UNIVERSITÉ DU QUÉBEC À MONTRÉAL

RÔLE DU GOÉLAND À BEC CERCLÉ (*LARUS DELAWARENSIS)* COMME BIOVECTEUR DE PATHOGÈNES EN RÉGION URBAINE EN LIEN AVEC L'EXPOSITION AUX RETARDATEURS DE FLAMME HALOGÉNÉS ET AUX SUBSTANCES PER- POLYFLUOROALKYLÉES

MÉMOIRE

PRÉSENTÉ

COMME EXIGENCE PARTIELLE

DE MAÎTRISE EN BIOLOGIE

PAR

SARAH VIGEANT

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DÉDICACE

À mon père, avec humour

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AVANT-PROPOS

Le présent mémoire de recherche présente le travail de maîtrise complété et déposé en janvier 2025 au département des sciences biologiques de l'Université du Québec à Montréal, dans le cadre des exigences partielles pour l'obtention du diplôme de maîtrise en biologie.

Peu de recherches ont été menées sur la caractérisation complète des bactéries et virus pathogènes présents dans le guano des goélands, et encore moins sur leurs rôles en tant que biovecteurs de ces micro-organismes. Le choix de se pencher sur ce sujet repose sur l'intérêt pour la prévention des risques sanitaires liés à la dispersion des agents pathogènes dans les environnements urbains densément peuplés. De plus, les polluants organohalogénés, tels que les retardateurs de flammes halogénés (RFH) et les substances per- et polyfluoroalkylées (SPFA), sont depuis longtemps identifiés comme des polluants d'origine anthropique présents dans les régions urbaines, pouvant avoir un impact sur la régulation du système immunitaire des espèces aviaires. Le goéland à bec cerclé (*Larus delawarensis*), nichant près de Montréal sur l'île Deslauriers dans le Fleuve Saint-Laurent, représente un modèle idéal pour étudier son rôle comme biovecteur de pathogènes dans différentes aires d'alimentation en raison de sa présence abondante, de son comportement alimentaire opportuniste et omnivore, et des niveaux élevés de bioaccumulation de SPFA et RFH rapportés dans des études antérieures.

Le but du présent mémoire fut d'étudier le rôle du goéland à bec cerclé comme biovecteur de pathogènes dans différentes aires d'alimentation de la région et d'identifier une possible relation entre la contamination aux RFH et SPFA et l'abondance de certains micro-organismes pathogènes. De ce fait, les résultats permettront d'appuyer l'hypothèse selon laquelle le temps passé dans une aire d'alimentation influence l'abondance de certains taxons microbiens, tout en établissant une corrélation entre les concentrations plasmatiques en RFH et SPFA et l'abondance de certains taxons bactériens pathogènes.

Ce projet représente une combinaison intéressante de toxicologie et microbiologie environnementale, résultat d'une collaboration enrichissante entre l'Université du Québec à Montréal et la Faculté de médecine vétérinaire de St-Hyacinthe (Université de Montréal). Il a aussi été pour moi une occasion unique de nourrir mon intérêt pour la recherche et la santé animale, un domaine qui m'a toujours passionnée et qui s'approche le plus de mon rêve de petite fille de devenir vétérinaire.

Ce travail, divisé en deux chapitres, a été rédigé avec l'assistance de mes professeurs Jonathan Verreault et Carl A. Gagnon, et sera soumis à des fins de publication dans un journal scientifique. Réaliser ce projet a représenté un défi personnel de taille, et, bien que je n'aie pas toujours cru avoir les compétences pour y parvenir l'avoir mené à terme me procure aujourd'hui une grande fierté et davantage de confiance en moi pour les projets futurs.

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LISTE DES ABRÉVIATIONS, DES SIGLES ET DES ACRONYMES

^{13}C-anti-DP	^{13}C-2,2',4,4'-tetrabromodiphényl éther/ *^{13}C-2,2',4,4'-tetrabromodiphenyl ether*
^{13}C-BDE-209	^{13}C-2,2',4,4',5,5'-hexabromodiphényl éther/ *^{13}C-2,2',4,4',5,5'-hexabromodiphenyl ether*
AIV	Virus d'influenza avaire/*Avian influenza virus*
ANOVA	Analyse de variance/*Analysis of variance*
Anti-DP	Anti Dechlorane plus
ASV	Variante de séquençage de l'amplicon/*Amplicon sequencing variant*
BDE	Bromodiphenyléther/*Bromodiphenylether*
BDE-30	2,2',4-bromodiphényl éther/*2,2',4-bromodiphenyl ether*
BDE-47	2,2',4,4'-tetrabromodiphényl éther/ *2,2',4,4'-tetrabromodiphenyl ether*
BDE-99	2,2',4,4',5-pentabromodiphényl éther/ *2,2',4,4',5-pentabromodiphenyl ether*
BDE-156	2,2',4,4',5-pentabromodiphényl éther/ *2,2',4,4',5-pentabromodiphenyl ether*
cDNA	ADN complémentaire/*Complementary DNA*
dbRDA	Analyse de redondance basée sur la distance/*Distance-based Redundancy Analysis*
DecaBDE	Décabromodiphényléther/*Decabromodiphenylether*
dsDNA	ADN double brins/*Double-stranded DNA*
ECNI	Capture d'électrons avec ionisation negative/ *Electron capture with negative ionisation*
FIB	Marqueurs de bactéries fécales/*Fecal index bacteria*
GC/MS	Chromatographe en phase gazeuse avec spectrométrie de masse/ *Gas chromatograph with mass spectrometry*
GPS	Système de positionnement global/*Global positioning system*
HBB	Hexabromobenzène/*Hexabromobenzene*
HBCD	Hexabromocyclododécane/*Hexabromocyclododecane*
HFR	Retardateurs de flamme halogénés/*Halogenated flame retardants*
HPLC	Chromatographie liquide à haute performance/ *High-performance liquid chromatography*
ILIS	Étalons internes marqués aux isotopes/ *Isotopically Labelled Internal Standards*
LDA	Valeur d'analyse linéaire discriminante/*Linear discriminant analysis value*
LEfSe	Analyse linéaire des tailles d'effet discriminantes/*Linear discriminant analysis effect size*
LOD	Limite de détection de la méthode/*Limite of detection of the method*
MRT-PCR	Transcription inverse multiplex de la réaction en chaîne de la polymérase/ *Multiplex reverse transcription polymerase chain reaction*

NDV	Virus de la maladie de Newcastle/*Newcastle disease virus*
NMDS	Échelonnement multidimensionnel non métrique/*Non-metric multidimensional scaling graphical representation*
PBDE	Polybromodiphenyléther/*Polybromodiphenylether*
PBEB	Pentabromoethyl benzène/*Pentabromoethyl benzene*
PCR	Réaction de polymérase en chaine/*Polymerase chain reaction*
PDD	Maladie de dilatation proventriculaire
PERMANOVA	Analyse de variance permutée/ *Permutational analysis of variance*
PFAS	Substances Per- polyfluoroalkylées/*Per- polyfluoroalkyl substances*
PFCA	Acides carboxyliques perfluorés/*Perfluorocarboxylic acids*
PFDA	Acide perfluorodécanoïque/*Perfuorodecanoic acid*
PFDoA	Acide perfluorododécanoïque/*Perfluorododecanoic acid*
PFEtCHXS	Perfluoro-4-ethylcyclohexane sulfonate
PFHpA	Acide perfluoroheptanoic/*Perfluoroheptanoic Acid*
PFHxS	Acide perfluorohexane sulfonique/*Perfluorohexane sulfonic acid*
PFNA	Acide perfluorononanoïque/*Perfluorononanoic acid*
PFOA	Acide perfluorooctanoïque/*Perfluorooctanoic acid*
PFOS	Sulfonate de perfluorooctane/*Perfluorooctane sulfonate*
PFSA	Sulfonate de perfluoroalkane/*Perfluoroalkane sulfonate*
PFTrDA	Acide perfluorotridécanoïque/*Perfluorotridecanoic acid*
PFUnDA	Acide pefluoroundécanoïque/*Perfluoroundecanoic acid*
QA/QC	Procédure d'assurance de qualité et contrôle de qualité/ *Quality Assurance and Quality Control Procedure*
RFH	Retardateur de flamme halogéné/*Halogenated flame retardants*
ROS	Régression d'ordre statistique/ Statistical order regression
SEM	Erreur standard de la Moyenne/*Standard error of the mean*
SPFA	Substances perfluoroalkyliques et polyfluoroalkyliques/ *Perfluoroalkyl and polyfluoroalkyl substances*
ssDNA	ADN simple brin/*Single-stranded DNA*
UHPLC-HRMS	Chromatographie liquide à ultra-haute performance couplée à la spectrométrie de masse à haute résolution/*Ultra-High Performance Liquid Chromatography coupled with High-Resolution Mass Spectrometry*

LISTE DES SYMBOLES ET DES UNITÉS

%	Pourcentage/*Percentage*
*	Astérisque/*Asterisk*
<	Moins que/*Lesser than*
>	Plus que/*Greater than*
±	Plus ou moins/*Plus or minus*
∑	Somme/*Sum*
°C	Degré Celsius/*Celsius degree*
µm	Micromètres/*Micrometers*
Adj-R^2	R^2 ajusté/*Adjusted R^2*
df	Degrés de liberté/*Degrees of freedom*
F	Valeur F/*F-value*
FWHM	Largeur totale à mi-maximum/*Full width at half maximum*
g	Gramme/*Gram*
G	Gauge
h	Heure/*Hour*
km	Kilomètre/*Kilometer*
L	Litre/*Liter*
m	Mètre/*Meter*
mL	Millilitre/*Milliliters*
mg	Milligramme/*Milligram*
min	Minute
mm	Millimètre/*Millimeter*
m/z	Rapport masse sur charge/*Mass to charge ratio*
n	Taille d'échantillon/*Sample size*
ng	Nanogramme/*Nanogram*
p	Valeur p/*p-value*
r	Valeur r de l'effet de taille/*r-value of effect size*
rpm	Révolution par minute/*Revolution per minute*

v	Volume
W	Valeur W/*W-value*
ww	Poids sec/*Wet weight*
α	Alpha
β	Bêta

RÉSUMÉ

Les goélands exploitent opportunément les habitats anthropiques pour se nourrir, et propagent des agents pathogènes via leur guano. Des études antérieures ont montré que les goélands à bec cerclé (*Larus delawarensis*) se reproduisant dans la région de Montréal (QC, Canada) sont fortement exposés aux substances per- et polyfluoroalkylées (SPFA) ainsi qu'aux retardateurs de flamme halogénés (RFH), contaminants pouvant être biotransportés dans les fèces. Cependant, les informations sur leur capacité à disséminer des bactéries et virus pathogènes, les risques associés pour la santé humaine et animale ainsi que leurs associations avec l'exposition aux contaminants sont encore peu documentés. Cette étude a examiné le rôle des goélands à bec cerclé comme biovecteurs de pathogènes en analysant divers micro-organismes fécaux et leurs liens avec les habitats d'alimentation fréquentés dans la région de Montréal. Le séquençage métagénomique d'échantillons de fèces de goélands a permis d'identifier plusieurs bactéries et virus, dont l'abondance était corrélée avec leurs déplacements de recherche alimentaire. Le genre bactérien *Enterococcus* ssp. et la famille virale *Picobirnaviridae* ont montré les plus fortes abondances relatives. Des corrélations positives ont été établies entre la probabilité de présence des goélands dans certains habitats et l'abondance des micro-organismes. Notamment *Clostridium* spp., *Helicobacter* spp., *Legionella* spp., *Mycoplasma* spp., *Pseudomonas* spp. et *Staphylococcus* spp. étaient significativement plus abondants chez les individus s'alimentant dans les champs agricoles. De plus, une différence de communauté bactérienne a été observée entre les goélands qui fréquentaient ou non les dépotoirs pour s'alimenter. L'abondance *d'Enterococcus* spp. était également associée à l'exposition aux SPFA et aux RFH, ainsi qu'à la présence de goélands dans les divers habitats. Cette étude souligne l'importance du rôle des goélands à bec cerclé comme biovecteurs de pathogènes viraux et bactériens dans la région fortement urbanisée de Montréal, soulignant ainsi l'importance de contrôler leur présence dans certains sites afin de réduire les risques pour la santé publique et animale.

Mots clés : Métagénomique; Virus; Bactérie; Utilisation de l'habitat; Contaminants environnementaux

ABSTRACT

Gulls are known to opportunistically exploit anthropogenic habitats for foraging and to spread pathogens via their guano. Previous studies have shown that ring-billed gulls (*Larus delawarensis*) breeding in the greater Montreal area (QC, Canada) accumulate elevated levels of per- and polyfluoroalkyl substances (PFAS) and halogenated flame retardants (HFRs), which can also be biotransported through their guano. However, there is limited information on the potential of ring-billed gulls to disseminate pathogenic bacteria and viruses that they carry, the associated risks for human and wildlife health, and the potential associations with contaminant exposure. This study examined the role of ring-billed gulls as pathogen biovectors by investigating a comprehensive list of microorganisms in their guano and relating their abundances with foraging habitat use in the greater Montreal area. Shotgun sequencing of individual gull guano samples identified several bacteria species and viral families for which the abundance was related to their foraging movements in this region. *Enterococcus* ssp. and viral family *Picobirnaviridae* exhibited the highest relative abundance. Positive correlations were found between the presence probability of gulls in certain foraging habitats and microorganism abundances. Specifically, *Clostridium*, *Helicobacter*, *Legionella, Mycoplasma*, *Pseudomonas* and *Staphylococcus* were significantly more abundant in gulls foraging predominantly in agricultural fields. Also, bacterial community difference was noted between gulls that frequented landfills for foraging and those that did not. Moreover, *Enterococcus* spp. abundance was significantly associated with PFAS and HFR levels in gull plasma, as well as the presence of gulls in various foraging habitats. This study highlights the role of ring-billed gulls as biovectors of viral and bacterial pathogens in the densely populated and highly urbanized Montreal area, thus emphasizing the importance of managing their presence in certain sites to reduce public and animal health risks.

Keywords : Metagenomic; Virus; Bacteria; Habitat use; Environmental Contaminants

INTRODUCTION

Les oiseaux généralistes, en particulier les espèces omnivores, dominent les écosystèmes urbains en raison de leur capacité à s'adapter aux environnements anthropisés et à exploiter efficacement les ressources variées disponibles dans ces milieux hétérogènes (Sorais et al., 2020). Il a été démontré que les habitats situés à proximité de la ville de Montréal (Qc, Canada), tels que les champs agricoles, les zones industrielles, les zones résidentielles, les milieux aquatiques, les dépotoirs et les stations d'épuration des eaux usées, sont fréquentés par plusieurs espèces aviaires pour s'alimenter, notamment par les goélands à bec cerclé (*Larus delawarensis* ; Gentes et al., 2015; Kerric et al., 2021; Sorais et al., 2021). Étant donné leurs déplacements sur de larges distances et leur cohabitation avec les humains, il est crucial de se pencher sur les enjeux microbiologiques qui en résultent. Parmi ces enjeux, la transmission de maladies, comme la grippe aviaire, peut toucher non seulement d'autres espèces animales ainsi que les humains. Ce type de transmission représente un risque particulier pour les élevages avicoles et, potentiellement, pour la santé humaine. Cependant, à ce jour, aucune étude axée sur l'ensemble des agents pathogènes du goéland à bec cerclé n'a été réalisée. Vu l'abondance des goélands à bec cerclé dans la région de Montréal, cette caractérisation est de grand intérêt pour des raisons sanitaires. En effet, il est connu que les laridés peuvent être porteurs asymptomatiques de plusieurs agents pathogènes (Ahlstrom et al., 2019, 2021; Alm et al., 2018). De surcroît, une étude réalisée en Andalousie (Espagne) chez divers oiseaux aquatiques donc certains utilisant des dépotoirs et d'autres non (i.e.d. *Ciconia ciconia*, *Larus fuscus*, *Grus grus* et *Anser anser*) a mis en évidence des différences interspécifiques dans la diversité et l'abondance microbienne, liées à cette utilisation d'habitat (Jarma et al., 2021). Ces auteurs ont démontré une plus grande abondance de micro-organismes potentiellement pathogéniques chez le goéland brun (*Larus fuscus*), en justifiant qu'il s'alimente fréquemment dans les sites d'enfouissement de déchets.

L'impact des déplacements et le rôle du goéland à bec cerclé en tant que biovecteur ont déjà été étudiés dans notre laboratoire, notamment en ce qui concerne la dissémination de retardateurs de flamme halogénés (RFH), mettant en lumière leur capacité à transporter ces contaminants (Desjardins et al., 2019). D'ailleurs, les laridés sont fréquemment utilisés comme espèces bio-indicatrices de la contamination environnementale. De précédents travaux réalisés sur des goélands à bec cerclé nichant également dans la région de Montréal ont démontré de fortes concentrations tissulaires et plasmatiques en RFH (Gentes et al., 2012, 2015; Kerric et al., 2021; Sorais et al., 2021). Il en est de même pour la contamination par d'autres contaminants organohalogénés tels que les substances per- et polyfluoroalkylées (SPFA), dont

des études ont montré une bioaccumulation dans les œufs de goélands argentés (*Larus argentatus)* dans la région des Grands Lacs du Canada (Letcher et al., 2015; Su et al., 2017). En revanche, il est bien établi que l'exposition aux RFH et aux SPFA peut engendrer une immunomodulation, diminuant ainsi l'efficacité du système immunitaire en réduisant la production d'anticorps (Fernie, Mayne, et al., 2005; Fernie, Shutt, et al., 2005; Guruge et al., 2009). À la lumière de ces études, il est probable que cette immunomodulation puisse entraîner une plus grande susceptibilité aux infections pathogènes. Bien qu'il ne semble pas y avoir d'études démontrant une relation entre l'exposition aux SPFA et aux RFH et l'abondance d'agents pathogènes chez les goélands à bec cerclé, l'exploration de cette relation est d'un grand intérêt pour mieux comprendre les mécanismes sous-jacents à la propagation des pathogènes dans les environnements anthropisés, particulièrement ceux fréquentés par cette espèce.

Ce projet de maîtrise avait donc pour objectif principal d'étudier la communauté microbienne pathogène présente chez les goélands à bec cerclé. Étant donné l'éloignement géographique des habitats d'alimentation et le fait que les oiseaux sont reconnus pour disperser des micro-organismes pathogènes via leurs fèces, un second objectif était de dresser un portrait du rôle du goéland à bec cerclé en tant que biovecteur, en établissant un lien avec ses déplacements à l'échelle du paysage dans la région de Montréal sous forme de signature pathogénique d'habitat. De plus, la tendance générale entre l'exposition aux SPFA et RFH et l'abondance de différents taxons pathogènes a été explorée.

Ce document est présenté sous la forme d'un mémoire par article, divisé en deux chapitres. Le premier chapitre comprend l'état actuel des connaissances sur les micro-organismes pathogènes aviaires ainsi que le concept d'organisme biovecteur. Le second chapitre prend la forme d'un article scientifique, rédigé en anglais, et porte sur les signatures pathogéniques d'habitats et les relations avec l'exposition aux SPFA et HFR ainsi que l'abondance microbienne pathogène.

CHAPITRE 1

État des connaissances

1.1 Problématique des biovecteurs aviaires

1.1.1 Micro-organismes pathogènes aviaires

Les espèces aviaires possèdent une communauté diversifiée de micro-organismes, dû au fait qu'ils occupent une multitude de niches écologiques (Waite & Taylor, 2015). Ces micro-organismes peuvent entrainer à la fois des répercussions positives pour la santé des hôtes aviaires, mais également générer des impacts potentiellement dangereux pour eux et pour les autres espèces avec lesquelles ils coexistent. Cependant, dans les habitats anthropisés, où certaines espèces opportunistes sont présentes en forte densité, celles-ci sont susceptibles d'être porteuses d'organismes pathogènes. En raison de la capacité des oiseaux à se déplacer sur de grandes distances, cela augmente également les risques de propagation des agents infectieux à l'échelle locale augmentent. La majorité des espèces aviaires ont une haute tolérance à une faible charge microbienne, mais les oiseaux asymptomatiques sont considérés comme des réservoirs en participant à la transmission de maladies infectieuses (Benskin et al., 2009 ; Clark, 2014).

Les oiseaux sont vulnérables aux infections pathogènes à tous les stades de leur cycle de vie, à la fois par exposition directe, via la nourriture et l'eau, ou indirecte par contact avec le matériel biologique d'autres individus de la même population ou d'espèces différentes (Benskin et al., 2009). L'alimentation est le principal facteur influençant l'exposition des espèces aviaires aux maladies infectieuses. Ces dernières peuvent provenir de diverses sources telles que les eaux usées, les mangeoires et les aires d'alimentation contaminées par les fèces comme les dépotoirs (Hubálek, 2004, 2021 ; Nelson et al., 2008). La majorité des études se concentrent sur les oiseaux s'alimentant dans des environnements susceptibles d'avoir des densités élevées d'agents pathogènes. Or, comme mentionné par Benskin et al. (2009) il est important de garder à l'esprit que la variation de la distribution spatiale des micro-organismes affectera l'exposition des hôtes à l'infection. C'est-à-dire que si les agents infectieux sont distribués de façon hétérogène dans l'environnement et que les individus utilisent l'environnement de manière différente, il en résultera probablement d'une hétérogénéité dans l'exposition des hôtes. De plus, le sexe des individus peut influencer l'exposition des oiseaux, car le comportement de quête alimentaire et le système immunitaire varient entre les femelles et les mâles de la majorité des espèces aviaires (Valdebenito et al., 2020 ; Monaghan et al., 1985). Il est également important de prendre en compte le facteur de virulence. En effet,

une fois ingérés, les microbes diffèrent dans leur capacité à provoquer une maladie et c'est pourquoi une faible charge microbienne peut parfois induire des symptômes sévères par rapport à une charge moins importante qui engendre moins d'effet (Thomas & Elkinton, 2004).

Les laridés sont fréquemment utilisés pour les études microbiologiques en raison de leur répartition mondiale et du fait qu'ils partagent souvent les mêmes habitats que les humains. En effet, il a été démontré dans des recherches de différents pays que les goélands représentent des réservoirs de bactéries résistantes aux antibiotiques (Alm et al., 2003; Araújo et al., 2014; Blagodatski et al., 2021; Brown et al., 2017; Schoen & Ashbolt, 2010) ainsi que de virus transmissibles à l'homme, tels que la grippe aviaire (Bergervoet et al., 2019; Blagodatski et al., 2021; Čížek et al., 2007). Étant majoritairement excrétés par le guano, ces agents pathogènes sont dispersés et contribuent à l'augmentation des risques sanitaires. Ainsi, les laridés suscitent souvent l'intérêt de la santé publique (Santé Canada, 2022).

À l'aide de la métagénomique, l'ensemble du matériel génétique d'un échantillon peut être isolé puis séquencé et les gènes rapporteurs générés peuvent être identifiés à l'aide d'une banque de données. Cependant, tous les micro-organismes ne sont pas forcément pathogènes ni zoonotiques. En effet, certains constituent le microbiome digestif alors que d'autres non (Kogut et al., 2020). Par exemple, chez l'humain, de nombreux mammifères et certaines espèces d'oiseaux, les genres *Enterococcus* spp. et *Escherichia* spp. font partie de la flore intestinale normale et exercent généralement un rôle commensal dans le tractus gastro-intestinal (Silva et al., 2012). Par contre, il existe des sous-populations portant des ilots de pathogénicité ou des plasmides codants pour des toxines étant ainsi pathogène (European Food Safety Authority, 2014). L'une des faiblesses de la méthode d'analyse par métagénomique est le manque de précision au niveau de l'identification jusqu'à la souche ou la variante du micro-organisme (Andersen & Hoorfar, 2018). Cela dit, l'identification de taxon des bactéries entériques ne signifie pas obligatoirement qu'ils sont pathogènes, mais plutôt qu'ils ont le potentiel de l'être. Les taxons ayant un potentiel de pathogénicité suscitant des préoccupations sont généralement ceux se transmettant par des voies multiples telles que l'eau, les aliments, le sol ou les contacts avec les animaux (Brouwer et al., 2018).

Avec l'augmentation importante des connaissances en métagénomique dans les dernières années, davantage d'études ont permis de caractériser les communautés bactériennes et virales présentes dans les fèces des oiseaux (Cao et al., 2020 ; Chung et al., 2018 ; Sarker, 2021). Puisque le but principal de la métagénomique est d'explorer l'ensemble des communautés microbiennes, les organismes sont

généralement regroupés en groupes ayant des caractéristiques communes, soit en classes pour les bactéries ou en familles pour les virus. Dans la plupart des cas, les résultats des études microbiologiques sont exprimés sous forme d'abondance relative ainsi que de diversité alpha (α) et bêta (β). Le concept d'abondance relative correspond à la quantité d'un type de micro-organisme retrouvé dans l'entièreté de l'échantillon, en prenant en compte la totalité des taxons qui s'y trouve. En ce qui concerne la diversité α, celle-ci mesure l'ensemble de taxons différents coexistant dans un échantillon et résume normalement les différentes espèces au sein d'un échantillon (Prober et al., 2015). Cette dernière est souvent quantifiée à l'aide d'indices, comme l'indice de Shannon, qui prend en compte à la fois la richesse des espèces et leur répartition dans l'échantillon (Kim et al., 2017). La richesse en espèces, quant à elle, peut être estimée à l'aide de méthodes comme le Chao1, qui ajuste les données pour estimer le nombre total d'espèces présentes, y compris celles qui sont peu fréquentes. Il est essentiel de considérer à la fois la richesse des espèces par le nombre total d'espèces et leur répartition, puisque cela permet de mieux comprendre la complexité de la communauté microbienne et permet de détecter des dominances ou déséquilibres de taxon qui pourraient influencer la dynamique écologique et les interactions pathogènes hôtes. En ce qui concerne la diversité β, celle-ci mesure plutôt les dissimilitudes des taxons dans la communauté bactérienne, c'est-à-dire entre les différents échantillons. Prendre en compte cette diversité permet de comprendre comment les communautés diffèrent entre des habitats ou conditions écologiques distinctes, offrant ainsi des informations sur les facteurs environnementaux qui modulent la composition microbienne. Cependant, contrairement aux communautés bactériennes pour lesquelles le gène 16S rRNA sert de marqueur universel facilitant l'identification taxonomique, les virus ne possèdent pas de gène commun conservé à l'ensemble des espèces, ce qui rend leur classification et la standardisation des analyses plus complexes. Pour cette raison, la diversité β est moins fréquemment utilisée pour étudier les communautés virales, bien que des approches basées sur la comparaison de profils métagénomiques ou de groupes fonctionnels émergent progressivement pour surmonter cette limite (Kosmopoulos & Anantharaman, 2024).

1.1.1.1 Les maladies zoonotiques

Les oiseaux peuvent être porteurs de nombreux agents pathogènes, dont certains sont dits zoonotiques, signifiant qu'ils peuvent être transmis à l'homme et à d'autres espèces animales (Cooper, 1990). Les pathogènes zoonotiques constituent une menace importante pour la santé publique et sont notamment étudiés chez les oiseaux migrateurs (Benskin et al., 2009 ; Chung et al., 2018 ; Hubálek, 2004). Étant donné leur grand nombre, il est possible de regrouper ceux-ci d'abord selon leur niveau taxonomique (ex. :

bactérie ou virus) puis en fonction de leurs espèces, genres ou souches, mais également selon des caractéristiques communes (ex. : entérobactéries, virus respiratoires, etc.)

Les études sur les infections entérobactériennes et leur niveau d'infection chez les oiseaux sauvages sont jusqu'à présent concentrées uniquement sur les espèces aviaires les plus susceptibles d'être infectées par des bactéries à partir de sources anthropiques, en particulier les goélands. Par exemple, plusieurs recherches décrivent les goélands comme réservoirs de salmonelles et de campylobactéries (Čížek et al., 2007; Migura-Garcia et al., 2017) et responsables de la dissémination d'*Escherichia coli* et de *Klebsiella pneumoniae* (Mukerji et al., 2023; Nelson et al., 2008). Il est également connu que les goélands peuvent être porteurs d'autres agents pathogènes tels que *Listeria* spp*., Streptococcus* spp*.*, ou *Clostridium* spp*.*, mais peu d'articles récents rapportent des infections (Quessy & Messier, 1992; Wood & Trust, 1972).

En ce qui concerne les études sur les infections virales zoonotiques aviaires, celles-ci sont majoritairement focalisées sur *l'influenza A* de souche hautement pathogène H5N1 (Deliberto et al., 2009 ; Munster et al., 2007 ; Velarde et al., 2010). En effet, les oiseaux sauvages migrateurs ont été impliqués dans la propagation et l'émergence de l'influenza humaine et animale qui est réputée pour être transportée à travers le monde (Gaidet et al., 2010 ; Ineson et al., 2022 ; Vijaykrishna et al., 2008). Cela constitue un enjeu environnemental et sanitaire important, car cette dispersion peut favoriser la transmission des maladies entre les habitats anthropiques et naturels, présentant des risques pour la faune sauvage, les élevages avicoles ainsi que pour la santé humaine. Par exemple, aux États-Unis, une épidémie de grippe aviaire hautement pathogène (HPAI A, H7N3) a touché de nombreuses exploitations avicoles commerciales en 2020 marquant une détection significative du virus HPAI A (Youk et al., 2020). Cette épidémie a été attribuée à une contamination par les oiseaux sauvages migrateurs en raison d'analyses phylogénétiques qui ont retracé les segments génétiques du virus à des lignées aviaires nord-américaines, particulièrement des oiseaux aquatiques, confirmant ainsi leur rôle de biovecteurs dans la dissémination du virus vers les exploitations avicoles. Une épidémie de la souche H5N1 a également frappé le Canada en 2021, qui depuis a affecté un total de 1,4 million d'oiseaux d'élevage au Québec (ACIA, 2024). D'autres virus peuvent également être transportés par les goélands et nuire aux élevages aviaires, tels que l'orthoavulavirus aviaire causant la maladie de Newcastle (NDV), ou le Bornavirus aviaire*,* causant la maladie de dilatation proventriculaire (Diel et al., 2012 ; Guo et al., 2015).

1.1.1.2 Concepts de biovecteur et biotransport

Le biotransport désigne le déplacement passif ou actif d'organismes, de nutriments ou de contaminants, qu'ils soient d'origine biologique ou chimique, entre différents milieux par l'entremise d'un vecteur vivant, appelé biovecteur (Blais et al., 2007). Parmi ceux-ci, les espèces aviaires jouent un rôle important en facilitant la dispersion de micro-organismes sur de longues distances géographiques, ce qui leur confère le statut de biovecteurs (Pulgarín-R et al., 2019). Plusieurs études ont montré la dispersion de pathogènes par les matières fécales des goélands s'alimentant dans des aires fortement anthropisées, telles que les dépotoirs ou les stations d'épuration des eaux usées (Ahlstrom et al., 2019, 2021 ; Alm et al., 2018). Parmi celles-ci, Alm et al. (2018) ont mis en évidence le rôle des goélands en tant que biovecteurs de coliformes humains. En s'intéressant au chevauchement entre le marqueur génétique spécifique à l'humain (i.e. Bactéroïde associé à l'humain, HF183) et les marqueurs de bactéries fécales associées aux goélands (FIB ; i.e. marqueurs moléculaires Gull2 et Gull4), les auteurs ont montré que les oiseaux se nourrissant dans les sites de traitement des eaux usées ou d'enfouissement de déchets de la région d'Ottawa (ON, Canada) peuvent transporter des micro-organismes sur les plages municipales. De plus, une étude portant sur diverses espèces d'oiseaux, dont la mouette rieuse (*Chroicocephalus ridibundus*), le goéland argenté (*Larus argentatus*) et le goéland brun (*Larus fuscus*) de la côte atlantique de l'Amérique du Nord, a montré une prévalence du virus de l'influenza aviaire chez les individus s'alimentant dans les plans d'eau, avec une détection dans les écouvillons cloacaux, soulignant leur rôle dans la transmission des pathogènes viraux (Ineson et al., 2022). Actuellement, rares sont les études qui combinent le suivi télémétrique d'oiseaux infectés avec l'analyse de la dispersion des pathogènes. L'une d'entre elles, menée par Navarro et al. (2019), a généré une carte de distribution des micro-organismes pathogènes tels que *Salmonella* spp., *Campylobacter* spp., et *Chlamydia* spp., dans le sud de la France chez le goéland leucophé (*Larus michahellis*), tout en examinant leur utilisation des différents habitats. Les résultats démontrent une plus grande proportion d'individus fréquentant les estuaires étant infectés à *Salmonella* spp. et *Campylobacter* spp., ainsi qu'une plus grande proportion de ceux fréquentant les habitats portuaires étant infectés à *Chlamydia* spp. Cependant, cette recherche illustre le potentiel de biotransport de seulement trois micro-organismes pathogènes. C'est-à-dire qu'aucune étude à ce jour n'a fait une analyse exhaustive d'agents infectieux et/ou potentiellementcr zoonotiques en utilisant des techniques de pointe en métagénomique et des analyses géospatiales (GPS) chez des oiseaux sauvages.

1.2 Contaminants organohalogénés

Puisque les oiseaux représentent des modèles idéaux pour étudier la présence de polluants dans les écosystèmes terrestres et aquatiques, les goélands sont fréquemment utilisés comme espèce bioindicatrice pour détecter la présence de contaminants organiques tels que les RFH (Gentes et al., 2012, 2015 ; Kerric et al., 2021 ; Sorais et al., 2020) et les SPFA (Lopez-Antia et al., 2021 ; Sebastiano et al., 2021 ; Thorstensen et al., 2021). D'ailleurs, une étude réalisée au sein de notre laboratoire par Gentes et al. (2015) a permis de documenter de fortes concentrations plasmatiques en RFH chez les goélands à bec cerclé en lien avec leur temps passé dans divers sites de gestion de déchets, notamment les dépotoirs. En ce qui concerne les SPFA, certains composés ont précédemment été détectés dans des œufs de la majorité des études qui se concentrent sur la détection dans les œufs de goélands argentés dans la région des Grands Lacs du Canada (Letcher et al., 2015 ; Su et al., 2017), mais peu d'étude réalisée au Canada se penchent sur le dosage plasmatique. En revanche, une récente étude réalisée chez trois espèces de laridés provenant du sud de la France (i.e. *Larus argentatus, Larus fuscus graellsii, Larus marinus*) a rapporté de fortes concentrations plasmatiques de L-sulfonate de perfluorooctane (L-PFOS), d'acide perfluorotridecanoïque (PFTrDA) et d'acide pefluoroundecanoïque (PFUnDA ; Sebastiano et al., 2021).

Lorsqu'exposées aux SPFA ou aux RFH, le système immunitaire des espèces aviaires peut être affecté. Plusieurs études ont montré une relation entre l'exposition à certains SPFA et une atteinte au niveau du système immunitaire chez les espèces aviaires (Castaño-Ortiz et al., 2019 ; Smits & Nain, 2013). Il en est de même pour les RFH, dont des études réalisées sur les crécerelles d'Amérique (*Falcos sparverius*) ont démontré une relation entre l'exposition et la modulation de la réponse immunitaire par une diminution de production d'anticorps (Fernie et al., 2005 ; Smits & Bortolotti, 2001). En revanche, très peu de recherches font un lien entre l'immunomodulation engendrée par les SPFA ou les RFH et la prévalence à une infection par un pathogène. L'une des rares études disponibles est celle de Guruge et al. (2009) réalisée sur des tissus embryonnaires de souris exposés au PFOS, visant à vérifier les réponses immunitaires avant et après une exposition à un virus de l'influenza, et ayant permis de constater une diminution importante de la production d'anticorps liée à l'exposition aux PFOS. Étant donné les impacts immunitaires démontrés par ces études, il est pertinent de s'intéresser à l'impact de l'exposition aux SPFA et RFH sur la communauté d'agents pathogènes présente chez le goéland à bec cerclé.

1.2.1 Retardateurs de flamme halogénés

Les RFH sont des composés ajoutés à une multitude d'objets d'utilisation courante (ex, rembourrage des meubles, textiles, véhicules, etc.) en raison de leurs propriétés ignifuges (Blum et al., 2011). Étant donné leur capacité de bioaccumulation et de leur persistance dans l'environnement (Birnbaum & Staskal, 2004 ; De Wit, 2002 ; Law et al., 2003), certains composés ont été interdits dans le cadre de la Convention Stockholm sur les polluants organiques persistants (UNEP, 2017). Afin de répondre au besoin d'inflammabilité, ces mesures restrictives ont mené à l'utilisation de retardateurs de flamme émergents ou alternatifs. Cependant, même avec ces efforts pour la réduction de leur utilisation, les RFH sont retrouvés à des concentrations élevées dans différents compartiments environnementaux ainsi que chez les organismes qui y vivent.

Dans la famille des RFH bromés se retrouvent les polybromodiphényléthers (PBDE), comportant 209 congénères nommés en fonction du nombre et de la position de la molécule de brome (Birnbaum & Staskal, 2004). Les PBDE ont été produits en trois formulations commerciales : les penta-BDE, octa-BDE et les déca-BDE (Jinhui et al., 2017). Ce dernier, le déca-BDE, a été la principale formulation mise en marché, représentant environ 80 % de la production de PBDE dans le monde (BSEF, 2023). Étant donné les risques pour l'environnement, ces contaminants ont été bannis en 2009 pour le penta et l'octa-BDE ainsi qu'en 2017 pour le déca-BDE (UNEP, 2017). À la suite de leur bannissement, de nouvelles familles de contaminants émergents ont été commercialisées en guise de remplacement ; tel que les pentabromoethyl benzène (PBEB), l'hexabromobenzène (HBB) et les Déchloranes (Dec ; Covaci et al., 2011 ; Sverko et al., 2011). Étant composé d'atomes de chlore, le Déchlorane Plus (DP), composé des isomères syn-DP et anti-DP, sont des molécules ayant une grande thermostabilité et sont généralement utilisés comme additifs (Sverko et al., 2011). D'autres RFH émergents ont été détectés dans l'environnement à des concentrations préoccupantes, comme le décabromodiphenyléthane (DBDPE), le 1.2-bis (2, 4, 6-tribromophenoxy) éthane (BTBPE), le 2-ethylhexyl-2, 3, 4, 5 - 14 tetrabromobenzoate (TBB) et le bis (2-ethylhexyl) -3, 4, 5, 6, - tetrabromo-phtalate (TBPH ; de Wit et al., 2010 ; Hou et al., 2021).

Les RFH ont été fréquemment étudiés chez les espèces aviaires et plus particulièrement chez les oiseaux sauvages, dont les goélands à bec cerclé. Les études de Gentes et al. (2012, 2015) réalisées au sein de notre laboratoire, ont montré une forte exposition de ces oiseaux dans la région de Montréal. En effet, des concentrations préoccupantes de RFH, ainsi que quelques composés émergents, ont été détectées dans le plasma et le foie de ces goélands. De surcroit, dans l'étude de Kerric et al. (2022) également réalisée

dans notre laboratoire, les auteurs ont identifié les différentes routes d'exposition des goélands aux PBDE. Parmi les résultats obtenus, ceux-ci suggèrent que les voies d'inhalation et alimentaires contribuent de manière significative à l'accumulation de PBDE dans le foie des goélands à bec cerclé. Sachant que ceux-ci sont fortement exposés dans la région de Montréal, il est pertinent de se questionner à savoir si une exposition élevée aux RFH peut avoir un impact sur l'infection aux agents pathogènes.

1.2.2 Contaminants per et polyfluoroalkyliques

Les SPFA sont des molécules organiques présentant une chaine de carbone liée à un ou plusieurs groupements fonctionnels per- ou polyfluorés. L'acide perfluorooctanoïque (PFOA) et PFOS, deux composés appartenant à la famille des perfluoroalkane sulfonate (PFSA), sont ceux ayant été les plus produits et étudiés au niveau de leur toxicité (Fenton et al., 2021). En raison de leurs propriétés oléophiliques, thermostables et antiadhésives, les SPFA sont utilisés à des fins de fabrication de produits antitaches, poêles antiadhésives ainsi que les emballages alimentaires (Glüge et al., 2020). Étant donné qu'ils posent des risques sévères pour la santé (ex. cancer, tératogénicité, perturbation endocrine) et pour l'environnement, deux composés ont été bannis lors de la Convention Stockholm sur les polluants organiques persistants, soit le PFOS en 2009 et le PFOA en 2017 (UNEP, 2010 & 2017). Les SPFA sont des molécules extrêmement stables et persistantes dans l'environnement. Couramment appelés les « *forever chemicals* » (composés éternels), leur demi-vie dans l'environnement est estimée à plus de 200 ans.

Les composés fluorés ont été confirmés comme bioaccumulables chez les oiseaux. En effet, l'étude de Sun et al. (2020) menée sur des faucons pèlerins (*Falco peregrinus*) d'Ontario (Canada), démontre une accumulation des SPFA dans les œufs ainsi qu'une exposition accrue des individus s'alimentant fréquemment dans les zones urbaines. L'étude de Letcher et al. (2015) démontre également une bioaccumulation des SPFA chez les goélands argentés de la même région, révélant dans les œufs des concentrations alarmantes de perfluorocarboxylates (PFCA), de PFSA, ainsi que du précurseur perfluoro-4-ethylcyclohexane sulfonate (PFEtCHXS).

1.3 Modèle d'étude : le goéland à bec cerclé

Le goéland à bec cerclé est l'espèce de la famille des laridés la plus abondante au Québec et en Amérique du Nord (Giroux et al., 2016 ; Morris et al., 2011). L'une des plus grandes colonies se situe d'ailleurs sur l'Île Deslauriers (Qc, Canada), composée en 2023, d'environ 18 000 couples (Service canadiens de la Faune, données non publ., 2023). Avec une superficie de 0,96 km^2, l'île est située dans le fleuve Saint-Laurent à

environ 3 km à l'est de l'île de Montréal (45 717° N et 73 433° W ; Brousseau et al., 1996). Cette localisation procure une large diversité d'habitats aux goélands où ils peuvent s'alimenter, tels que les champs agricoles, les zones industrielles, les zones résidentielles, les milieux aquatiques, les dépotoirs et les stations d'épuration des eaux usées. En raison de la taille de sa colonie, de sa proximité avec divers habitats et de sa position trophique relativement élevée, le goéland à bec cerclé est choisi comme espèce bio-indicatrice de RFH depuis plusieurs années afin d'en étudier l'exposition et les effets toxiques (Brown et al., 2019 ; Desjardins et al., 2019 ; Gentes et al., 2012, 2015 ; Kerric et al., 2021, 2022 ; Sorais et al., 2020, 2021)

Étant omnivore et opportuniste, le goéland à bec cerclé s'est adapté de façon à privilégier l'exploitation des ressources dans des paysages anthropisés (Brousseau et al., 1996; Patenaude-Monette et al., 2014). D'après Patenaude-Monette et al. (2014), en période de nidification, la distance de déplacement réalisée dans un but de recherche alimentaire peut s'élever à 63 km autour de la colonie de l'Île Deslauriers. Selon Caron-Beaudoin et al. (2013), en ville, sa diète est principalement composée de déchets, alors qu'en zone rurale les goélands s'alimentent majoritairement d'annélides et de grains.

En raison de la distance parcourue lors de ses déplacements, le goéland à bec cerclé est une espèce jouant un rôle important en tant que biovecteur. En effet, ce rôle a été mis en évidence par Desjardins et al. (2019), qui ont démontré qu'environ 1 g de retardateurs de flamme halogénés (RFH) est excrété dans la grande région de Montréal durant la période d'incubation par l'ensemble des goélands nicheurs de l'île Deslauriers (Qc, Canada). Toutefois, cette estimation ne tient pas compte de la dispersion géospatiale réelle de ces contaminants à travers les différents habitats fréquentés par les individus.

Objectifs et hypothèses

1.4 Objectif général

À l'égard des connaissances actuelles, l'objectif principal de ce projet de maîtrise était d'étudier le rôle du goéland à bec cerclé comme biovecteur de pathogènes, en dressant un portrait global des agents pathogènes présents ainsi qu'en établissant un lien avec ses déplacements à l'échelle du paysage dans la région de Montréal. Cet objectif avait pour but d'enrichir les connaissances concernant la propagation fécale microbienne dans les endroits fréquentés par les goélands pour s'alimenter. Également, des associations ont été étudiées entre l'exposition aux RFH et aux SPFA ainsi que l'abondance relative des pathogènes dans un but exploratoire, afin de vérifier si la charge plasmatique de ces contaminants chez le goéland peut avoir une incidence sur leur taux d'infection à différents pathogènes.

1.5 Objectifs spécifiques et hypothèses

Objectif A : Établir un lien entre l'abondance relative des bactéries pathogènes dans le guano des goélands et leur comportement de quête alimentaire (sites principaux d'alimentation visités).

Hypothèse A : Les goélands à bec cerclé ayant une plus grande probabilité de présence dans les dépotoirs présentent une corrélation positive avec l'abondance de bactéries pathogènes d'origine alimentaire, telles que *Salmonella spp.* et *Campylobacter spp.* (Migura-Garcia et al., 2017). De même, les individus fréquentant fréquemment les milieux aquatiques sont associés à une abondance accrue de la famille *Orthomyxoviridae*, incluant le virus de l'influenza aviaire (Ineson et al., 2022). Enfin, une forte utilisation des stations d'épuration des eaux usées est liée à une plus grande abondance d'*Escherichia spp.*

Objectif B : Établir un lien entre l'abondance relative et la diversité β des pathogènes dans le guano des goélands selon le sexe des individus.

Hypothèse B : Les goélands à bec cerclé femelles, en raison de leur comportement distinct de recherche de nourriture dans les dépotoirs, caractérisé par une préférence pour les sources de nourriture fraîchement jetées (Monaghan et al., 1985), présentent une plus grande diversité β des communautés bactériennes et virales que les mâles.

Objectif C Explorer le lien entre les concentrations plasmatiques de RFH et de SPFA chez les goélands à bec cerclé ainsi que l'abondance relative des pathogènes dans leur guano.

Hypothèse C : Les concentrations plasmatiques totales de RFH et de SPFA sont positivement corrélées à l'abondance des micro-organismes pathogènes dans le guano des goélands, en raison de leurs effets immunosuppresseurs documentés (Fernie, Mayne, et al., 2005; Smits & Nain, 2013).

CHAPITRE 2

ROLE OF RING-BILLED GULLS AS BIOVECTOR OF VIRAL AND BACTERIAL PATHOGENS IN HIGHLY URBANIZED AREAS IN RELATION WITH ORGANOHALOGEN CONTAMINANT EXPOSURE

Sarah Vigeant[a], Carl A. Gagnon[b], Jonathan Verreault[a]*

[a] Centre de recherche en toxicologie de l'environnement (TOXEN), Département des sciences biologiques, Université du Québec à Montréal, P.O. Box 8888, Succursale Centre-ville, Montréal, QC, H3C 3P8, Canada

[b] Molecular Diagnostic Laboratory, Centre de diagnostic vétérinaire de l'Université de Montréal (CDVUM), Faculté de médecine vétérinaire, St-Hyacinthe, QC, J2S 2M2, Canada

2.1 Abstract

Gulls are known to opportunistically exploit anthropogenic habitats for foraging and to spread pathogens via their guano. Previous studies have shown that ring-billed gulls (*Larus delawarensis*) breeding in the greater Montreal area (QC, Canada) accumulate elevated levels of per- and polyfluoroalkyl substances (PFAS) and halogenated flame retardants (HFRs), which can also be biotransported through their guano. However, there is limited information on the potential of ring-billed gulls to disseminate pathogenic bacteria and viruses that they carry, the associated risks for human and wildlife health, and the potential associations with contaminant exposure. This study examined the role of ring-billed gulls as pathogen biovectors by investigating a comprehensive list of microorganisms in their guano and relating their abundances with foraging habitat use in the greater Montreal area. Shotgun sequencing of individual gull guano samples identified several bacteria species and viral families for which the abundance was related to their foraging movements in this region. *Enterococcus* ssp. and viral family *Picobirnaviridae* exhibited the highest relative abundance. Positive correlations were found between the presence probability of gulls in certain foraging habitats and microorganism abundances. Specifically, *Clostridium*, *Helicobacter*, *Legionella, Mycoplasma*, *Pseudomonas* and *Staphylococcus* were significantly more abundant in gulls foraging predominantly in agricultural fields. Also, bacterial community difference was noted between gulls that frequented landfills for foraging and those that did not. Moreover, *Enterococcus* spp. abundance was significantly associated with PFAS and HFR levels in gull plasma, as well as the presence of gulls in various foraging habitats. This study highlights the role of ring-billed gulls as biovectors of viral and bacterial pathogens in the densely populated and highly urbanized Montreal area, thus emphasizing the importance of managing their presence in certain sites to reduce public and animal health risks.

Keywords: Metagenomic; Virus; Bacteria; Habitat use; Environmental Contaminants

2.2 Introduction

Several generalist and omnivorous bird species, including larids, thrive in urban environments by exploiting the diverse resources available in industrialized and heterogeneous landscapes (Sorais et al., 2020). These urban areas provide a variety of foraging opportunities, from food wastes in landfills and residential areas to insects and seeds in agricultural fields. Such environments allow birds to adapt their feeding strategies and exploit new niches that are highly competitive in natural habitats. However, foraging in urbanized habitats may expose wild birds to various pathogens, including bacteria and viruses that can be pathogenic to humans, domestic animals, and other wildlife species. For instance, bacteria such as *Campylobacter jejuni*, *Clostridium perfringens* and *Enterococcus faecium*, as well as viruses like avian orthoavulavirus and avian influenza virus (AIV) can be acquired from contaminated food sources or habitats such as landfills and agricultural fields (Hubálek, 2004, 2021).Because birds can travel long distances between foraging habitats, they can act as biovectors by dispersing these pathogens within their home range (Pulgarín-R et al., 2019). This behavior may be of high environmental and health concern as it can lead to the spread of diseases across anthropogenic and natural habitats, posing risks to wildlife, domestic animals, and human health. For example, an outbreak of highly pathogenic AIV (HPAI A) caused by the H7N3 subtype affected numerous commercial poultry farms in 2020 (Youk et al., 2020). This epidemic was attributed to contamination by migratory birds. Phylogenetic analyses traced the genetic segments of the virus to North American avian lineages, particularly migratory waterfowl, confirming their role as biovectors in the spread of the virus to poultry farms.

When birds forage in urban and rural habitats, they may encounter microorganisms originating from human or animal feces. For instance, enterobacteria can originate from wastewater treatment settling ponds, landfills, and agricultural fields treated with organic fertilizers such as manure. For example, a study in New Hampshire (USA) found that herring gulls (*Larus argentatus*) and great black-backed gulls (*Larus marinus*) feeding in landfills and wastewater treatment settling ponds carried *E. coli* strains similar to those found in these environments, later transferring them to recreational waters (Nelson et al., 2008). Similarly, in Western Michigan (USA), ring-billed gulls (*Larus delawarensis*) and herring gulls frequenting landfills have been found to carry human-associated microorganisms such as *Campylobacter jejuni* and *Salmonella enterica* to municipal beaches (Alm et al., 2018). Additionally, a study on various gull species including black-headed gulls (*Chroicocephalus ridibundus),* herring gulls, and lesser black-backed gulls *(Larus fuscus*) from the Atlantic coast of North America showed a prevalence (cloacal swab) of avian influenza virus in individuals foraging in waterbodies, highlighting their role in the transmission of viral pathogens (Ineson

et al., 2022). Furthermore, sex-based differences in foraging behaviors can influence pathogen exposure and susceptibility to infection. In England, during the non-breeding season, female herring gulls were shown to carry *Salmonella* spp. twice as much as males due to their preference for selecting freshly dumped foods in landfills (Monaghan et al., 1985).

Larids frequently forage in anthropogenic habitats and their microbiome has been studied to investigate enterobacteria contamination(Zeballos-Gross et al., 2021). A significant concern with bird foraging activities in human-associated habitats is the high risk of contracting bacteria with antibiotic-resistant genes. For instance, in Andalusia (Spain), lesser black-backed gulls feeding in landfills were shown to harbor higher loads of bacterial pathogens with antibiotic-resistant genes compared to those feeding in more natural habitats (Jarma et al., 2021). Similarly, a study along the northeastern Iberian coast (Spain) identified yellow-legged gulls (*Larus michahellis*) as reservoirs of multidrug-resistant *Salmonella* and *Campylobacter* strains, particularly those that foraged in landfills (Migura-Garcia et al., 2017). In this study, gulls carried bacteria with resistance to critical antibiotics including fluoroquinolones and tetracyclines, thus underscoring their role in the dissemination of antimicrobial-resistant pathogens. However, many avian species tolerate low microbial loads and asymptomatically harbor pathogens, allowing these diseases to go undetected in the host and facilitating their transmission across habitats before any symptoms appear or management interventions can be implemented (Benskin et al., 2009; Clark, 2014). Moreover, the uneven spatial distribution of microorganisms at the landscape level can lead to varying levels of host exposure, complicating efforts to predict and manage disease risks of contamination following their excretion (Benskin et al., 2009). As such, a study in southern Spain used global positioning system (GPS) tracking on yellow-legged gulls to monitor their movements, creating pathogen risk maps that allowed the identification of critical habitats for transmission of bacteria like *Salmonella* spp. and *Campylobacter* spp. *(Navarro et al., 2019).*

Frequently used as sentinel species for biomonitoring environmental contaminants, larids benefit from their widespread distribution and omnivorous diet, which often includes anthropogenic wastes. By foraging in urbanized areas, larids may also be exposed to a suite of organohalogen contaminants including halogenated flame retardants (HFR) and per- and polyfluoroalkyl substances (PFAS). In the greater Montreal (QC, Canada) area, ring-billed gulls (*Larus delawarensis*) frequently forage in agricultural fields, residential areas, industrial areas, landfills, wastewater treatment facilities and waterbodies such as the St. Lawrence River (Gentes et al., 2015; Kerric et al., 2021; Sorais et al., 2021). Studies have documented

significant levels of HFRs in the plasma of ring-billed gulls breeding in this region, underscoring the risk of contaminant exposure in these birds (Gentes et al., 2012, 2015; Kerric et al., 2021, 2022; Sorais et al., 2020). HFRs are compounds added to various everyday objects (e.g., furniture upholstery, textiles, vehicles, etc.) for their flame-retardant properties (Blum et al., 2011). Polybrominated diphenyl ethers (PBDE) and other HFRs are frequently detected in wastewater treatment plants and landfills and are known to resist conventional treatment processes (Gentes et al., 2015; Tongue et al., 2019; Xu et al., 2021) demonstrated that male ring-billed gulls that visited various waste management facilities exhibited significantly higher plasma concentrations of the PBDE mixture decabromodiphenylether (DecaBDE) compared to those that did not. PFAS are used in the production of stain-resistant materials, non-stick pans and food packaging (Glüge et al., 2020) due to their oleophilic, thermostable, and non-stick properties. PFAS have also been found in high concentrations in landfill leachates and wastewater effluents, where they persist due to their resistance to degradation (Helmer et al., 2022).

Exposure to environmental contaminants such as HFR and PFAS has been linked to immunosuppression in various bird species. For example, HFRs have been associated with decreased antibody production in broiler chicks (*Gallus gallus domesticus,* Arbor Acres strain) exposed to various doses of decabromodiphenyl ether (BDE-209, Cheng et al., 2022). Similarly, PFAS have shown immunomodulatory effects in avian species such as decreased expression of key immune genes in chicken embryo fibroblasts exposed to perfluorooctane sulfonate (PFOS, Castaño-Ortiz et al., 2019)and suppression of T-cell-mediated immunity in Japanese quails *(Coturnix coturnix japonica*) exposed to perfluorooctanoic acid (PFOA, Smits & Nain, 2013). Although studies linking immunosuppression and increased pathogen load are limited, evidence from other species suggests that exposure to these contaminants can heighten susceptibility to infections. For instance, mice embryos exposed to PFOS showed reduced antibody production and increased vulnerability to influenza virus infection (Guruge et al., 2009). These findings underscore the need to investigate the impact of contaminant exposure on pathogen prevalence in ring-billed gulls.

The main objective of this study was to investigate the pathogenic microbial community in ring-billed gull's guano and explore its relationships with their use of foraging habitats and assess their role as biovectors. This study was conducted in the greater Montreal area, where one of the largest North American colonies of ring-billed gulls breed on Deslauriers Island (Qc, Canada) (Kerric et al., 2021). The role of this species as a biovector of organic contaminants has already been underscored by Desjardins et al. (2019), who estimated that approximately 1 g of halogenated flame retardants is excreted into the Montreal region

during the 28-day incubation period by the entire breeding colony on this island. However, while this estimate highlights their role in contaminant transfer, little is known about how their movements across heterogeneous habitats influence the composition and distribution of microbial pathogens they carry. We hypothesized that gulls foraging in landfills have higher abundance of foodborne pathogens (e.g. *Salmonella* spp*., Campylobacter* spp*.*) in guano, that individuals foraging in wastewater treatment plant settling ponds have higher abundance of *Escherichia* spp*.,* and that birds foraging in waterbodies have higher abundance of virus from the *Orthomyxoviridae* family such as avian influenza virus. A second hypothesis was that females exhibit higher bacterial β-diversity than males, likely due to their distinct foraging behavior in landfills, where they prefer freshly discarded food sources, leading to greater exposure to a wider variety of microbes. A more exploratory objective was to investigate the associations between plasma levels of HFRs and PFAS and pathogen abundance and diversity. As such, we hypothesized that gulls exhibiting greater plasma concentrations of PFAS and HFR have higher enterobacterial abundance. This study provides critical insights into how ring-billed gulls act as biovectors of microbial pathogens across diverse foraging habitats while also exploring how PFAS and HFR exposure in these habitats influence their pathogenic load. These findings provide new perspectives on how contaminant dynamics influence the spread of pathogens within human-impacted environments.

Material and Methods

2.2.1 Field sampling

Ring-billed gulls were sampled on Deslauriers Island (QC, Canada) in the St. Lawrence River, located 3 km downstream of Montreal, where approximately 18,000 pairs breed annually (Canadian Wildlife Service, unpubl. data, 2023). A total of 53 incubating gulls (36 males and 17 females) were randomly captured in May and June 2021 and 2022 using a remotely triggered leg trap placed on the nest. Miniature GPS dataloggers (Axy-Trek, TechnoSmArt, Guidonia, Rome, Italy) were attached to the birds' central tail feathers using waterproof tape (TESA, Charlotte, NC, USA) following methods described by (Gentes et al., 2015), and the birds were released into the colony immediately after. The GPS dataloggers (total weight: 8 g) accounted for less than 2% of the mean body mass of ring-billed gulls (mean ± SEM: 476 ± 6 g; n = 53). Approximately 10 days later (mean ± SEM: 11.6 ± 0.3 days) the birds were recaptured using the same method, and the GPS dataloggers were retrieved. Morphological measurements (e.g., body mass, tarsus and wing length, and bill height) were recorded, and blood samples were collected from the brachial vein using a 10 mL syringe and a 25G 5/8 needle rinsed with heparin. The blood samples were kept in a cooler

throughout the field day. The birds were then euthanized and feces (guano) samples were collected directly from the cloaca under dissection along with other tissues, which were used in companion studies, and stored in liquid nitrogen. The sex of the birds was confirmed by examining the gonads. In the laboratory, blood samples were centrifuged (2,500 x *g*, 7 min), and the resulting plasma (~2 mL) was stored at -30 °C until chemical analysis (section 2.4). The guano samples were kept at -80 °C until metagenomic analyses (section 2.3). The capture and handling methods with ring-billed gulls were approved by the Institutional Animal Care Committee of the Université du Québec à Montréal (permit no. 885) in accordance with the guidelines of the Canadian Council on Animal Care (Ottawa, ON, Canada).

2.2.2 Spatial analysis

The geographical positions of individual gulls (± 5-10 m precision) were recorded at 10-min intervals and projected onto a high-resolution map (ArcGIS, ESRI 2009; Redlands, CA, USA) following the methodology described by (Sorais et al., 2020). Data recorded under poor satellite coverage (horizontal dilution of precision > 10) were filtered out. To focus on foraging activities, GPS positions within the colony and those of birds in flight (speed > 4 km/h) were excluded from the dataset (Gentes et al., 2015). The probability of presence in specific foraging habitats (e.g., agricultural fields, industrial areas, residential areas, waterbodies, landfills, and wastewater treatment plant settling ponds) was then calculated using the methods by (Sorais et al., 2020).

2.2.3 Metagenomic analyses

High-throughput sequencing analyses were performed on ring-billed gull guano samples at the Molecular Diagnostic Laboratory of CDVUM, Université de Montréal (Saint-Hyacinthe, QC, Canada).

2.2.3.1 Virome

For the isolation and purification of viral genomes, the total nucleic acids were extracted from guano samples using the Quick-DNA/RNA Viral Kit and the Quick-RNA Viral Kit (Zymo Research Corporation, Irvine, CA, USA), containing inhibitor removal reactives and column to concentrate the total RNA from the viral DNA. The product was then quantified using the Qubit 3.0 Fluorometer (Invitrogen, Waltham, MA, USA). Isolation and purification of viral genomes for RNA virus sequencing was done using Zymo's Quick-RNA Viral extraction kit. Complementary DNA (cDNA) was then synthesized using random primers from New England Biolabs (NEB, Ipswich, MA, USA) using the NEBNext Ultra II RNA First Strand Synthesis Module. Random primers were first added to the RNA, heated at 80 °C for 2 minutes, and then held at 25 °C. First-

strand cDNA synthesis was performed using the following thermal profile: 25 °C for 10 minutes, 42 °C for 50 minutes, and 70 °C for 15 minutes, followed by storage at 4 °C. Double-stranded DNA (dsDNA) was generated using the NEBNext Ultra II Non-Directional RNA Second Strand Synthesis Module (New England Biolabs), with incubation at 16 °C for 150 minutes and subsequent refrigeration at 4 °C. The resulting dsDNA was purified with 1.8× PCR product wash beads (Quantabio, Beverly, MA, USA) and quantified using a Qubit 3.0 Fluorometer (Invitrogen, Waltham, MA, USA) along with the total DNA previously extracted. Regarding high-throughput sequencing and bioinformatic analyses, libraries were prepared using the Illumina Nextera XT kit (Illumina Inc., San Diego, CA, USA), purified with 1.0× PCR product wash beads, and size-selected using a 0.6× ratio. Quality was assessed on a Bioanalyzer 1200 using the High Sensitivity DNA kit (Agilent Technologies, Santa Clara, CA, USA). Sequencing was carried out on an Illumina MiSeq platform using a V3 600-cycle kit.

2.2.3.2 Microbiome

Total DNA was first extracted using ZymoBIOMICS™ DNA/RNA kit then was quantified using the Qubit 3.0 Fluorometer. Samples with DNA concentrations exceeding 1 ng/µL were diluted accordingly. Amplicon libraries targeting bacterial and fungal communities were prepared using the QIAseq 16S/ITS Region Panel (Qiagen, Hilden, Germany), which performs 16S rRNA gene and ITS region amplification across six hypervariable regions (V1V2, V2V3, V3V4, V4V5, V5V7, and V7V9) in three multiplex PCR reactions, each containing a distinct primer set. This approach allows for broader taxonomic resolution across bacterial and fungal lineages. PCR amplification was performed with the following thermal cycling conditions: initial denaturation at 95 °C for 2 minutes; 20 cycles of denaturation at 95 °C for 30 seconds, annealing at 50 °C for 30 seconds, and extension at 72 °C for 2 minutes; followed by a final elongation at 72 °C for 7 minutes. The resulting amplicons from the three PCRs were pooled for each sample and purified twice using 1.1× QIAseq purification beads. Indexing was then performed by PCR using the following conditions: 95 °C for 2 minutes; 18 cycles of 95 °C for 30 seconds, 60 °C for 30 seconds, and 72 °C for 2 minutes; with a final elongation at 72 °C for 7 minutes. The reaction was held at 4 °C before cleanup. Indexed products were purified using 0.9× QIAseq purification beads. Library quality and fragment size were assessed using the Agilent High Sensitivity DNA Kit on a Bioanalyzer 1200 (Agilent Technologies, Santa Clara, CA, USA). Libraries were sequenced on an Illumina MiSeq platform using a V3 600-cycle kit (2×300 bp paired-end reads).

2.2.3.3 Bioinformatic and statistical analyses

The reads were analyzed using CLC Genomic Workbench program (version 23.0.4, Qiagen, Hilden, GER). For bacterial sequencing, paired-end V3 600 was performed with 275 cycles for each read and 8 cycles for each index. Raw read sequences across the 53 samples ranged from 10.5 millions to 26 millions. for the six amplified 16S regions. For viral sequencing, paired-end V3 600 was performed with 300 cycles for each read. The fungal ITS sequences obtained were excluded from downstream analyses. The dataset for bacterial 16S rRNA was classified using the SILVA database r138 v.138.1 with a confidence threshold value of >50%, while metagenomic viral sequences were classified using the NCBI Virus database. Statistical analyses were performed with RStudio (v2022.07.2, RStudio Core Team, Boston, USA) and deemed statistically significant when $p \leq 0.05$. Rarefaction was carried out using the median sequencing depth method (Villeneuve et al., 2023). The total abundance for each amplicon sequencing variant (ASV) lower than the rarefaction threshold was removed. Consequently, the analysis included 37 genera of bacteria and 9 families of viruses. Since metagenomic sequencing does not allow for the specification of microorganism strains (Andersen & Hoorfar, 2018) only bacterial genera and viral families containing potentially pathogenic members were considered in the statistical analyses. Furthermore, taxa with no pathogenic potential, such as those comprising the normal gull intestinal flora, were excluded.

The global ASV community was analyzed using relative rarefied abundance following the methodology of (Villeneuve et al., 2023) utilizing the Phyloseq package (v.1.46.0; Fig.S1 & S2). The α-diversity indice such as the Shannon index and ASV richness was calculated from rarified data to compare diversity between gull males and females (Fig. S3). To assess the α-diversity between sexes, Wilcoxon rank sum test was used on the rarefied abundance of ASV for both bacteria genera and virus species. Co-occurrence patterns in the abundance of bacteria genera and virus species in the different foraging habitats were examine using the Spearman correlation between their rarefied ASV abundance, with the Benjamini-Hochberg correction. To assess the impact of landfills and wastewater treatment plant settling ponds on microbial community composition, β-diversity of individuals that foraged at least once in these habitats versus those that did not was visualized using non-metric multidimensional scaling (NMDS) based on Bray-Curtis dissimilarity from rarefied abundance data, for both bacteria and viruses. Permutational analysis of variance (PERMANOVA) was used to determine the significance of clusters. NMDS and PERMANOVA analyses were conducted with the *vegan* package (v2.6-4) employing a default 999 permutations, specifically using the *vegdist* and *adonis2* function. Results were visualized using the *plotly* package (v4.10.4). Linear discriminant analysis effect size (*LEfSe*) was applied to rarefied data (LDA > 2, $p \leq 0.05$) with the

*microbiomeMarke*r package (v.1.9.0) to identify microbial taxa distinguishing individuals associated with landfills and wastewater treatment plant settling ponds.

2.2.4 Chemical analyses

2.2.4.1 HFR

A total of 35 PBDE congeners and 14 other HFR (complete list in supporting informations in Table 2.3) were analyzed in plasma samples of ring-billed gulls at the Université du Québec à Montréal (Montreal, QC, Canada) following methods described by (Gentes et al., 2015). Briefly, plasma samples were homogenized with diatomaceous earth (JT. Baker, NJ, USA). To this, 100 µL of an internal standard solution (BDE-30, BDE-156, ^{13}C-BDE-209, and ^{13}C-*syn*-DP; 200 ppb, Wellington Laboratories, Guelph, ON, CA) was added. The HFRs were then extracted using a pressurized liquid extraction system (Fluid Management System, Billerica, MA, USA) with 50:50 (v:v) dichloromethane and n-hexanes. The final clean-up of samples was performed using acid-basic-neutral silica column followed by a neutral alumina column, both certified without PBDE (Fluid Management System). Analytes were identified and quantified using a gas chromatograph (GC) coupled to a mass spectrometer (MS; Agilent Technologies, Santa Clara, CA, USA) operating in negative chemical ionization mode (GC/MS-ECNI). An apolar capillary column DB-5 HT (J & W Scientific, Brockville, ON, Canada) was used for the separation of analytes.

Quality assurance (QA) and quality control (QC) procedures included the analysis of blanks, duplicates of ring-billed gull plasma samples, and standard reference material (NIST 1947; Lake Michigan fish tissues, National Institute of Standards and Technology, Gaithersburg, MD, USA). The mean (± SEM) recoveries of internal standards in the samples were: BDE-30 (91.5 ± 1.5%), BDE-156 (94 ± 1.3 %), ^{13}C-BDE-209 (61.5 ± 1.7%), and ^{13}C-anti-DP (95.5 ± 1.2%). Quantification of analytes was performed using an internal standard approach, and thus all HFRs were inherently recovery-corrected. Limits of detection (LODs) determined for each PBDE and other HFR analytes are listed in Table 2.3.

2.2.4.2 PFAS

The analysis of 60 PFAS (complete list in Appendix in Table S2) was performed on ring-billed gull plasma samples at the Université de Montréal (Montreal, QC, Canada). Plasma samples (40 µL) were transferred into a polypropylene centrifuge tube and 120 µL of an isotope-labeled standard (6 ng/mL in acetonitrile) was added to each tube along with 72 µL of methanol and 8 µL of HPLC-grade water. The samples were then centrifuged (10 min, 6,000 rpm) and an aliquot of 160 µL of supernatant was transferred to a

polypropylene injection vial. The samples prepared for injection thus had a composition of 50:30:20 acetonitrile:methanol:water (v/v/v). The analysis was carried out by ultra-high-performance liquid chromatograph coupled with high-resolution MS (UHPLC-HRMS; Thermo Q-Exactive Orbitrap, Waltham, MA, USA) with an injection volume set at 20 µL. The PFAS were scanned in both negative and positive ion modes and acquired simultaneously in 14.5 min using an electrospray ionization source operated in fast polarity-switching mode. A Thermo Hypersil Gold C18 column (100 mm × 2.1 mm, particle size 1.9 µm) was used for analyte separation. To offset potential PFAS exposure from the mobile phase, a Thermo Hypercarb column (20 mm × 2.1 mm, particle size 7 µm) was positioned downstream of the mixing point of the pathways, but upstream to the injector. The scanning range was m/z 150-1000 in full scan mode and the resolution was set at 70,000 FWHM at m/z 200.

QA/QC procedures included the analysis of blanks, duplicates of ring-billed gull plasma samples, commercial bird plasma spiking (chicken plasma from Sigma-Aldrich in 3.8% trisodium citrate as anticoagulant, P3266, sterile duck plasma from Cedarlane in sodium heparin, D514-06 (Rockland Inc, Limeric, PA, USA), and standard reference material (NIST 1947; Lake Michigan fish tissues, National Institute of Standards and Technology, Gaithersburg, MD, USA). The average recoveries (± SEM) of the internal standards in the samples were: perfluoroheptanoic acid (PFHpA: 77.9 ± 0.04%), PFOA (97.1 ± 0.02 %), PFNA (100.8 ± 0.1 %), perfluorodecanoic acid (PFDA: 64.6 ± 0.3%), perfluoroundecanoic acid (PFUnDA: 63.6 ± 0.5%), and perfluorohexane sulfonic acid (PFHxS: 91.9 ± 0.01%), therefore a blank correction was applied. The LODs listed in Table S2 were derived from the variation of the procedure blank (undoped chicken plasma) or signal intensity in chicken plasma matrices spiked with low-concentration PFAS.

2.2.4.3 Statistical analyses

The presence probability of ring-billed gulls in each foraging habitat was calculated for both males and females, with differences between sexes assessed using an analysis of variance (ANOVA). If more than 60% of the gull plasma samples had concentrations above the LODs for individual PFAS and HFRs, the sums of the analytes within their respective chemical classes were calculated. For those analytes meeting this criterion, concentrations of non-detects (<LOD) were generated using NDExpo version 1.0 (http://expostats.ca/site/app-local/NDExpo/). This tool estimates censored data (<LOD) based on a log-normal distribution using a semi-parametric method to generate a regression on order statistics (Lavoué et al., 2019). HFR and PFAS concentrations were a-priori tested for normality with the Shapiro-Wilk test

and for homoscedasticity with the Bartlett's test. Since the data was not normally distributed, even after transformation, the differences in plasma concentrations for each analyte sum between individuals that were present or absent in landfills and wastewater settling ponds for foraging were verified using non-parametric tests (Wilcoxon and KruskalWallis).

The associations between gull plasma concentrations of PFAS and HFRs and the abundance of various pathogenic taxa were tested along with the presence probability of gulls in foraging habitats as covariable. To test this, a distance-based redundancy analysis (dbRDA) model was used with the *vegan* package in R. Finally, an ANOVA-like permutational test was applied on the dbRDA model to assess the significance of the models, applying a Benjamini-Hochberg correction to adjust for multiple comparisons. All statistical analyses were performed with RStudio (v2022.07.2, RStudio Core Team, Boston, USA).

2.3 Results

2.3.1 Foraging habitat use

While engaging in foraging activities away from the colony, ring-billed gulls had the highest probability of presence in residential areas (mean ± SEM: 34.8 ± 1.6%), followed by agricultural fields (27.9 ± 1.7%), waterbodies (26.4 ± 1.6%), industrial areas (8.3 ± 0.7%), landfills (1.6 ± 0.3%), and wastewater treatment plant settling ponds (0.7 ± 0.1%). All foraging habitats were visited by gulls at least once, except for landfills and wastewater treatment plant settling ponds, which were frequented by only 50.9% and 45.3% of the individuals, respectively. The sex of the gulls did not influence the probability of presence in any foraging habitats (Figure 2.1).

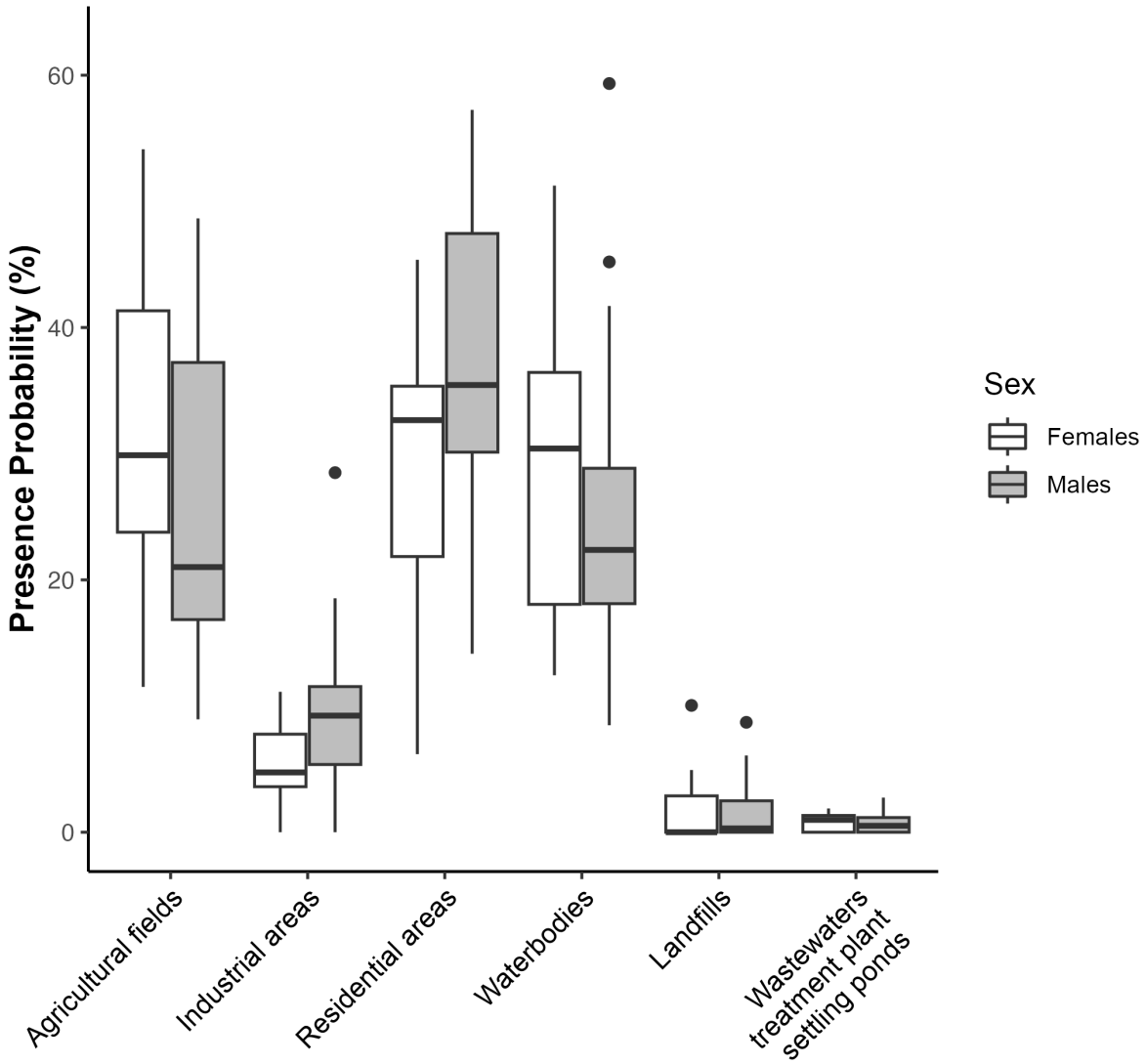

Figure 2.1 Presence probability (%) of ring-billed gulls (*n* = 17 females and *n* = 36 males) in the six major foraging habitats in the Montreal area (Qc, Canada). Horizontal bars across each box represent the median, the boxes represent the interquartile range (25th to 75th percentiles), vertical bars the range, and circles the outliers.

2.3.2 Bacterial and viral abundances

The bacterial genera in ring-billed gull guano were mainly dominated by *Enterococcus* spp. (mean ± SEM: 65.5 ± 5.2%), *Clostridium* spp. (8.5 ± 2.9%), and *Escherichia* spp. (6.0 ± 1.6%; Fig. 2.2 A). Among bacterial species, *Enterococcus cecorum* (59.9 ± 5.1%) was the most abundant, followed by *Escherichia coli* (5.9 ±

1.6%) and *Clostridium perfringens* (5.5 ± 2.4%; Fig. 2.10). Specific strains were not determined. For viral family abundance, the *Picobirnaviridae* family (37.3 ± 5.6%) was the most abundant, with species displaying genomes similar to human picobirnavirus being detected. The second most abundant viral family was *Herpesviridae* (24.0 ± 5.2%; Fig. 2.2 B), with species like colombid alphaherpesvirus and gallid alphaherpesvirus. Other virus families with potentially zoonotic taxa were detected such as *Paramyxoviridae* (17.0 ± 3.5%), including species such as avian *orthoavulavirus* genus, and *Adenoviridae* family (12.8 ± 3.4%).

Differences in the relative abundance between gull males and females were observed for the bacterial genera *Campylobacter* spp. (female : 0.1 ± 0.1%, male: 3.1 ± 0.9%; $p = 0.002$, $df = 45$, $n = 47$), *Helicobacter* spp. (female : 0.4 ± 0.2%, male: 2.9 ± 1.0%; $p = 0.01$, $df = 45$, $n = 47$), *Serratia* spp. (female : 5.2 ± 1.8%, male: 0.7 ± 0.4%; $p = 0.02$, $df = 45$, $n = 47$), and *Fusobacterium* spp. (female : 6.7 ± 3.2%, male: 0%; $p = 0.04$, $df = 45$, $n = 47$). However, no statistical differences were observed between males and females in the relative abundance of viral families.

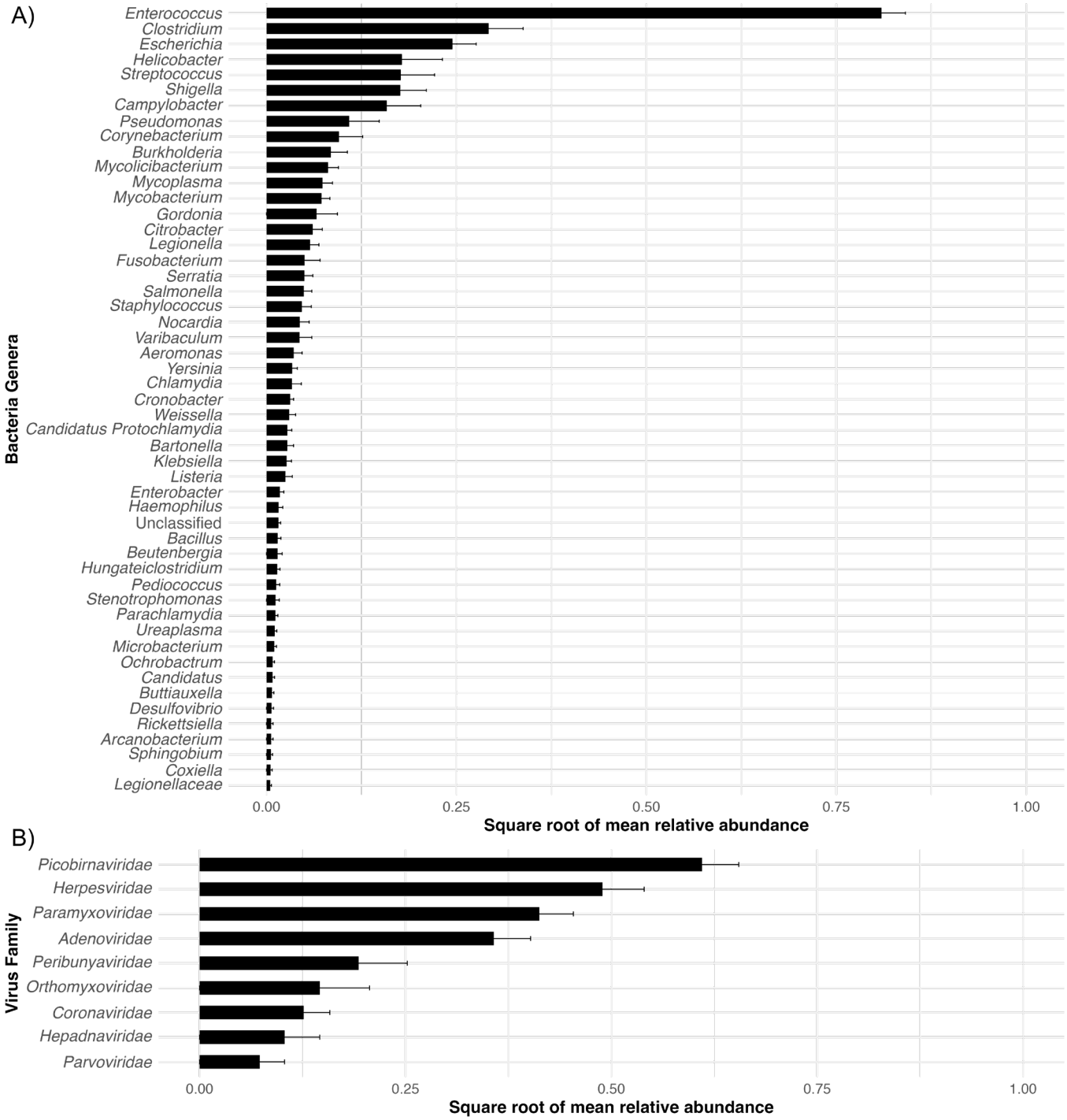

Figure 2.2 Square root of mean of relative abundance (± SEM) of (A) bacterial genera and (B) virus families in guano samples of ring-billed gulls (*n* = 53) from the Montreal area (QC, Canada).

2.3.3 Bacterial and viral profiles in foraging habitats

The presence probability of combined male and female ring-billed gulls in different foraging habitats was associated with the abundance of pathogenic micro-organisms in their guano (Fig. 2.3). Both positive and negative correlations were observed, reflecting varying relationships between gull presence and pathogen abundance across different habitats.

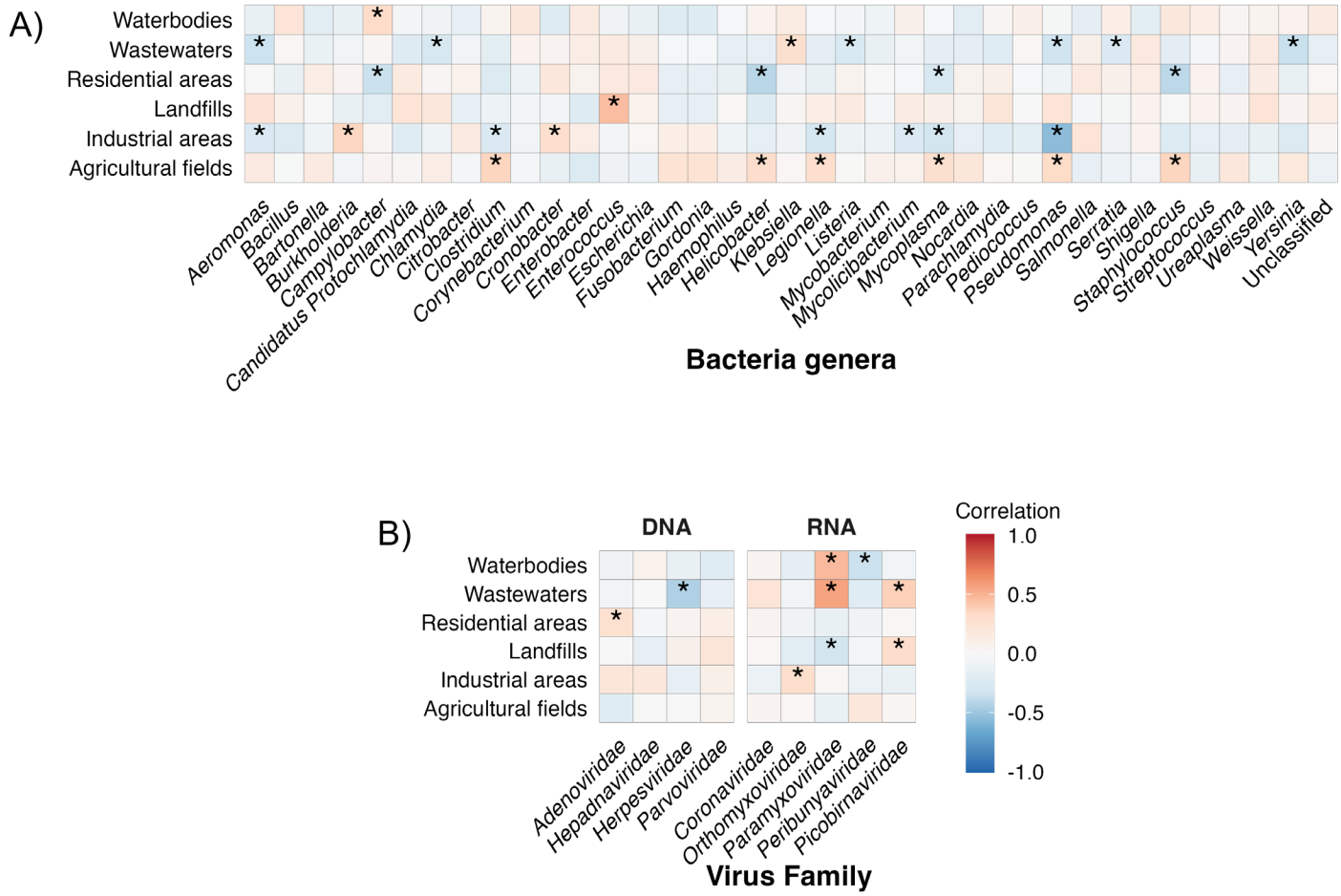

Figure 2.3 Spearman correlations between the rarified abundance of (A) bacteria genera and (B) virus families in guano samples ring-billed gulls (n_A = 47, n_B = 48) and their presence probability (%) in different foraging habitats in the Montreal area (QC, Canada). Wastewater stands for wastewaters treatment plant settling ponds. The strength of positive (red) and negative (blue) correlations are shown. Correlations are significant (*) when $p \leq 0.05$ after multiple pairwise testing using the Benjamini-Hochberg correction.

Positive correlations were more informative to delineate foraging habitats of higher potential pathogen abundance in relation with gull presence, and thus to understand their role as biovector. Presence of gulls

foraging in agricultural fields was positively correlated with the abundance of several bacterial genera such as *Clostridium* spp. (adjR^2 = 0.35, p = 0.04), *Helicobacter* spp. (adjR^2 = 0.30; p = 0.04), *Legionella* spp. (adjR^2 = 0.29, p = 0.05), *Mycoplasma* spp. (adjR^2 = 0.27, p = 0.03), *Pseudomonas* spp. (adjR^2 = 0.32; p = 0.05), and *Staphylococcus* spp. (adjR^2 = 0.35, p = 0.02). Moreover, *Enterococcus* spp. abundance showed a positive correlation with gull's landfill usage (adjR^2 = 0.45, p = 0.04). Also, *Campylobacter* spp. was positively correlated with the presence of gulls in waterbodies (adjR^2 = 0.31, p = 0.03) and *Klebsiella* spp. with wastewater treatment plant settling ponds (adjR^2 = 0.28, p = 0.04). Regarding bacterial species, two pathogenic bacteria showed positive correlations with frequent gull presence in specific habitats (Fig. 2.11). Specifically, *Escherichia albertii* was significantly associated with residential habitats (adjR^2 = 0.32, p = 0.03), while *Enterococcus faecium* was linked to wastewater treatment plant settling ponds (adjR^2 = 0.33, p = 0.04).

For the virus family, *Paramyxoviridae* abundance was correlated with the presence of ring-billed gulls in waterbodies (adjR^2 = 0.47, p = 0.007) and wastewater treatment plant settling ponds (adjR^2 = 0.56, p = 0.0001). Moreover, *Picobirnaviridae* showed positive correlation with presence probability of gulls in wastewater treatment plant settling ponds (adjR^2 = 0.37, p = 0.02) and landfills (adjR^2 = 0.30, p = 0.05). *Adenoviridae* and *Orthomyxoviridae* respectively showed correlation with time spent in residential areas (adjR^2 = 0.26, p = 0.01) and industrial areas (adjR^2 = 0.28, p = 0.05).

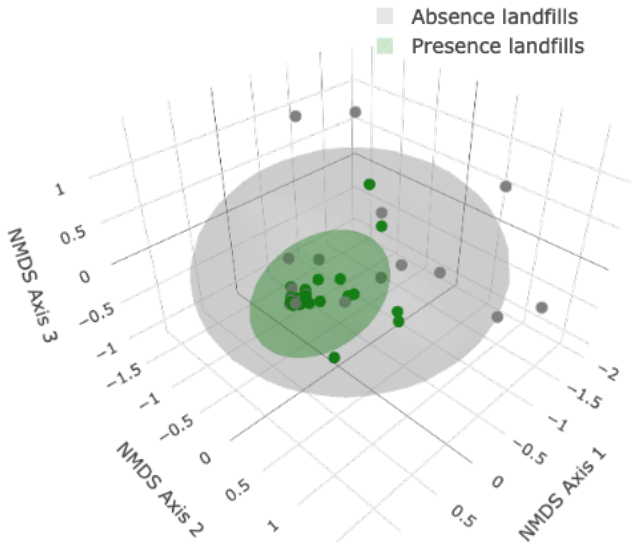

Figure 2.4 Non-metric Multidimensional scaling (NMDS) ordination of bacterial community composition in guano samples of ring-billed gulls that visited (presence, *n* = 25) or not (absence, *n* = 22) landfills in the Montreal area (Qc,

Canada), generated with a Bray-Curtis dissimilarity matrix based on the rarefied abundance matrix. Adonis test was significant ($p \leq 0.05$) after multiple pairwise comparisons corrected using the testing by Benjamini-Hochberg method.

The bacterial communities in the guano, between ring-billed gulls that foraged or not in landfills revealed two distinct clusters (Fig. 2.4). Notably, the cluster representing gulls that visited landfills was smaller and fell within the larger cluster of individuals that did not visit landfills, and this cluster was statistically significant as confirmed by a PERMANOVA test ($adjR^2 = 0.07$ $F = 0.73$, $df = 1$, $p = 0.006$). This pattern suggested that gulls foraging in landfills had a less diverse microbial community compared to those that did not. Further analysis investigating the differences in microbial community between gulls that visited or not landfills for foraging was conducted using the Linear Discriminant Analysis (LDA) effect size (LEfSe) method (Fig. 2.5). The LDA scores provided a quantitative measure of the impact size, where genera such as *Enterococcus* spp. and *Helicobacter* spp. showed strong associations with gulls foraging in landfills as indicated by their positive LDA scores. Conversely, genera such as *Campylobacter* spp., *Streptococcus* spp., *Corynebacterium* spp., *Shigella* spp., and *Escherichia* spp. displayed negative LDA scores, indicating an association with gulls that did not visit landfills.

No significant differences were observed in the community composition of the microbial communities in the wastewater environment ($p > 0.05$).

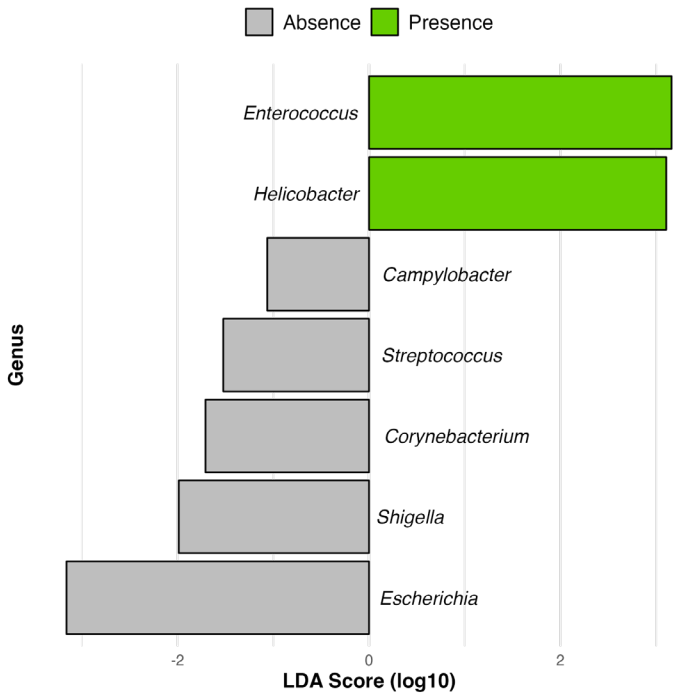

Figure 2.5 Linear discriminant analysis (LDA) score comparing different bacteria genera community between ring-billed gulls that visited (presence, *n* = 25) or not (absence, *n* = 22) landfills in the Montreal area (Qc, Canada). Model calculated using the LEfSe analysis. Genera with a LDA score > 1 are displayed.

2.3.4 Contaminant concentrations

Among Σ_{12}PFSA, PFOS exhibited the highest plasma concentrations (66.5 ± 11.8 ng/g ww) in combined male and female ring-billed gulls, contributing to 92.2 % of Σ_{12}PFSA and 63.7% of Σ_{76}PFAS (Table 2.1). The next highest concentrations in gull plasma was perfluorododecanoic acid (PFDoA; 14.5 ± 2.0 ng/g ww), a long chain perfluorocarboxylic acid (PFCA), with a percentage of contribution of 28.4% of (Σ_{18}PFCA) and 0.42% of Σ_{76}PFAS. Moreover, among HFR concentrations, BDE-99 and BDE-47 were the PBDE congeners with the highest plasma concentrations in ring-billed gulls (BDE-99: 3.1 ± 0.3 ng/g ww; BDE-47: 2.3 ± 0.3 ng/g ww) with a percentage of contribution to Σ_{49}HFR of 31.4% and 29.5%, respectively (Table 2.1). For Σ_{17}Other HFRs detected (i.e., hexabromobenzene: HBB, heptabromodiphenyl ether and dechlorane-604 mixture: BDE-183/Dec-604, syn-dechlorane plus: syn-DP, anti-dechlorane plus: anti-DP), their concentrations were lower with a percentage of contribution of the Σ_{49}HFR of 8.9%. There was no significant difference in plasma concentrations for PFAS between gulls that visited or did not visit landfills (PFAS: W = 6128, r = 404.1, p = 0.38) or wastewater treatment plant settling ponds (PFAS: W = 6343, r =

418.2, p = 0.75). However, a significant difference in HFR concentrations was found between gulls visiting and not visiting landfills (HFR: W = 3679, r = 268.3, p = 0.05), while no significant difference was detected for gulls foraging at wastewater treatment plant settling ponds (HFR: W = 4648, r = 339, p = 0.51).

Table 2.1 Plasma concentrations (mean ± SEM, ng/g ww) of organohalogen contaminants determined in combined male and female ring-billed gulls from the Montreal area (17 females and 36 males).

Analytes groups	Concentrations (ng/g ww)
Σ_{76}PFAS	104 ± 15.0
Σ_{12}PFSA	73.7 ± 13.5
Σ_{18}PFCA	27.2 ± 3.2
Σ_{14}FTP	2.6 ± 1.0
Σ_{5}Miscellaneous PFAS	0.60 ± 0.04
Σ_{49}HFR	9.9 ± 0.8
Σ_{32}PBDE	8.8 ± 0.7
Σ_{17}Other HFRs	0.88± 0.1

2.3.4.1 Relationship between contaminant concentrations and relative bacterial abundances

The db-RDA provides a multivariate perspective that considers the combined influence of environmental factors and contaminants, complementing the findings from Spearman correlations. This analysis revealed complex relationships between abundance of specific taxa and both plasma contaminant concentrations and habitat presence probability of ring-billed gulls. Specifically, a positive association was found between *Enterococcus* spp. abundance and plasma levels of Σ_{49}HFR and Σ_{76}PFAS, as the vector points in the direction of the abundance of *Enterococcus* spp. of each individual (Fig. 2.6). For Σ_{76}PFAS, the proximity of the landfills vector indicates an association between gull presence in this habitat and *Enterococcus* spp. abundance (Fig. 2.6A). Similarly, for Σ_{49}HFR, the overlapping vectors for wastewater treatment plant settling ponds and residential habitats suggest that these habitats may influence the association, possibly due to elevated HFR levels (Figure 2.6B).

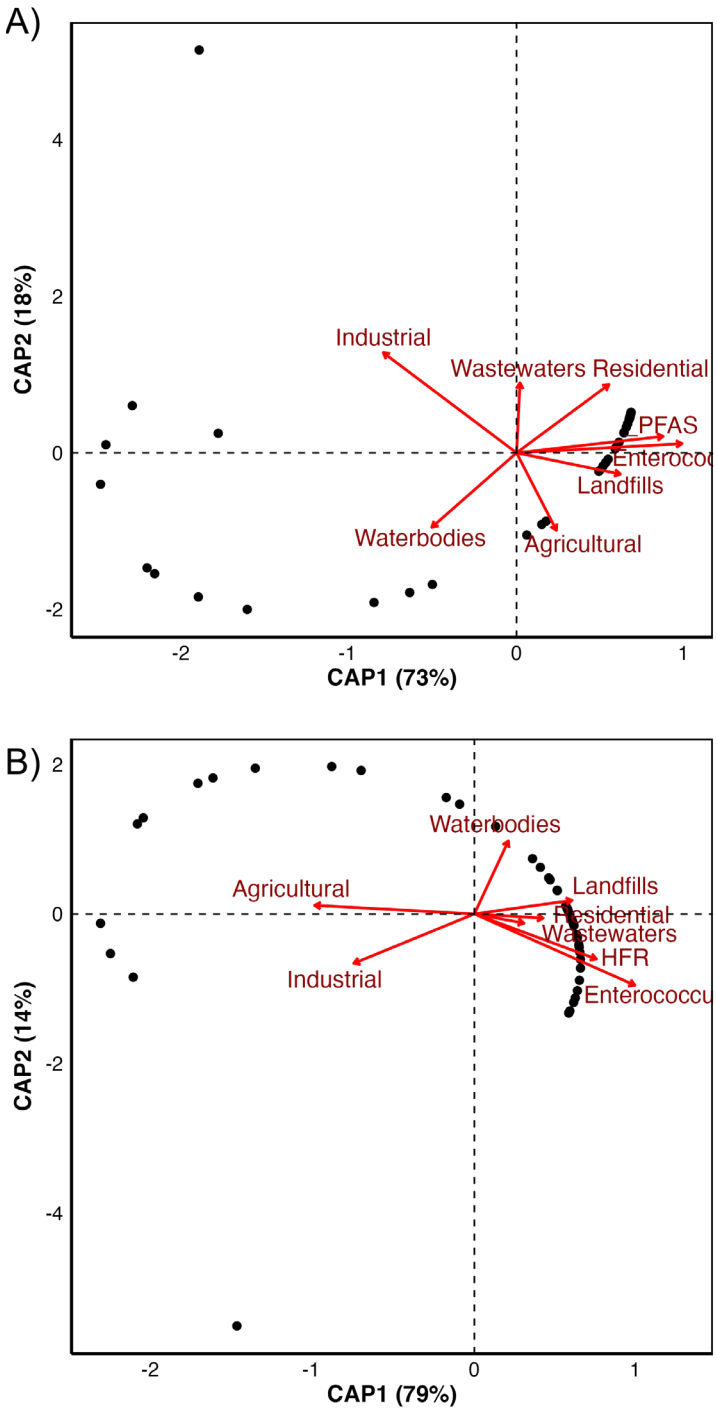

Figure 2.6 Distance-based redundancy analysis (db-RDA) correlating *Enterococcus* spp. abundance in ring-billed gull guano samples with explanatory factors, including presence probability (%) in foraging habitats and plasma concentrations (log ng/g w/w) of (A) Σ_{76}PFAS and (B) Σ_{49}HFR. Points represent the abundance of *Enterococcus* spp. in each sample.

The db-RDA analysis revealed significant associations between the abundance of *Enterococcus* spp. in ring-billed gull guano and plasma concentrations of Σ_{76}PFAS and Σ_{49}HFR , as well as presence probability in

various foraging habitats (Table 2). *Enterococcus spp.* was positively correlated with both Σ_{76}PFAS and Σ_{49}HFR concentrations (Σ_{76}PFAS: $F = 0.83$, $p = 0.04$; Σ_{49}HFR: $F = 2.55$, $p = 0.03$). Furthermore, correlations were observed between plasma Σ_{76}PFAS concentrations ($F = 0.8$, $p = 0.04$) and presence probability of gulls in agricultural fields ($F = 3.4$, $p = 0.03$), industrial areas ($F = 4.2$, $p = 0.01$), residential areas ($F = 3.1$, $p = 0.03$), and waterbodies ($F = 3.4$, $p = 0.03$). Similarly, correlations were found between plasma Σ_{49}HFR concentrations ($F = 2.6$, $p = 0.03$) and presence probability in agricultural fields ($F = 3.4$, $p = 0.03$), industrial areas ($F = 4.3$, $p = 0.01$), residential areas ($F = 3.06$, $p = 0.04$), and wastewater treatment plant settling ponds ($F = 0.2$, $p = 0.05$). The abundance of Landfills, however, did not show any correlations in this multivariate analysis. This contrasts with the Spearman correlation results, which highlighted a significant relationship between *Enterococcus* spp. abundance and landfill presence when considered independently of other factors.

Table 2.2 Correlations between the *Enterococcus* spp. abundance and plasma levels of Σ_{76}PFAS and Σ_{49}HFR and presence probability of ring-billed gull males and females in foraging habitats using db-RDA.

	PFAS				**HFR**			
	Df	SumOfSqs	F	Pr(>F)	Df	SumOfSqs	F	Pr(>F)
Σ_{49}HFR	NA	NA	NA	NA	1	0.37	2.55	0.03*
Σ_{76}PFAS	1	0.11	0.83	0.04*	NA	NA	NA	NA
Agricultural fields	1	0.44	3.36	0.03*	1	0.52	3.39	0.03*
Industrial areas	1	0.54	4.15	0.01*	1	0.65	4.27	0.01*
Residential areas	1	0.40	3.09	0.03*	1	0.47	3.06	0.04*
Waterbody	1	0.45	3.44	0.03*	1	0.51	3.31	0.30
Landfills	1	0.15	1.13	0.32	1	0.14	0.90	0.41
Wastewaters treatment plant settling ponds	1	0.029	0.22	0.92	1	0.03	0.17	0.05*
Residuals	38	5.58			39	5.96		

Statistical parameters used to evaluate the significance of the db-RDA results; Df: degrees of freedom, F: F-statistic of Anova test, and Pr(>F): *p,* NA: data no applicable. Correlations are significant (*) when $p \leq 0.05$ as determined using Anova test.

2.4 Discussion

2.4.1 Bacterial community

Although the focus was solely on potentially pathogenic microorganisms, two of the three most abundant bacterial genera (i.e., *Enterococcus* and *Escherichia* spp.) identified in ring-billed gull guano are integral components of the normal intestinal flora of many vertebrates, including birds and mammals (Aarestrup et al., 2002; Souillard et al., 2022), and were therefore expected. Among members of *Enterococcus* spp., the high abundance of *Enterococcus cecorum* that may be present in guano suggests that Montreal-breeding ring-billed gulls may serve as reservoirs and potential biovectors for this bacterium. This may pose a potential health risk as *E. cecorum* is a major concern in the poultry industry, causing serious locomotor diseases such as myositis, arthritis, osteomyelitis, tenosynovitis, spondylitis, femoral head necrosis, and even death (Jung et al., 2018; Souillard et al., 2022). However, its pathogenicity varies depending on the strain (Jung et al., 2018), which was not determined in the present study.

Since most studies report bacterial abundance at the phylum level without focusing on potential pathogenic taxa, direct comparisons at the genus level are limited. Regardless, our results align with those of Jarma et al. (2021), where *Enterococcus* spp. was a predominant genus in the gut microbiota of the lesser black-backed gull from coastal areas in Norway during the breeding season. In contrast, *Pseudomonas* spp. and *Staphylococcus* spp. showed higher relative abundance in that study, whereas these genera were less abundant in the guano of the present ring-billed gulls. Additionally, our results contrast with those of Liao et al.(2023), who reported low *Enterococcus* spp. abundance in feces samples of black-headed gulls from inland areas in China. The differences between our results and those reported in these two studies may be due to the geographical location and species-specific factors including life or reproductive cycle, seasonality, degree of habitat urbanization, and dietary preferences.

2.4.1.1 Relationship between bacterial abundances and foraging habitats

Several correlations between bacterial genus abundance and presence probability of ring-billed gulls in foraging habitats were found. The presence probability of gulls in agricultural fields showed the highest number of positive correlations, including with *Clostridium* spp., *Helicobacter* spp., *Legionella* spp., *Mycoplasma* spp., *Pseudomonas* spp., and *Staphylococcus* spp. This combination of bacterial genera, found in the guano of ring-billed gulls foraging predominantly in agricultural fields, can be attributed to nutrient runoffs and livestock-derived organic matter, facilitating bacterial proliferation and the input of a variety of bacteria originating from organic fertilizers such as manure and compost (Li et al., 2020; Liu et

al., 2020; Loss et al., 2019). Both organic and inorganic fertilizers can alter the bacterial community in the soil of agricultural fields (Lin et al., 2019; Ye et al., 2019, 2021). As such, inorganic fertilizers can influence microbial composition indirectly by altering soil pH and increasing the availability of nitrogen and phosphorus, which favors certain bacterial taxa. On the other hand, organic inputs (e.g., manure) may introduce a wide range of bacteria, including both beneficial and pathogenic species. Genera of *Salmonella* spp., *Clostridium* spp., *Escherichia coli*, and *Listeria* spp., all having potentially pathogenic risks, typically originate from manure produced by pigs, poultry and cattle, and have been reported to be widely distributed into soil (Li et al., 2020; H. Zhang et al., 2020). Additionally, manure-amended soil may harbor antibiotic-resistant bacteria including *Staphylococcus aureus* with methicillin-resistant strains and *Enterococcus* spp. with vancomycin-resistant strains, as documented in various studies on antibiotic resistance in agricultural settings (Huygens et al., 2021; L. Zhu et al., 2022; Y. G. Zhu et al., 2013).

Enterococcus spp. also showed positive correlations with the presence of ring-billed gulls in landfills for foraging. Landfills receive various types of wastes including household, industrial, construction, clinical, and organic refuse. The percolation of water through waste materials produces leachate, which can contain antibiotic residues and turn active landfilling areas into breeding grounds for antibiotic-resistant bacteria. Although ring-billed gulls are primarily exposed to food remains in the dry active landfilling or compost areas, the entire landfill site may contribute to the proliferation of these bacteria (Chen et al., 2017; Mondragón-Quiguanas et al., 2022). Moreover, as landfills age, the concentrations of human bacterial pathogens in soil may increase, turning these areas into potential hotspots for enterococcal infections, as observed by Wu et al. (2021) in landfills from China. As such, our findings align well with the study of Alm et al. (2018),where landfill-foraging herring gulls in Michigan (USA) exhibited higher *Enterococcus* spp. abundance than those foraging in wastewater treatment plant settling ponds. Furthermore, Sacristán-Soriano et al. (2024) reported similar results for black-backed gulls from Spain, where landfill-foraging individuals carried more *Enterococcus* spp. in their guano than those feeding primarily, though not exclusively, in paddy fields. Additionally, our study demonstrated that gulls visiting landfills at least once for foraging had a more restricted bacterial diversity, dominated by *Enterococcus* and *Helicobacter* genera, while *Campylobacter* spp., *Streptococcus* spp., *Corynebacterium* spp., *Shigella* spp., and *Escherichia* spp. were more associated with gulls that did not use landfills. These findings further support those of Sacristán-Soriano et al. (2024), who found a higher abundance of *Campylobacter* spp. and *Streptococcus* spp. in gulls feeding in paddy fields rather than landfills.

A positive correlation between *Enterococcus* spp. abundance and gull foraging time in wastewater treatment plant settling ponds was predicted, but our results did not support this prediction. Nevertheless, the abundance of a species within this genus (*Enterococcus faecium*) was positively correlated with the presence probability of gulls in wastewater treatment plant settling ponds. Typically, wastewater treatment plant settling ponds are known to harbor high concentrations of fecal bacteria (Fouz et al., 2020) including antibiotic-resistant strains (Auguet et al., 2017; Lépesová et al., 2018; Makowska et al., 2021). The presence of *enterococci*, which is part of the normal flora of birds and mammals, in wastewater was expected. Indeed, several studies have reported high detection rates of multi-resistant strains in wastewater treatment plant settling ponds and biofilms (Gouliouris et al., 2019; Makowska et al., 2021; Nishiyama et al., 2017). Among *Enterococcus* spp., *E. faecium*, which is zoonotic (Arredondo-Alonso et al., 2020), its association with ring-billed gulls foraging in wastewater treatment plant settling ponds raises important concerns regarding the potential health risks for waterfowl and other wildlife. This bacterial species is a known pathogen that may cause infections such as gastroenteritis, urinary tract infections, endocarditis, and meningitis (Stępień-Pyśniak et al., 2018; Venkataraman et al., 2023). Gulls are attracted to wastewater treatment facilities due to predictable resources, including organic wastes and invertebrates present in settling or aeration ponds, and can be exposed to pathogens through ingestion of contaminated water or biofilms. The presence of *E. faecium* in these environments, combined with the foraging behavior of gulls, suggest that they could act as biovectors, posing a potential risk to public health (Stępień-Pyśniak et al., 2018).

The lack of correlation between the abundance of *Escherichia* spp. and the time gulls spent in any foraging habitats in the present study contrasts with other studies that associated *Escherichia* spp. with fecal contamination in landfills and wastewater treatment plant settling ponds (Ahlstrom, Bonnedahl, et al., 2019a; Ahlstrom, Ramey, et al., 2019; Umar et al., 2015). Despite this, one member of *Escherichia* spp., *E. albertii*, showed a positive correlation between its abundance and gulls frequently foraging in residential habitats. Unlike other species within the *Escherichia* genus, *E. albertii* is zoonotic, causing gastroenteritis in humans and birds. Although less studied than other *Escherichia* species, its significance is increasingly recognized due to its pathogenic potential, particularly through the production of the Shiga toxin (Gomes et al., 2020). Transmission of *E. albertii* typically occurs through contact with animal feces or ingestion of contaminated water or food, particularly chicken and processed meat products such as pork and duck, for which outbreaks have been linked to restaurant foods (Leszczyńska et al., 2023; Maeda et al., 2015; Maheux et al., 2014; Ooka et al., 2015). Hence, the correlation between *E. albertii* abundance and

residential areas likely reflects gulls foraging in residential or commercial garbage bins, where improper food waste disposal creates favorable conditions for bacterial proliferation. These results therefore illustrate how residential areas can act as interface for the exchange of pathogenic bacteria between humans and urban-adapted birds.

2.4.2 Viral community

In terms of viral read abundance, the high presence of picobirnaviruses was expected since waterfowl is known to be its natural reservoir (Wille et al., 2019). Picobirnaviruses have been detected in a wide range of wildlife species and most of the time in asymptomatic healthy individual. They are considered to be potentially zoonotic and can cause gastroenteric symptoms in immunocompromised humans (Ganesh et al., 2014). Picobirnavirus infection has been reported in various species of wild birds as well as domestic (farmed) birds including turkey (*Meleagridea)*, chicken (*Gallidea)*, and American ostrich (*Struthio americanus;* Day et al., 2010; Ganesh et al., 2014; Masachessi et al., 2012).

The second most abundant viral reads uncovered in ring-billed gulls was related to the *Herpesviridae* viral family. Herpesviruses are prevalent in many vertebrate species and are known to be highly adapted to their hosts, often establishing latent infections that may persist subclinically throughout the host's life, depending on the immune status of the bird (Sebastiano et al., 2020). Indeed, the host can remain asymptomatic, becoming clinically evident only under conditions of stress or immune suppression. Although each herpesvirus has evolved to specifically infect certain host species, they can also be acquired by other species. For instance, Columbid herpesvirus-1 was detected in herring gulls and peregrine falcons (*Falco peregrinus*) in Poland, as reported by Woźniakowski et al. (2013). This virus, typically found in urban wild pigeons, was present in both raptors and gulls, illustrating the cross-species transmission in urban environments where pigeons and these other species often interact. In addition, highly pathogenic herpesviruses such as Gallid alphaherpesvirus 1 (GaHV-1), the responsible agent of infectious laryngotracheitis and Gallid alphaherpesvirus 2 (GaHV-2), which causes Marek's disease, can lead to bird mortality and be responsible for economic loss in poultry industry (Sogut *et al.,* 2021).

The third most abundant viral reads found were related to the *Paramyxoviridae* family, a diverse group of viruses known to infect a wide range of bird species. This family predominantly includes viruses from the *Orthoavulavirus* genus, also called Avian orthoavulavirus, which has many serotypes (Yin *et al.,* 2017). The Avian orthoavulavirus species, commonly known as Newcastle disease virus (NDV), is particularly notable

due to its ability to cause Newcastle disease. As waterfowl and aquatic birds are natural reservoirs of this virus (Jindal et al., 2009; Meng et al., 2016), this result was expected. The presence of numerous ducks and geese on and around this breeding colony (Deslauriers Island) suggests they could be likely sources of this virus. NDV strains vary in virulence, with velogenic strains causing severe gastroenteritis or neurological symptoms, mesogenic strains leading to milder neurological or respiratory symptoms, and lentogenic strains being the least virulent, causing mild respiratory issues (Boykin, 2020; Diel et al., 2012). This disease may pose high risk to large seabird colonies. As such, previous studies have detected Newcastle disease in ring-billed gulls from this region in Canada (Diel et al., 2012; Wobeser et al., 1993). Importantly, NDV can cause a high mortality rate and severe economic losses in commercial poultry birds. Therefore, Newcastle is a reportable disease in Canada under the *Health Animals Act* (Canadian Food Agency, 2024).

The *Adenoviridae* family, which includes several avian pathogens such as Fowl adenovirus (FAdV) and Duck adenovirus, and the *Peribunyaviridae* family, which comprises viruses like Bunyamwera virus, were the fourth and fifth most abundant viral families, respectively. FAdV have been increasingly reported in wild birds without clinical signs, suggesting that asymptomatic individuals may serve as reservoirs and contribute to the environmental persistence and transmission of these viruses (Brochu et al., 2019). Their high prevalence in guano samples is therefore consistent with the capacity of adenoviruses to establish subclinical, persistent infections in avian hosts, leading to continuous viral shedding over time. In contrast, some members of the *Peribunyaviridae* family are maintained in bird–mosquito transmission cycles and possess receptor-binding proteins that are compatible with human cell entry factors, highlighting their potential to cross species barriers and emerge as zoonotic pathogens (Dufloo et al., 2025). The detection of *Peribunyaviridae* family in guano may reflect seasonal dynamics, as the study was conducted during the summer months, when mosquito activit, and consequently virus circulation, is typically elevated.

Although *Orthomyxoviridae* was only the sixth most abundant viral family, it included the Avian Influenza A virus (AIV), a reportable disease in many countries including Canada (CFIA, 2024). The AIV, commonly referred to as bird flu, has been a significant concern for both wildlife and the poultry industry due to its potential to cause widespread outbreaks. Gulls in particular are important hosts for certain subtypes of AIV such as H5, H13 and H16, and they have been documented as both carriers and disseminators of these viruses over long distances, with evidence linking them to the contamination of poultry farms. For instance, H13N2 viruses isolated from Canadian ring-billed gulls have been found to infect domestic poultry such as domestic turkeys (*Meleagris gallopavo*) in North America, often without causing symptoms (Benkaroun et

al., 2016). Moreover, AIV undergoes rapid evolution, allowing the virus to adapt swiftly to new hosts and environmental conditions, which can lead to the emergence of highly pathogenic strains capable of causing severe disease. For example, a severe outbreak happened with a highly pathogenic strain of Avian influenza virus A (H5N1 clade 2.3.4.4b) in eastern Canada during the breeding season of 2022, affecting mainly seabirds (Avery-Gomm et al., 2024). Since gulls from our study were breeding in the same area, the detection of Avian Influenza A in the guano sample was expected. However, because the metagenomic analysis does not provide precision identification of the virus strain, it remains unclear whether the detected AIV sequences correspond to highly pathogenic strains or not.

2.4.2.1 Relationship between viral abundances and foraging habitats

Correlations were found between the abundance of a few virus species and ring-billed gull foraging time in certain habitats. Notably, the abundance of *Paramyxoviridae* showed correlations with waterbodies and wastewater treatment plant settling ponds. These correlations provided interesting insights into the dynamics of this virus family among aquatic birds, corroborating findings reported elsewhere. Indeed, Avian orthoavulavirus for example, is generally transmitted via inhalation or ingestion of contaminated feces or nasal secretions of birds such as gulls, ducks, and other waterfowl (V. R. Brown & Bevins, 2017). Its stability in aquatic environments also allows for fomite transmission (Masachessi et al., 2012). The correlation of *Paramyxoviridae* reported herein with waterbodies near cities, such as lakes, rivers or ponds, may be due to fecal-oral transmission route, where water becomes contaminated by infected birds' feces, allowing others to ingest the virus while foraging (Rahman et al., 2018). Also, Avian orthoavulavirus can survive in water for long periods due to its lipid envelope, which protects its RNA from degradation and makes it resistant to temperature changes (Ruan et al., 2020), enhancing its transmission potential and explaining the correlation found with waterbodies and wastewater treatment plant selling ponds.

Moreover, our results revealed a significant association between *Picobirnaviridae* abundance and the time gulls spent foraging in wastewater treatment plant settling ponds and in landfills. This finding was expected as this virus family is prevalent in fecal matter of various mammal species, thriving due to the rich organic matter and nutrient content found in these environments (Adriaenssens et al., 2018; Hamza et al., 2011; S. Zhang et al., 2015). The fecal-oral transmission route of this virus is the primary infection pathway for most animals and especially birds (Ganesh et al., 2014). Specifically, gulls likely contract *Picobirnaviridae* by feeding on floating particles in settling or aeration ponds. This virus family is known for its resilience in most aquatic environments due to its non-enveloped structure, which allows it to

persist in fecal matter and contaminated water for extended periods of time and to resist in low pH environments, enhancing its potential for transmission (Ganesh et al., 2012; Vaz et al., 2020). This highlights the role of wastewater treatment plants settling ponds and landfill soils in maintaining or possibly amplifying this virus family in the environment, which gulls may acquire while foraging in this habitat.

Other correlations were expected, such as *Adenovirideae* abundance that was correlated with residential areas and *Orthomyxoviridae* that was correlated with industrial areas. The presence of adenovirus in residential zones was anticipated due to the frequent interaction of urban-adapted bird species with human environments, which may facilitate environmental contamination and viral transmission in areas of high bird density and anthropogenic activity. *Adenoviridae* contains five genera and the most common one infecting bird is *Aviadenovirus* (Vaz et al., 2020). Indeed, adenovirus has been detected in silver gulls (*Larus novaehollandiae*) from urban environment in Australia including a variant closely related to pigeon adenovirus 2 *(Vaz et al., 2020)*. As for *Orthomyxoviridae*, the most frequent transmitted species between birds is the Avian Influenza and specifically the A subtype, with Charadriiformes such as gulls being considered the major natural reservoirs (Joseph Domenech & Bernard Vallat, 2009; Munster et al., 2007b). Avian influenza A contamination has been reported frequently in industrial areas such as poultry processing factories, where a high concentration of birds facilitates viral mutation and rapid transmission of viruses both within farms and across different geographical locations(Leibler et al., 2009). This spread is exacerbated by inadequate biosecurity measures during the transportation and shipment of poultry products, which can carry the virus to new areas.

2.4.3 Relationship with organohalogen contaminants

Plasma Σ_{32}PBDE concentrations in ring-billed gulls from the present study were approximately three times lower than those reported by (Gentes et al., 2015) in gulls sampled between 2010 and 2012, and four times lower than those reported by (Desjardins et al., 2019) in individuals sampled in 2014, both from the same breeding colony. The significantly lower plasma concentrations determined in the present individuals compared to previous sampling campaigns can be attributed to regulations and environmental management of PBDE mixtures in North America over the last decade. Indeed, the designation of the major PBDE mixtures (penta- and octa-BDEs) in 2009 and deca-BDE in 2017 as persistent organic pollutants under the Stockholm Convention (UNEP United Nations Environment Programme, 2017) has led to stricter controls on their usage.

Plasma Σ_{76}PFAS concentrations in present ring-billed gulls were three to four times lower than those reported by (Sebastiano et al., 2021) in various gull species from southern France, depending on the sex of the birds. Several factors may explain this difference, including higher PFAS emissions from domestic usage and wastewater effluents in Europe compared to North America (Johnson et al., 2022; Rankin et al., 2016). Also, species-specific feeding behavior, trophic level, and biotransformation contribute to variations in blood contaminant concentrations (Borgå et al., 2004; Haarr et al., 2018). PFAS have also been detected in eggs of herring gulls nesting in the Great Lakes region of Canada (Letcher et al., 2015; Su et al., 2017), but at lower concentrations compared to those found in the plasma of ring-billed gulls from this study. For example, in the study of Su *et al.* (2017), the sum of PFSA concentrations and PFCA in herring gull eggs were approximately two times lower compared to the Σ_{12}PFSA and the Σ_{18}PFCA concentrations found in ring-billed gull plasma from this study.

For both Σ_{76}PFAS and Σ_{49}HFR, plasma concentrations were positively correlated with *Enterococcus* spp. abundance and showed significant associations with the probability of gulls foraging in specific habitats. This likely reflects the combined influence of environmental contamination and where and on what the gulls forage. Few studies have examined the microbiota of wildlife exposed to environmental PFAS levels, but our findings align with a study on freshwater turtles (*Emydura macquarii macquarii*), which showed an increased abundance of *Enterobacteriaceae* when exposed to PFAS (Beale et al., 2022). Currently, no studies have explored the effects of PFAS on avian microbiota. Regarding HFRs, our results differ from those of (Gomez et al., 2020), who found that early-life exposure to BDE-47 (penta-BDE) in rats led to a decreased abundance of the *Enterococcus* genus in their gut microbiota. Further research is needed to better understand this relationship in birds.

2.5 Conclusions

The findings of this study provided a comprehensive insight into the microbial community in guano of ring-billed gulls breeding in the densely populated and urbanized Montreal area. This study highlighted the role of this urban-adapted gull in the dispersal of several bacterial potentially pathogenic genera and the possible influence of environmental contaminant exposure on microbial abundance. We found a strong correlation between the gulls' foraging behavior and pathogen load, particularly with the use of agricultural fields and abundance of the pathogenic bacteria *Enterococcus* spp., *Clostridium* spp., and *Helicobacter* spp. Contrary to our predictions, gulls foraging in wastewater treatment plant settling ponds exhibited higher abundance of the viral families *Picobirnaviridae* and *Paramyxoviridae*. Additionally, while

positive correlations were observed between *Enterococcus* spp. abundance and plasma concentrations of PFAS and HFRs, these correlations were relatively weak and were associated with the presence probability in various habitats, suggesting that the observed relationships with contaminant levels may not be directly linked to exposure alone but could be influenced by the gulls' foraging behavior and habitat use patterns. However, our study design did not allow for the investigation of the effects of seasonal variations of foraging behaviors on pathogen load, which could provide deeper insights into the temporal aspects of pathogen transmission. Further research would be necessary to assess whether these relationships vary across different times of the year and to explore the potential interactions between contaminant exposure and immune response in gulls. Identifying strain-level differences in pathogens carried by gulls could also help clarifying public health risks posed by the presence of these birds in urbanized environments.

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CONCLUSION GÉNÉRALE

L'objectif principal de ce mémoire était d'étudier le rôle du goéland à bec cerclé comme biovecteur de micro-organismes pathogènes, en établissant un lien avec ses déplacements dans les différentes aires d'alimentation de la région de Montréal. À l'aide d'analyses métagénomiques et géospatiales, il a été possible de dresser un portrait de l'ensemble des bactéries et virus fécaux et de corréler leur abondance avec la probabilité de temps passé dans différents types d'habitats. Le second axe de ce projet visait à étudier, de façon exploratoire, l'impact de la concentration plasmatique en contaminants organohalogénés, soit les RFH et SPFA, sur l'abondance microbienne.

Les observations de cette étude ont démontré que les individus s'alimentant fréquemment dans les champs agricoles présentaient une plus grande abondance de plusieurs genres bactériens contenant des espèces/souches potentiellement pathogènes, telles que *Enterococcus* spp., *Clostridium* spp., *Staphylococcus* spp., et *Helicobacter* spp., pouvant être associées avec l'épandage d'engrais organiques comme le fumier ou le compost. Contrairement aux prédictions, les individus fréquentant les dépotoirs fréquemment présentaient un corrélation positive avec l'abondance d'*Enterococcus* spp. et non d'*Escherichia* spp. Ce phénomène pourrait s'expliquer par la présence d'une charge élevée de matières organiques spécifiques et des conditions environnementales favorables pour la prolifération d'*Enterococcus* spp. dans les dépotoirs. De plus, des différences de communautés bactériennes ont été observées entre les individus fréquentant et ceux ne fréquentant pas les dépotoirs.

Cette étude a également démontré que les individus s'alimentant fréquemment dans les stations d'épuration d'eaux usées présentaient une grande abondance de virus des familles *Paramyxoviridae* et *Picobirnaviridae*. Également, les goélands s'alimentant fréquemment dans les milieux aquatiques présentaient une forte abondance de *Paramyxoviridae*, contrairement à ce qui était hypothétisé. Ces résultats, pouvant être liée aux contaminations fécales des oiseaux aquatiques, soulignent la stabilité de ces micro-organismes dans les environnement aquatiques ainsi que les risques de transmission par les organismes biovecteurs.

En ce qui concerne la concentration plasmatique en RFH et SPFA, des corrélations ont été observées avec l'abondance d'*Enterococcus* spp. dans le guano des goélands. Cependant, ces corrélations étaient relativement faibles et présentaient également des associations avec la probabilité de présence dans

divers habitats. Ces résultats suggèrent donc que les relations observées entre l'abondance relative *d'Enterococci* et les niveaux de contamination pourraient ne pas être directement liées à l'exposition seule, mais pourraient être influencées par l'alimentation dans les habitats contaminés.

Ainsi, les résultats de cette étude soulignent l'importance de prendre en compte les interactions complexes entre les comportements de recherche alimentaire des goélands, leurs différentes aires d'alimentation et leur exposition aux contaminants environnementaux pour mieux cerner leur rôle en tant que biovecteurs de pathogènes. En effet, la diversité des environnements fréquentés expose les goélands à une grande variété d'aliments contaminés en contaminants organohalogénés ou en micro-organismes pathogènes. Cette ingestion d'aliments contaminés modifie leurs communautés microbiennes et le risque de dissémination de divers pathogènes dans les écosystèmes urbains.

Cette étude confirme l'importance du goéland à bec cerclé en tant que biovecteur de pathogènes dans des environnements urbains. Effectivement, ces résultats appuient l'idée que les interactions écologiques et les sources d'alimentation influencent non seulement la composition microbienne intestinale des goélands, mais aussi leur rôle dans la transmission de pathogènes. Ce constat pourrait avoir des implications pour la gestion des espèces aviaires en milieu urbain, en incitant à une meilleure compréhension des risques sanitaires associés à leurs comportements alimentaires et à leurs habitats privilégiés.

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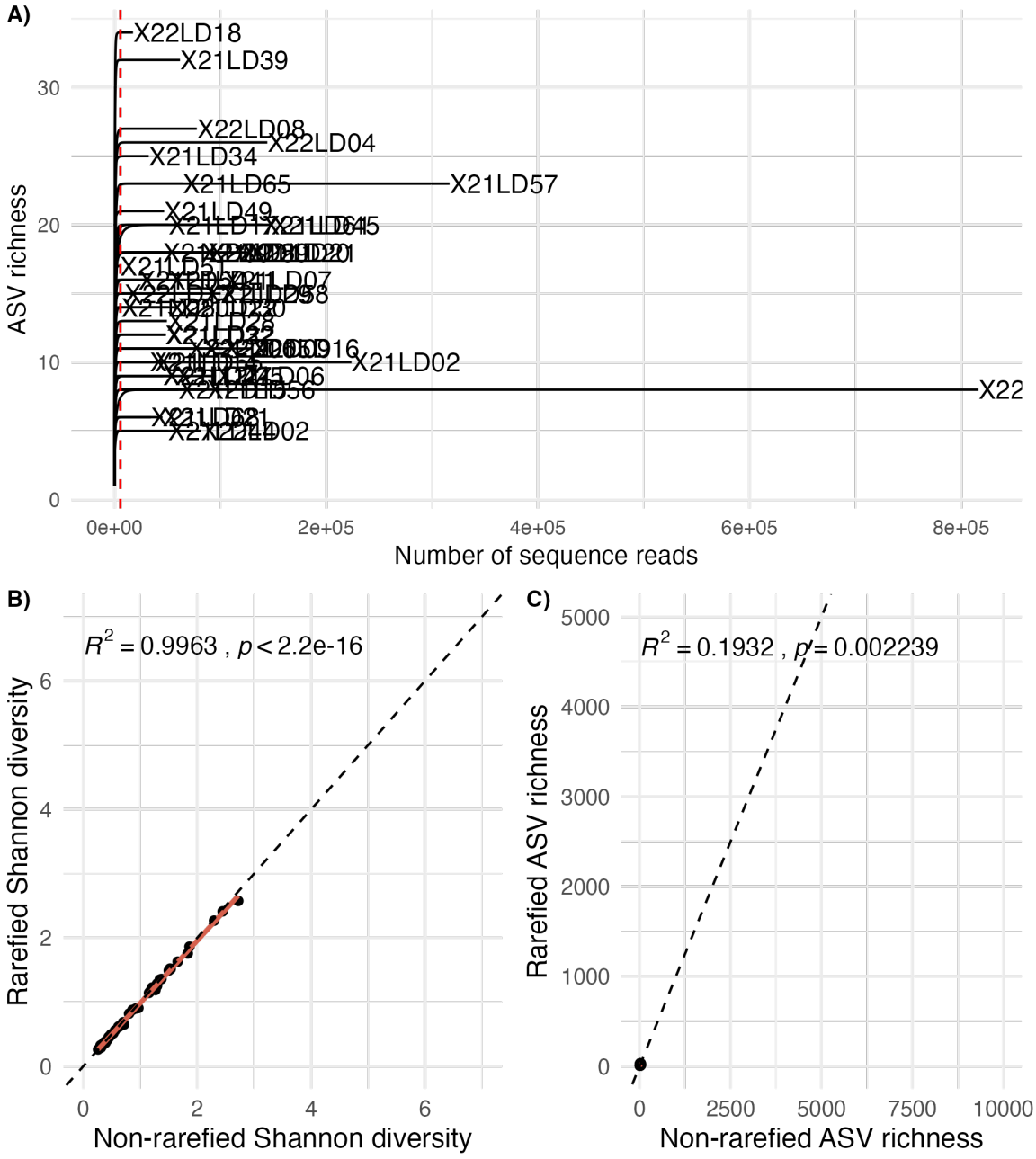

Figure 2.7 Rarefaction curve and Spearman correlation between diversity and richness of both rarefied and non-rarefied samples of bacterial ASV from ring-billed gulls feces (n = 53). (A) Rarefaction curve illustrating the relationship between the number of bacterial ASV and the sequence count for each sample. The vertical dashed red line denotes the rarefaction depth applied across all samples (i.e. 5430 reads per sample). (B) Correlation of Shannon diversity calculated for rarefied versus non-rarefied samples. (C) Correlation of ASV richness between rarefied and non-

rarefied samples. Linear regression is indicated in red, while the dotted line represents a theorical relationship where rarefaction has no impact on diversity and richness. The R^2 represents the Spearman correlation coefficient

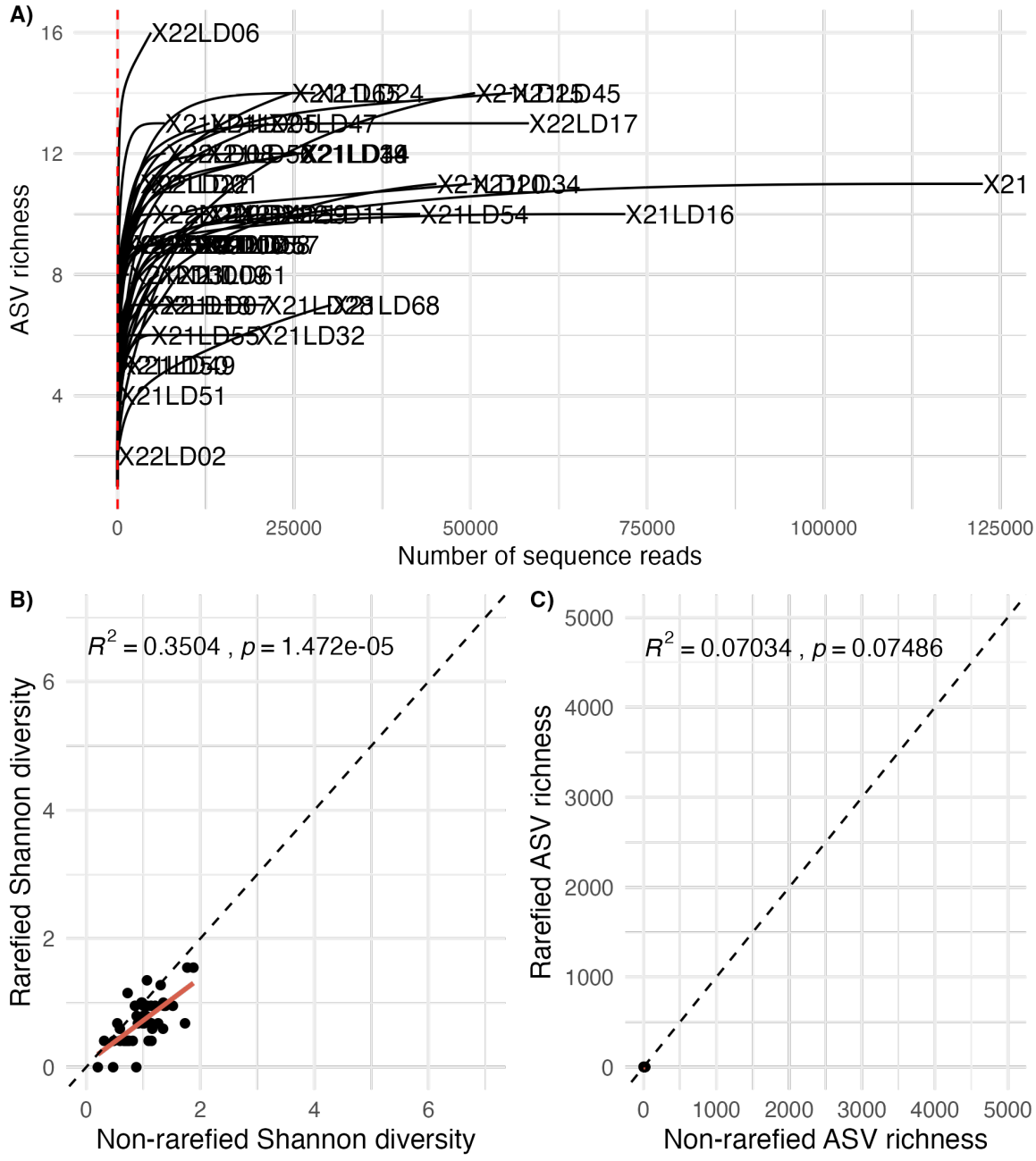

Figure 2.8 Rarefaction curve and Spearman correlation between diversity and richness of both rarefied and non-rarefied samples of viral ASV from ring-billed gulls feces (n = 53). A) Rarefaction curve illustrating the relationship between the number of viral ASV and the sequence count for each sample. The vertical dashed red line denotes the rarefaction depth applied across all samples (i.e. 230 reads per sample). B) Correlation of Shannon diversity calculated for rarefied versus non-rarefied samples. C) Correlation of ASV richness between rarefied and non-rarefied samples. Linear regression is indicated in red, while the dotted line represents a theorical relationship where rarefaction has no impact on diversity and richness. The R^2 represents the Spearman correlation coefficient.

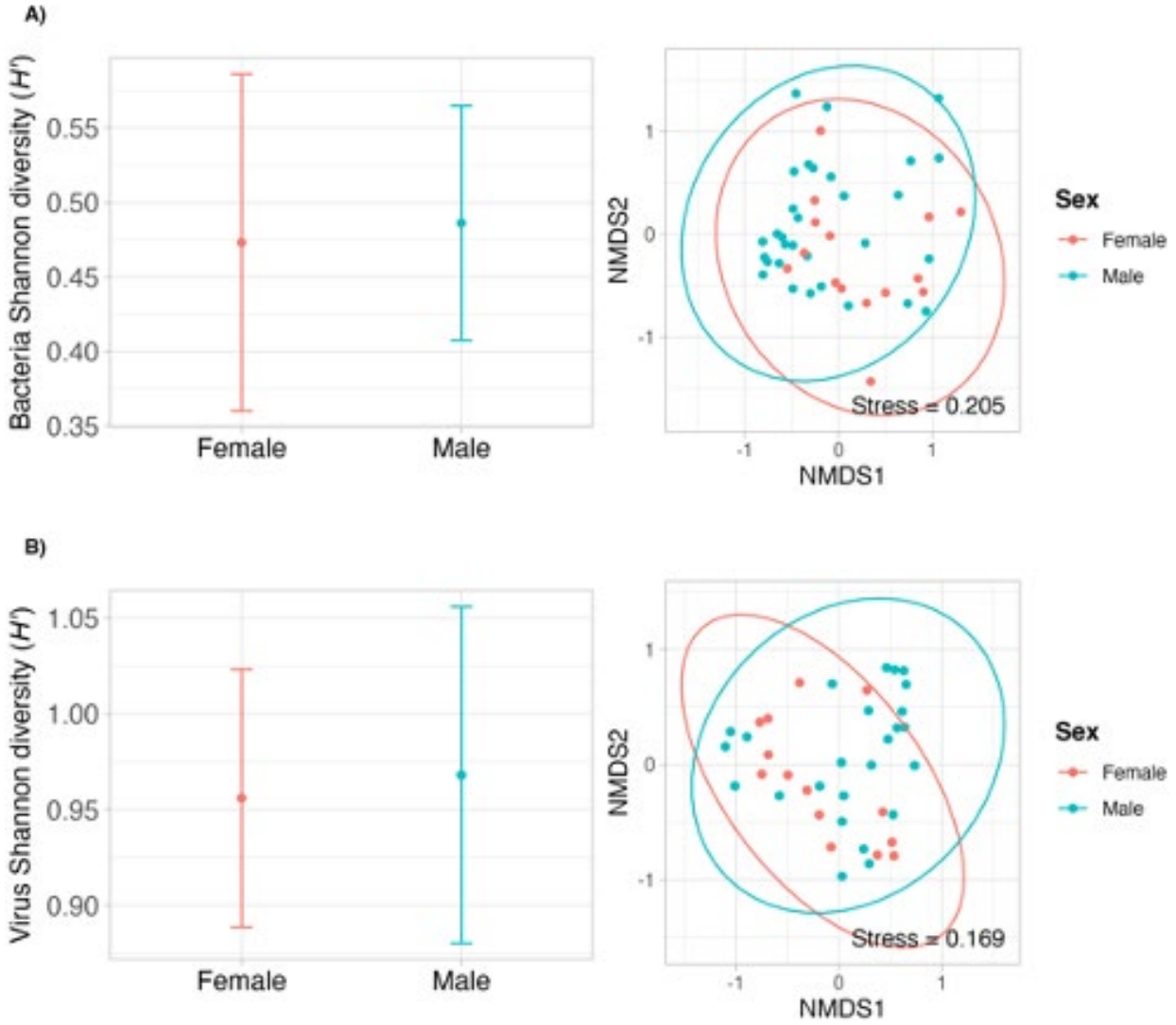

Figure 2.9 Alpha diversity of (A) bacterial and (B) viral communities in guano samples of ring-billed gulls (n = 53) by sex. Each group's diversity is represented by the rarefied Shannon index and Chaos1 richness, where significance of difference was tested by Wilcox rank sum test ($p < 0.05$). For both bacteria and virus, the difference between male and female for richness and diversity was not significative.

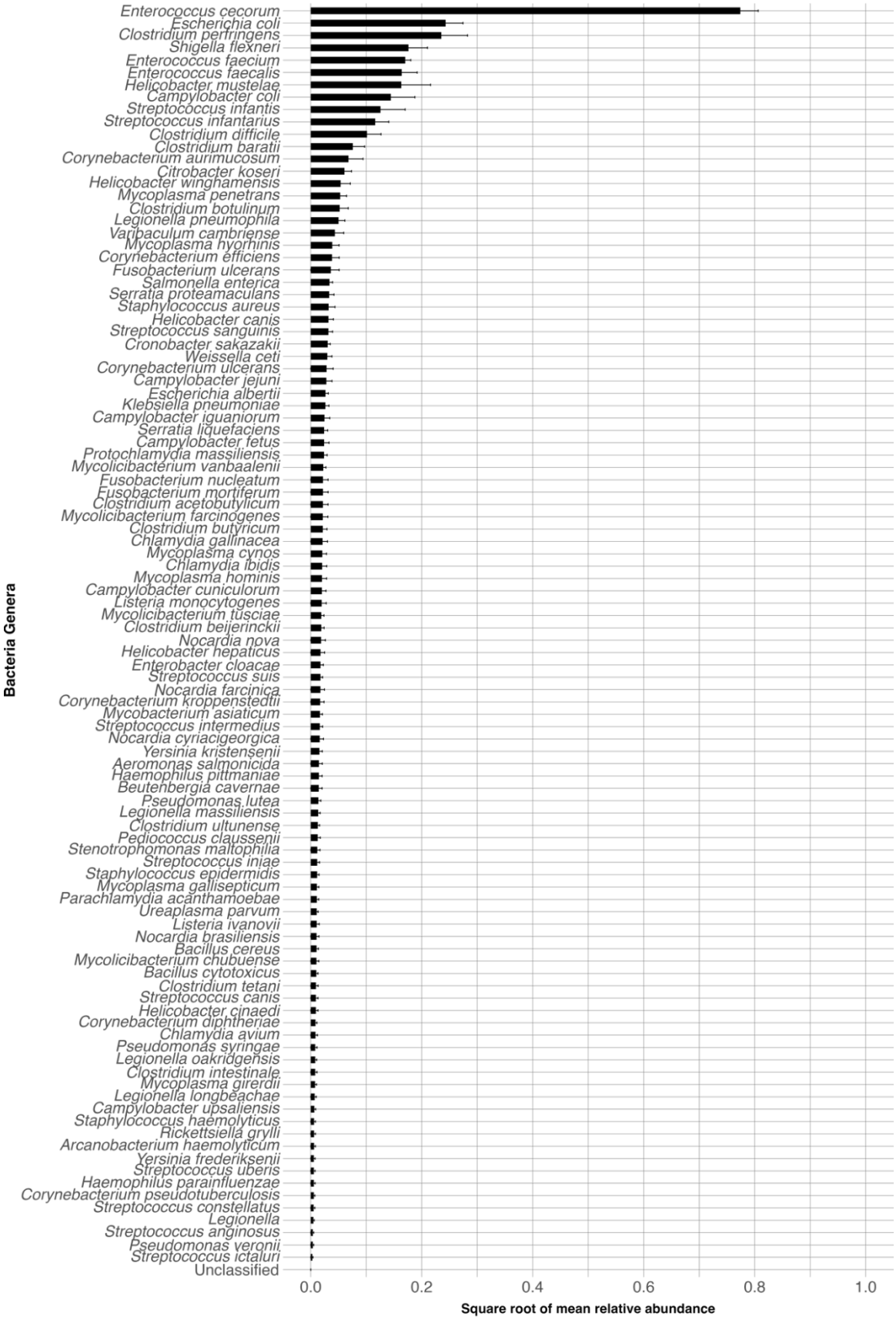
Enterococcus cecorum
Escherichia coli
Clostridium perfringens
Shigella flexneri
Enterococcus faecium
Enterococcus faecalis
Helicobacter mustelae
Campylobacter coli
Streptococcus infantis
Streptococcus infantarius
Clostridium difficile
Clostridium baratii
Corynebacterium aurimucosum
Citrobacter koseri
Helicobacter winghamensis
Mycoplasma penetrans
Clostridium botulinum
Legionella pneumophila
Varibaculum cambriense
Mycoplasma hyorhinis
Corynebacterium efficiens
Fusobacterium ulcerans
Salmonella enterica
Serratia proteamaculans
Staphylococcus aureus
Helicobacter canis
Streptococcus sanguinis
Cronobacter sakazakii
Weissella ceti
Corynebacterium ulcerans
Campylobacter jejuni
Escherichia albertii
Klebsiella pneumoniae
Campylobacter iguaniorum
Serratia liquefaciens
Campylobacter fetus
Protochlamydia massiliensis
Mycolicibacterium vanbaalenii
Fusobacterium nucleatum
Fusobacterium mortiferum
Clostridium acetobutylicum
Mycolicibacterium farcinogenes
Clostridium butyricum
Chlamydia gallinacea
Mycoplasma cynos
Chlamydia ibidis
Mycoplasma hominis
Campylobacter cuniculorum
Listeria monocytogenes
Mycolicibacterium tusciae
Clostridium beijerinckii
Nocardia nova
Helicobacter hepaticus
Enterobacter cloacae
Streptococcus suis
Nocardia farcinica
Corynebacterium kroppenstedtii
Mycobacterium asiaticum
Streptococcus intermedius
Nocardia cyriacigeorgica
Yersinia kristensenii
Aeromonas salmonicida
Haemophilus pittmaniae
Beutenbergia cavernae
Pseudomonas lutea
Legionella massiliensis
Clostridium ultunense
Pediococcus claussenii
Stenotrophomonas maltophilia
Streptococcus iniae
Staphylococcus epidermidis
Mycoplasma gallisepticum
Parachlamydia acanthamoebae
Ureaplasma parvum
Listeria ivanovii
Nocardia brasiliensis
Bacillus cereus
Mycolicibacterium chubuense
Bacillus cytotoxicus
Clostridium tetani
Streptococcus canis
Helicobacter cinaedi
Corynebacterium diphtheriae
Chlamydia avium
Pseudomonas syringae
Legionella oakridgensis
Clostridium intestinale
Mycoplasma girerdii
Legionella longbeachae
Campylobacter upsaliensis
Staphylococcus haemolyticus
Rickettsiella grylli
Arcanobacterium haemolyticum
Yersinia frederiksenii
Streptococcus uberis
Haemophilus parainfluenzae
Corynebacterium pseudotuberculosis
Streptococcus constellatus
Legionella
Streptococcus anginosus
Pseudomonas veronii
Streptococcus ictaluri
Unclassified
Bacteria Genera
0.0
0.2
0.4
0.6
0.8
1.0
Square root of mean relative abundance

Figure 2.10 Square root of mean (± SEM) relative abundance of bacterial species in guano samples of ring-billed gulls (n = 53) from the Montreal area (QC, Canada).

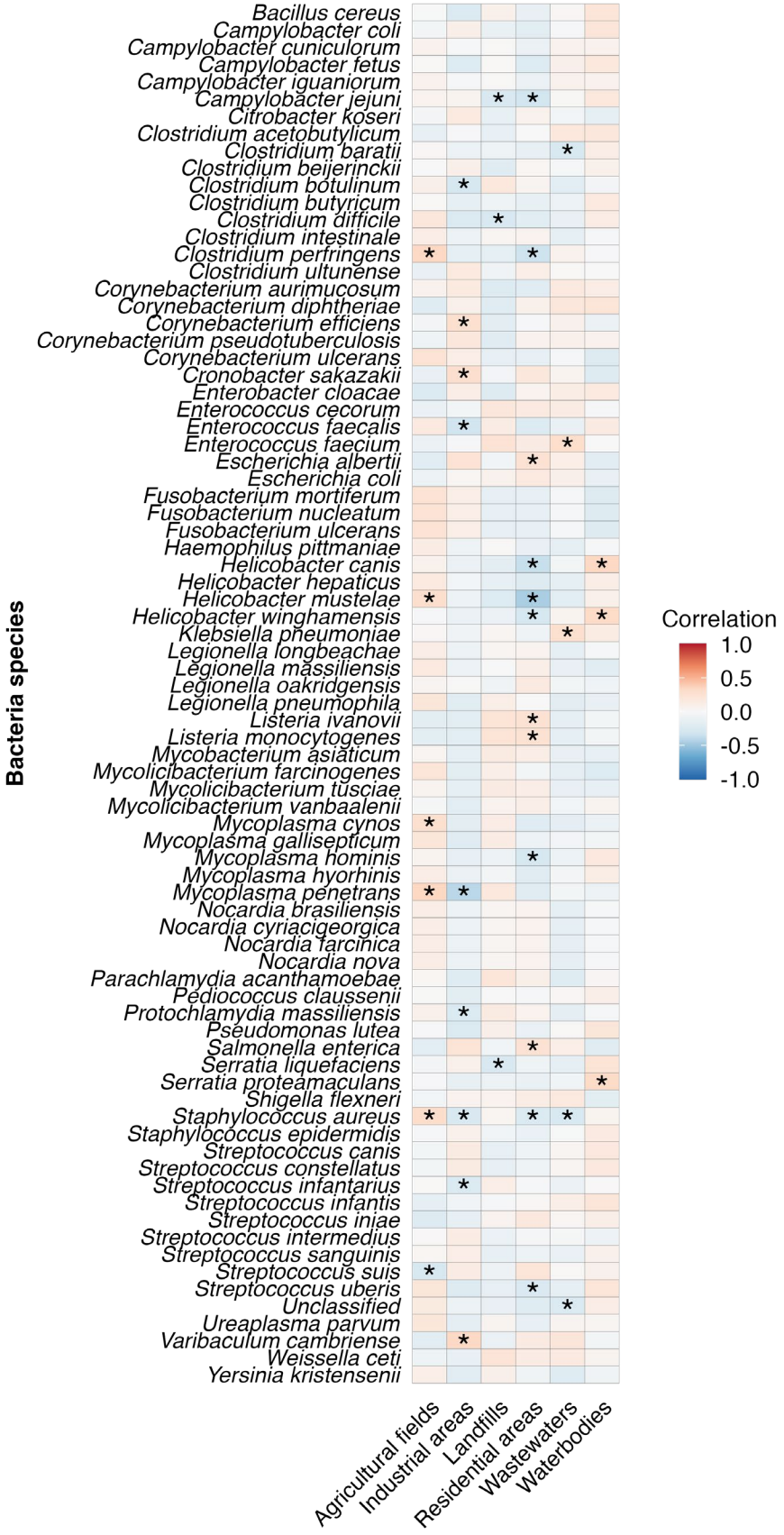
Bacteria species
Bacillus cereus
Campylobacter coli
Campylobacter cuniculorum
Campylobacter fetus
Campylobacter iguaniorum
Campylobacter jejuni
Citrobacter koseri
Clostridium acetobutylicum
Clostridium baratii
Clostridium beijerinckii
Clostridium botulinum
Clostridium butyricum
Clostridium difficile
Clostridium intestinale
Clostridium perfringens
Clostridium ultunense
Corynebacterium aurimucosum
Corynebacterium diphtheriae
Corynebacterium efficiens
Corynebacterium pseudotuberculosis
Corynebacterium ulcerans
Cronobacter sakazakii
Enterobacter cloacae
Enterococcus cecorum
Enterococcus faecalis
Enterococcus faecium
Escherichia albertii
Escherichia coli
Fusobacterium mortiferum
Fusobacterium nucleatum
Fusobacterium ulcerans
Haemophilus pittmaniae
Helicobacter canis
Helicobacter hepaticus
Helicobacter mustelae
Helicobacter winghamensis
Klebsiella pneumoniae
Legionella longbeachae
Legionella massiliensis
Legionella oakridgensis
Legionella pneumophila
Listeria ivanovii
Listeria monocytogenes
Mycobacterium asiaticum
Mycolicibacterium farcinogenes
Mycolicibacterium tusciae
Mycolicibacterium vanbaalenii
Mycoplasma cynos
Mycoplasma gallisepticum
Mycoplasma hominis
Mycoplasma hyorhinis
Mycoplasma penetrans
Nocardia brasiliensis
Nocardia cyriacigeorgica
Nocardia farcinica
Nocardia nova
Parachlamydia acanthamoebae
Pediococcus claussenii
Protochlamydia massiliensis
Pseudomonas lutea
Salmonella enterica
Serratia liquefaciens
Serratia proteamaculans
Shigella flexneri
Staphylococcus aureus
Staphylococcus epidermidis
Streptococcus canis
Streptococcus constellatus
Streptococcus infantarius
Streptococcus infantis
Streptococcus iniae
Streptococcus intermedius
Streptococcus sanguinis
Streptococcus suis
Streptococcus uberis
Unclassified
Ureaplasma parvum
Varibaculum cambriense
Weissella ceti
Yersinia kristensenii
Agricultural fields
Industrial areas
Landfills
Residential areas
Wastewaters
Waterbodies
Correlation
1.0
0.5
0.0
-0.5
-1.0

Figure 2.11 Spearman correlations between the abundance of bacteria species in guano samples ring-billed gulls, and their presence probability (%) in different foraging habitats in the Montreal area (QC, Canada). Wastewaters stands for wastewaters treatment plant settling ponds. The strength of positive (red) and negative (blue) correlations are shown. Correlations are significant (*) when $p < 0.05$ after multiple pairwise testing using the Benjamini-Hochberg correction.

Table 2.3 Method limits of detection (MLODs), method limits of quantification (MLOQs), and mean (± standard deviation) concentrations (ng/g) of Σ_{35}PBDE congeners and Σ_{12} other HFR

Congener	MLOD	MLOQ	Procedural blank
BDE-7	0.01	0.01	< MLOD
BDE-10	0.01	0.01	< MLOD
BDE-15	0.01	0.03	< MLOD
BDE-17	0.01	0.01	< MLOD
BDE-28	0.01	0.01	0.04 ± 0.03
BDE-47	0.01	0.02	0.11 ± 0.08
BDE-49	0.01	0.02	0.04 ± 0.06
BDE-66	0.01	0.02	< MLOD
BDE-71	0.01	0.01	< MLOD
BDE-77	0.01	0.02	< MLOD
BDE-85	0.01	0.02	< MLOD
BDE-99	0.01	0.04	0.03 ± 0.02
BDE-100	0.01	0.04	0.01 ± 0.00
BDE-119	0.01	0.03	< MLOD
BDE-126	0.01	0.02	< MLOD
BDE-138	0.01	0.05	< MLOD
BDE-139	0.01	0.03	< MLOD
BDE-140	0.01	0.03	< MLOD
BDE-153	0.01	0.02	< MLOD
BDE-154/BB-153	0.01	0.01	< MLOD
BDE-171	0.01	0.05	< MLOD
BDE-180	0.01	0.02	< MLOD
BDE-183/Dec-604	0.01	0.03	< MLOD

BDE-184	0.01	0.04	< MLOD
BDE-191	0.01	0.01	< MLOD
BDE-196	0.01	0.03	< MLOD
BDE-197/-204	0.02	0.07	< MLOD
BDE-201	0.01	0.03	< MLOD
BDE-203	0.02	0.06	< MLOD
BDE-205	0.04	0.13	< MLOD
BDE-206	0.23	0.76	< MLOD
BDE-207	0.03	0.10	< MLOD
BDE-208	0.04	0.14	< MLOD
BDE-209	0.09	0.29	< MLOD
Cplus	0.01	0.05	< MLOD
DBCD	0.20	0.6	< MLOD
DBDPE	0.36	1.21	< MLOD
***syn*-DP**	0.04	0.12	< MLOD
***anti*-DP**	0.01	0.05	0.01 ± 0.03
Dec-601	0.02	0.08	< MLOD
Dec-602	0.02	0.08	< MLOD
Dec-603	0.14	0.47	< MLOD
Dec-604 CB	0.01	0.01	0.00 ± 0.00
HBB	0.01	0.01	0.01 ± 0.02
OBIND	0.06	0.19	< MLOD
PBEB	0.01	0.01	0.02 ± 0.03

Table 2.4 Method limits of detection (MLODs) and mean (± standard deviation) concentrations (ng/g) of Σ_{76}PFAS compounds

Compounds	MLOD	Procedural blank
PFBA	2,46	0.05 ± 0.02
PFPrA	6,04	< MLOD
PFMPA	2,89	< MLOD
PFMBA	0,91	< MLOD
PFEESA	0,11	< MLOD
PFPeA	0,11	< MLOD
PFHxA	0,72	< MLOD
PFHpA	0,43	< MLOD
FHpSA	0,07	< MLOD
PFOA	0,12	< MLOD
PFNA	0,37	< MLOD
PFDA	0,12	< MLOD
FDSA	0,07	< MLOD
PFUdS	0,05	< MLOD
PFUnA	0,18	< MLOD
PFDoA	0,08	< MLOD
PFTrDA	0,07	< MLOD
PFTeDA	0,04	< MLOD
PFPrS	0,24	< MLOD
PFEtS	0,63	< MLOD
PFBS	0,07	< MLOD
PFPeS	0,08	< MLOD
PFHsSAmS	0,12	< MLOD

PFHxS	0,08	< MLOD
PFHpS	0,06	< MLOD
PFOS	0,04	< MLOD
PFNS	0,07	< MLOD
PFTrDS	0,08	< MLOD
PFDS	0,04	< MLOD
PFDoS	0,07	< MLOD
FBSA	0,09	< MLOD
FHxSA	0,08	< MLOD
FOSA	0,05	< MLOD
6,2-FTUCA	0,70	< MLOD
8,2-FTUCA	0,31	< MLOD
10,2-FTUCA	0,16	< MLOD
MeFBSA	0,11	< MLOD
MeFOSA	0,06	< MLOD
EtFOSA	0,06	< MLOD
FOSAA	0,09	< MLOD
MeFOSAA	0,32	0.332 ± 0.007
EtFOSAA	0,20	< MLOD
3,3-Acid	1,00	< MLOD
4,3-Acid	0,95	< MLOD
5,3-Acid	0,61	< MLOD
7,3-Acid	0,62	< MLOD
6,6-PFPi	0,05	< MLOD
6,8-PFPi	0,07	< MLOD

8,8-PFPi	0,07	< MLOD
6,2-FTCA	3,03	< MLOD
8,2-FTCA	1,54	< MLOD
10,2-FTCA	0,08	< MLOD
4,2-FTS	0,28	< MLOD
6,2-FTS	0,10	< MLOD
8,2-FTS	0,17	< MLOD
10,2-FTS	0,27	< MLOD
Gen-X	42,59	< MLOD
ADONA	0,33	< MLOD
6,2-Cl-PFESA	0,07	< MLOD
8,2-Cl-PFESA	0,04	< MLOD
PFECHS	0,08	< MLOD
PFHxDA	0,08	< MLOD
PFOcDA	0,19	< MLOD
PFHxPA	0,27	< MLOD
PFOPA	0,12	< MLOD
PFHxSAm	0,07	< MLOD
PFOSAmS	0,11	< MLOD
PFHxSAm	0,07	< MLOD
PFOANO	0,14	< MLOD
PFOSNO	0,17	< MLOD
PFOAB	0,37	< MLOD
PFOSB	0,31	< MLOD
5,3-FTB	0,20	< MLOD

5,1,2-FTB	0,13	< MLOD
6,2-FTAB	0,78	< MLOD
PFOSAm	0,17	< MLOD