet material, which was then swabbed for cultivable bacteria. We assumed that the material at the inner core of the pellet was not contaminated by bacteria residing in the buccal cavity while the pellet was cast. We dipped swabs in sterile water (Fisher Scientific, Fair Lawn, New Jersey, USA) and swabbed target sites for 15 s (CDC 2010, Landers et al. 2010). We placed the tip of each swab into bar coded 2 mL cryotubes with 1.0 mL of tryptic soy broth (TSB/25% glycerol solution; FAO 2007) and stored the samples at –20 °C until plating. In total, TSB broth from 28 cloacal, 28 buccal, 22 fecal, and 13 pellet samples were used to inoculate 465 plates of selective culture media for point sample testing, 5 plates/sample.

Bacterial culture

We thawed TSB samples at room temperature and plated aliquots on general and selective media to assess target bacteria levels. We used tryptic soy agar (TSA) as a control to estimate total culturable bacteria and 4 selective agars, Cetrimide (CA), eosin methylene blue (EMB), mannitol salt (MSA), and Xylose Lysine Deoxycholate (XLD), were used to isolate *Pseudomonas aeruginosa*, *E. coli*, *Staphylococcus aureus*, and *Salmonella* spp., respectively. We followed manufacturer's (Sigma-Aldrich) recommendations to sterilize CA, EMB, MSA, TSA, and XLD agar, and poured the agar into sterile plates under a biologically sterile safety cabinet (hood) that utilizes HEPA filters, according to standard microbiological procedure.

We conducted pilot tests using TSA media to estimate concentration levels and used laboratory grade sterile water (Fisher Scientific) to dilute (10^{-1}, 10^{-2}) aliquots for testing to ensure the number of bacteria colony forming units (CFU) were not too numerous to count, and multiplied the colony number by the dilution levels to normalize the data for comparisons. We selected dilution levels by the number of CFUs in the initial solution and from the number of CFUs in subsequent serial dilutions that tested positively. We assumed that the levels we selected would include all bacteria types that were represented in the initial solution. However, we recognize that diluting samples may cause a false negative result for bacteria that are at extremely low concentrations in the initial solution and, thus, note that our results are possibly a conservative representation of infections. We used the same sterile hood in which the media was prepared to dispense 100 μL of TSB on general and selective media and used a sterile glass wand to spread the sample across the media. We incubated culture plates at 39 °C for 48 h to mimic the body temperature of the bird (Ligon 1969). Verification of bacteria type was 2-fold. The first part was the use of selective media, which, according to the manufacturer, is very specific to target bacterial strains. The second part was visual identification based on colony morphology and color indicators. For example, EMB is selective for gram-negative bacteria but includes a color indicator to distinguish bacteria that ferment lactose (*E. coli*) from those that do not (*Salmonella*). Cetrimide agar is not known to isolate species other than *Pseudomonas*. MSA contains high salt concentrations that selects for gram-positive bacteria, but only colonies that turned the media yellow were counted because when *Staphylococcus aureus* ferments mannitol, acid is produced. *Salmonella* was distinguished from *Shigella* on XLD plates by a similar color change since *Salmonella* can ferment sugar, lowering pH of the plate and turning colonies yellow. CFU were counted by hand when colonies were few and with the aid of photographic imagery and the multi-point tool of ImageJ 1.51s when colonies were numerous. We calculated CFU/mL at sample sites per individual by estimates of total selective bacteria load and by individual species isolated. We included a negative control with each sample set to test for contamination.

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Table 1. Percent infection across 4 sample points in the gastrointestinal tract of Northern Saw-whet Owls captured on the Mohonk Mountain Preserve near New Paltz, New York, during October and November of 2017. Each sample was used to inoculate 4 selective media plates to test for the prevalence of *E. coli*, *P. aeruginosa*, *Salmonella* spp., and *S. aureus*.

Site	*n*	*E. coli*	*P. aeruginosa*	*Salmonella* spp.	*S. aureus*
Buccal	28	79.31	3.45	0.00	93.10
Cloacal	28	75.86	3.45	6.9	17.24
Feces	22	81.82	4.55	36.36	4.55
Pellet	13	92.31	7.69	15.38	23.08

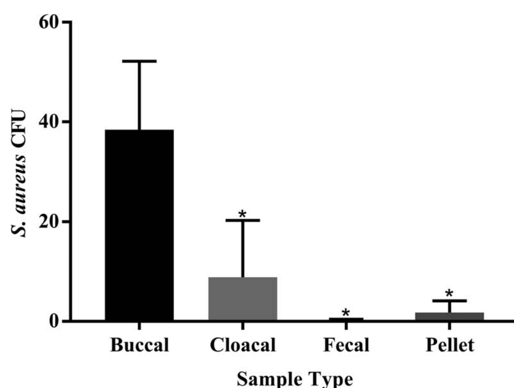

Figure 1. Distribution of positive *Staphylococcus aureus* results at 4 sample point locations in the gastrointestinal tract of Northern Saw-whet Owls ($n = 28$) captured on the Mohonk Preserve near New Paltz, New York, during October and November of 2017. Selective media was used to test for the amount of *S. aureus* colonization. Bacterial load was calculated through colony count. Results are presented as the mean of the square root of CFU/mL. * = $P < 0.05$ when compared to buccal colonization. Error bars: 95% CI.

Blood samples

We examined blood smears at 200× for 100 fields to determine prevalence of *Leucocytozoon*, the most common haemosporidian found in migrating saw-whet owls (Young and Proudfoot 2014, Warne et al. 2015, Carlson et al. 2018). We used the same blood smears to estimate H:L ratios by microscopy at 1,000× to approximate stress levels in migrating saw-whet owls (Gross and Siegel 1983, Harmon 1998, Vleck et al. 2000), and used those H:L ratios to assess the influence of bacterial infections, *Leucocytozoon* prevalence, and a combination of the 2 on the owls' health.

Data analyses

We used SPSS 21.0 (IBM Corp., Armonk, New York, USA) for all comparisons, and applied square-root transformation to the raw data to control for outliers. When comparing colonization levels of bacteria species to H:L ratios, BCI, and *Leucocytozoon* data, only buccal and cloacal counts were considered to maximize sample number. When analyzing relationships between total fecal bacteria, only buccal, cloacal, and fecal samples were analyzed, and when total pellet bacteria was studied only data from individuals with complete sets (buccal, pellet, fecal, cloacal) were considered.

A multivariate general linear model (GLM) was used to determine if species-specific bacterial load was different across sample sites. Data were not normally distributed and had unequal sample sets across sites so a Dunnett's T3 post hoc analysis was used with GLM analysis. The relationship between bacterial species, BCI, H:L ratios, and *Leucocytozoon* infection was determined through a Spearman's rho correlation test to account for non-normality.

Results

We found general TSA culture media, used as a positive control, yielded fewer CFU/mL than selective media, and all negative controls were free of bacteria. All saw-whet owls sampled showed positive results for presence of at least 1 of 4 target species of bacteria, and *S. aureus*, *E. coli*, *Salmonella* spp., and *P. aeruginosa* were present in 89.66%, 86.21%, 21.03%, and 6.9% of individuals tested, respectively.

All 28 buccal cultures and 13 pellet samples analyzed showed growth of at least one type of target bacteria. Of the 28 cloacal and 22 fecal samples, 82.14% and 77.27% were positive for target bacteria, respectively. *S. aureus* was the most abundant species isolated from buccal swabs, and *E. coli* was the most abundant species isolated from cloacal, fecal, and pellet samples (Table 1).

Results from GLM analysis revealed no association between *E. coli*, *Salmonella* spp., and *P. aeruginosa* distribution by GI sample location. The bacterial load of *S. aureus*, however, proved to differ across GI locations (Fig. 1; Table 2) with statistically significant differences between buccal samples and the remaining sample points (Table 3).

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Table 2. Results of Dunnett's T3 post hoc analysis of mean differences of *S. aureus* colonization in buccal swabs and 3 other sample locations within the gastrointestinal tract of Northern Saw-whet Owls banded on the Mohonk Preserve near New Paltz, New York, during October and November of 2017.

Site	Mean	SE	*n*	*P*
Cloaca	29.517	8.720	28	0.008
Feces	38.259	6.722	22	<0.001
Pellet	36.621	6.805	14	<0.001

Table 3. Results of general linear model used to assess the relationship between bacterial species present at 4 sample locations (buccal, cloacal, fecal, and pellet) within the gastrointestinal tract of Northern Saw-whet Owls banded on the Mohonk Preserve near New Paltz, New York, during October and November of 2017.

Variable	df	MS	*F*	*P*
P. aeruginosa	3	2.644	1.203	0.313
E. coli	3	1575.327	2.148	0.100
S. aureus	3	8031.494	11.540	<0.001
Salmonella	3	76.112	2.110	0.105

We found no correlations between H:L ratios or BCI and bacterial presence. However, positive correlations were found between site-specific bacteria, and between H:L ratios and *Leucocytozoon* infection (Table 4).

Discussion

We sampled 28 Northern Saw-whet Owls during autumn migration of 2017 and tested them for the presence of 4 common strains of cultivable bacteria: *E. coli*, *P. aeruginosa*, *Salmonella* spp., and *S. aureus*. Each individual sampled yielded positive results for at least one strain of target bacteria across GI sample locations. TSA plates yielded less CFU/mL of bacteria than selective media, which we assumed was due to competition between target and nontarget bacteria.

GLM analysis revealed that only *S. aureus* was dependent on the GI sample location with significantly higher levels occurring in the buccal cavity. Results also revealed no significant relationship between H:L ratios or BCI and bacterial load, but there was a positive correlation between H:L ratios and *Leucocytozoon* infection. In addition, Spearman's rho correlations indicate positive relationships between GI location and colonizations, including *E. coli* colonies and *Salmonella* spp. colonies between sample points, point colonizations of *S. aureus* and *E. coli*, Salmonellae and *E. coli*, and Salmonellae and *P. aeruginosa*. Furthermore, we found positive relationships between total *E. coli* colonies and pellet/cloacal load, *S. aureus* colonies and total buccal load, total pellet load and total fecal load. These relationships cannot be classified as causative, but they open new research topics for saw-whet owl studies.

E. coli is a facultatively anaerobic bacterium that can survive anaerobic intestines by using nitrate as its terminal electron acceptor. When gut *E. coli* is excreted in the feces, it can survive in the oxygenated environment and will increase 2–4 logs within the first 3 d, but the life cycle will terminate if it does not re-enter the GI tract (Russell and Jarvis 2001). The oral–fecal life cycle of *E. coli* can explain the lack of significant difference between sample points and the positive relationship between fecal and buccal *E. coli* colonization. The positive relationship between buccal and cloacal *E. coli* can be explained for this same reason, as the coprodeum of the cloaca stores feces before defecation (Orosz 2015).

Positive correlations between buccal and cloacal/fecal *E. coli* could also be indicative of an ability to survive gastric juice acidity. We would have assumed that the highly acidic stomach would provide a hostile environment for ingested microbes that aim to colonize the intestines. The optimum pH of Salmonellae is 4–9, with certain strains able to live as low as pH 3.7 (Tajkarimi 2007). Previous studies show that *E. coli* are able to survive the acidic environment of the human stomach which, in healthy individuals, can range from 1.3 to 5.8 (Kong and Singh 2008). Owl stomachs tend to be less acidic with Barn Owls (*Tyto alba*) ranging from 1.9 to 6.2, and rising to pH 4–6 after feeding and shortly before pellet egestion (Smith and Richmond 1972). It is possible that Salmonellae and *E. coli* ingested by adult birds could survive the low pH of the stomach to reach the lower digestive tract. Therefore, ingested Salmonellae on or in prey that survived the GI tract would account for the positive relationship between pellet and cloacal Salmonellae. Alterna-

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Table 4. Spearman rho correlations between site-specific bacteria and infection of *Leucocytozoon*, and heterophil to lymphocyte ratios in Northern Saw-whet Owls banded on the Mohonk Preserve near New Paltz, New York, during October and November of 2017. Bacteria were isolated using selective media. *Leucocytozoon* prevalence and H:L ratios were determined through microscopy.

Variable 1	Variable 2	*n*	df	r_s	*P*
Fecal *E. coli*	Buccal *E. coli*	22	20	0.280	0.024
Cloacal *S. aureus*	Cloacal *E. coli*	28	26	0.242	0.025
Fecal *S. aureus*	Cloacal *E. coli*	22	20	0.427	0.048
H:L	*Leucocytozoon*	28	26	0.432	0.022
Fecal Salmonellae	Fecal *E. coli*	22	20	0.467	0.028
Cloacal *E. coli*	Buccal *E. coli*	28	26	0.475	0.011
Pellet *E. coli*	Cloacal *S. aureus*	14	12	0.554	0.040
Pellet bacteria	Fecal bacteria	13	11	0.649	0.016
Total *S. aureus*	Buccal bacteria	28	26	0.732	$<$0.001
Pellet Salmonellae	Fecal *P. aeruginosa*	14	12	0.734	0.003
Pellet Salmonellae	Pellet *P. aeruginosa*	14	12	0.734	0.003
Pellet Salmonellae	Cloacal Salmonellae	14	12	0.734	0.003
Pellet bacteria	Total *E. coli*	13	11	0.765	0.002
Cloacal bacteria	Total *E. coli*	28	26	0.832	$<$0.001

tively, as proposed by Houston and Cooper (1975), these species could colonize the gut when birds are very young and gastric juices are not as acidic.

We speculate that the positive correlations between *E. coli* and Salmonellae, and *E. coli* and *S. aureus*, are due to common external variables. Competition for respiration resources, like oxygen or nitrogen, could explain the positive relationships between cloacal *S. aureus* and cloacal *E. coli*, fecal Salmonellae and fecal *E. coli*, and total *E. coli* load and total cloacal load. *E. coli* is found in the gut of virtually all mammals, but is usually outnumbered by obligate anaerobes 100 or more fold (Russell and Jarvis 2001). Therefore, higher *E. coli* presence would indicate lower levels of competition with obligate anaerobic bacteria, creating a more optimum environment for other facultative anaerobes such as *S. aureus* and *Salmonella*. Since both *Salmonella* and *E. coli* have evolved mechanisms to survive the GI tract, the pellet *E. coli* and cloacal *S. aureus* relationship can indicate decreased prevalence of obligate anaerobes that influence both bacteria. The relationship between fecal *S. aureus* and cloacal *E. coli* can also be explained through respiration competition, again, because feces are stored in the cloaca (Orosz 2015). However, this hypothesis is speculative and further research on anaerobes present in the GI tract would need to be done to support or refute this claim.

The high bacterial load of buccal swabs, specifically *S. aureus* colonization, is in accordance with previous studies of cultivable bacteria in the pharynx of the Eurasian Griffon Vulture (*Gyps fulvus*; Vela et al. 2015) and Peregrine Falcon (*Falco peregrinus*; Cooper 1980). Assuming that birds acquire most of their microbiome from food, it is expected that buccal bacteria would be higher than cloacal or fecal bacteria since the low pH of the stomach would kill some or most bacteria that cannot withstand extreme pH environments. In regard to significantly higher levels of *S. aureus* in the buccal cavity, Aly and Levit (1987) proposed that teichoic acid of the cell walls of *S. aureus* binds to cellular fibronectin of the squamous epithelium that characterizes the avian buccal cavity (Johnston 2014). Binding allows the bacteria to successfully adhere to the cell surface and to colonize the cavity. These results further support the homology between mammalian and avian host/microbe relationships.

Salmonella and *P. aeruginosa* are both opportunistic pathogens, which could account for their positive correlations in pellet samples. *Staphylococcus aureus* is also an opportunistic pathogen known to colonize the nose and throat of humans and some strains are antibiotic resistant, like MRSA, a common hospital or community acquired infection (Misawa et al. 2015). However, we did not find bacterial loads to correlate with H:L ratios or BCI, nor *Leucocytozoon* infection.

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Leucocytozoon and H:L ratios, however, were positively correlated, which was expected as phagocytic heterophils would be upregulated in response to blood infection (Young and Proudfoot 2014). These results are beneficial for future studies because they indicate H:L ratios may provide a more informative metric than the BCI, albeit slightly more invasive. H:L ratios may pick up subtle differences in fitness that inhibit or delay migration, resulting in late arrival to breeding grounds. And, as mentioned above, establishing baseline data on H:L ratios of infected and noninfected owls may ultimately allow researchers to assess the organism's ability to resist infections, microbial and erythrocytic.

We suggest 2 competing hypotheses for why health indicators and bacteria were not correlated. First, as stated above, the birds sampled were all migratory birds and data collected indicate chronic bacteria. Clinical signs of *Salmonella* infection include lethargy, fluffed up plumage, and a tendency to remain near feeding areas (Benskin et al. 2009). Clinical signs of *E. coli*, *Staphylococcus*, *P. aeruginosa*, and *Salmonella* infections in humans include abdominal pain, lethargy, and vomiting, so we expect clinical infections of any of these microbes would result in similar symptoms in saw-whet owls. If all birds sampled were observably healthy, it is expected that the bacteria detected are part of the normal microflora of saw-whet owls and that these specific species do not aid in immunological function. Second, it is possible that since the birds are presumably early on in their migration while passing through New York, the full effects of migration have not yet kicked in.

Beyond the scope of chronic infection, the methods employed by this study should be expanded to add to the findings of de Jesus et al. (2019) in which researchers examined *E. coli* isolates from the intestines of nonmigratory wild birds for antibiotic resistance expression. Saw-whet owls, as migratory birds that span North America, have the potential to act as reservoir hosts of multi drug-resistant bacteria, potentially allowing researchers to track transcontinental patterns of drug resistance.

The limitations in this study lie in the culturability of bacteria. It is difficult to give an exact percentage, but it is estimated that only about 2% of all bacteria are able to be cultivated in the laboratory (Wade 2001). The gold standard of characterizing bacterial diversity is 16s rDNA analysis (Kohl 2012). Thus, research using genetic sequencing should be done to detect unculturable bacteria. Furthermore, to investigate if bacteria are commensal or infectious, birds should also be studied on the breeding grounds during nesting, just before the onset of migration, during migration, and during mid-winter in the northern portions of their distribution where nonmigratory individuals may remain (Beckett and Proudfoot 2012). Should bacteria be infectious, controlled experiments in a laboratory setting must be done to evaluate the mechanism(s) of coinfection (Johnson and Buller 2011). This initial survey of bacterial presence of the saw-whet owl serves as an important stepping stone for further studies and is the first of its kind to characterize the microflora of this species.

Acknowledgments

We thank D.J. Esteban for use of his laboratory and for reviewing an early draft of this manuscript; S. Kovari and C. Belardo assisted in capture and banding of saw-whet owls. Funding was provided by the John Burroughs Natural History Society, Vassar College, and J.E. Walter. Logistical support was provided by the Mohonk Mountain House and the Mohonk Preserve. Compliance with Ethical Standards: Conflict of Interest—the authors declare that they have no conflict of interest; Ethical Approval—research was conducted under the authorization of USGS permit 23081, USFWS permit MB72487B-0, and NYSDEC licenses 6 and 355. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All experiments were approved under Vassar IACUC protocol No.15-13B.

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