

The movement ecology and physiological health of glaucous-winged gulls wintering in the Salish Sea

**by
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B.Sc.(Biology), Vancouver Island University, 2018

Thesis Submitted in Partial Fulfillment of the
Requirements for the Degree of
Master of Science

in the
Department of Biological Sciences
Faculty of Science

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Fall 2022

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Abstract

Gulls (Laridae) use natural and urban environments and are useful ‘biomonitors’ of coastal ecosystem health. I studied movement ecology (using GPS tags) and physiological health (from blood samples) in glaucous-winged gulls (*Larus glaucescens*; GWGU) during winter in the Salish Sea, a region undergoing rapid anthropogenic change. GWGUs exhibited high wintering region fidelity within the Salish Sea and exploited landfills and Pacific herring spawn activity. These resources were especially important for migrant GWGUs (22% of the population). Daytime habitat use varied among individuals, but all GWGUs roosted on water at night. There was marked individual variation in, and covariation among, physiological traits (glucose, triglycerides, hemoglobin, hematocrit, and oxidative state). However, region and habitat type where birds were captured did not predict variation in health biomarkers, even though these included varying levels of urban development and anthropogenic activity. This study establishes reference values for health biomarkers for long-term monitoring of future anthropogenic impacts in this region.

Keywords: Anthropogenic activity; Coastal ecosystem health; Biomonitor; Conservation

Acknowledgements

I would like to express my deep appreciation for the many people who have contributed time and energy to this research and have supported my journey throughout graduate school. First, I am grateful to my supervisor, Dr. Tony Williams, for his enthusiasm, insightful mentorship, and patience. He has been endlessly encouraging and always helped me keep 'my eyes on the prize.' I would like to thank my committee members, Dr. Mark Hipfner and Dr. David Green who generously provided their knowledge and expertise throughout the project, as well Dr. John Reynolds, for his insightful feedback as examiner, and Dr. Isabelle Côté for chairing the defence.

This research was funded by and conducted in collaboration with Environment and Climate Change Canada. I am grateful to all ECCC collaborators involved in this work, especially Dr. Mark Hipfner, for organizing the larger project and facilitating my involvement. My deep thanks to Alice Domalik for sharing her time and knowledge during various phases of planning, field work, and analysis. A special thanks to the 'gull crew' who were instrumental to the field work and coordination of this project, including Anneka Vanderpas, Nik Clyde, Vivian Pattison, Josh Green, Sarah Hudson, and Kristina Hick, as well as Allison Patterson for advice on tracking data analysis. Thank you also to Dr. Theresa Burg, and her lab at the University of Lethbridge, for providing genetic analysis of gull sex, and to all other collaborators for advice and support including Drs. Keith Hobson, John Elliot, Sandi Lee, and Amy Wilson.

I am extremely grateful to all my colleagues in the Williams Lab and the Centre for Wildlife Ecology for their knowledge, feedback, and friendship. I am especially grateful to Connie Smith for help with logistics and Mason King for sharing his expertise in all things related to assays and lab equipment. Thank you to Laurie Sutterlin, and all staff in the Biological Sciences Department at Simon Fraser University, for academic and logistical support.

A special thanks to those who have provided mentorship throughout my education, including Drs. Jane Watson, Erin Foster, Tim Goater, Eric Demers, Chris Darimont, and Genevieve Singleton. I would not have found myself here without your inspiration!

Finally, thank you to my family and friends for the wealth of wisdom, encouragement, and enthusiasm they have provided throughout this process. The levity and joy you bring to my life have made all the difference. Thank you to Trevor for his patience, proof-reading, and moral support, and last but not least, thanks to Iggy for all of the laughs!

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List of Acronyms

AIC	Akaike's Information Criterion
AICc	Akaike's Information Criterion corrected for small sample sizes
AK	Alaska
BC	British Columbia
CC	Central Coast of British Columbia
CV	Coefficient of variation (%)
DNA	Deoxyribonucleic acid
dROMs	Reactive oxygen metabolites
ECCC	Environment and Climate Change Canada
GPRS	Global Penetrating Radar Systems
GPS	Global Positioning System
GSM	Global System for Mobile Communications
GWGU	Glaucous-winged gull
LAC	Library and Archives Canada
OXY	Total antioxidant titres
PC1	First principal component
PC2	Second principal component
PCA	Principal components analysis
PCR	Polymerase chain reaction
PCV	Packed cell volume (%)
SFU	Simon Fraser University
USA	United States of America
VI	Vancouver Island
WA	Washington
WC	'West Coast' of Vancouver Island

Chapter 1. Winter movement ecology and habitat use of glaucous-winged gulls

1.1. Abstract

Gulls (Laridae) use natural and urban environments and are useful ‘biomonitors’ of coastal ecosystem health. I conducted a preliminary investigation of winter movement ecology (using GPS tags) of glaucous-winged gulls (*Larus glaucescens*; GWGU) to better inform management of this species and their utility as a biomonitor in the Salish Sea, a region undergoing rapid anthropogenic change. GWGUs exhibited high intra and inter-annual regional fidelity during winter, indicating potential susceptibility to localized environmental changes which could be tracked with long-term monitoring. Most gulls utilized forage fish activity (75%) and landfills (59.3%) and these resources were especially important for migrant GWGUs (22% of the population). Daytime habitat use varied among individuals, but all GWGUs roosted almost exclusively on water at night, posing challenges to protecting this species from anthropogenic threats such as oil spills. Evidence of potential individual variation in habitat use, and its relationship to diet, contaminant exposure, and health should be further investigated to better understand the impact of human activity on this species and increase the utility of GWGUs as biomonitors of the Salish Sea.

1.2. Introduction

Animal movements and the habitats they utilize are often considered a reflection of their resource needs and availability. Globally, humans are causing significant changes in the landscape (Dirzo et al., 2014; Gibson et al., 2007) with implications for the distribution of resources and, hence, the movement behaviours of many species (Doherty et al., 2021). While sensitive species are often excluded from human-dominated areas, opportunistic generalists readily utilize these environments (Ducatez et al., 2018), benefiting from anthropogenic food subsidies and exclusion of apex predators (Isaksson, 2018). However, areas with high levels of human activity are not without risks including higher abundances of meso-predators (Fischer et al., 2012; Kettel et al., 2018), invasive competitors (Gallardo et al., 2015; Molnar et al., 2008), and pollution (Shahidul Islam & Tanaka, 2004). Additionally, there can be physical obstacles preventing ease of

movement, leading to habitat fragmentation, as well as the risk of entanglement, vehicle or window collisions, and chronically elevated levels of stress due to disturbances by human presence or noise pollution (Isaksson, 2018).

Increasing urbanization is especially pronounced in coastal environments (Bishop et al., 2017; Halpern et al., 2019). To assess ecosystem health in marine and coastal areas, seabirds are often studied as they can sensitively reflect changes in such environments (Bond et al., 2015; Driscoll et al., 2013). Gulls (Laridae) are particularly adaptable to human activity, making them useful biomonitors of urban coastal environments (Davis et al., 2017; Hebert et al., 1999; Laranjeiro et al., 2020; Thorstensen et al., 2021; Zorrozua, Monperrus, et al., 2020). Although gulls are characterized as opportunistic and generalist consumers, studies utilizing lightweight, automated tracking devices have shown that many species exhibit individual foraging specializations including marine, terrestrial (agricultural and urban), or mixed resource use (Caron-Beaudoin et al., 2013; Masello et al., 2013; Mendes et al., 2018; O'Hanlon et al., 2020). Anthropogenic food subsidies can offer high efficiency energy gain (Patenaude-Monette et al., 2014). In some gull species, these are associated with positive impacts on reproductive success and population sizes (Duhem et al., 2008; Gyimesi et al., 2016; Real et al., 2017; Weiser & Powell, 2010), but negative impacts have been reported in others (Auman et al., 2008; Bennett et al., 2017; Blight et al., 2015; O'Hanlon et al., 2017; Svagelj et al., 2015). Further, the use of urban environments may have negative health impacts on gulls related to poor nutrition, exposure to pollution, and other urban stressors (Isaksson, 2018; Laranjeiro et al., 2020).

Given the array of human activities present in coastal environments, assessing anthropogenic impacts on the health of free-living wildlife populations can be challenging. Understanding these relationships can be even more complex for opportunistic and omnivorous gulls. For instance, if individuals are highly transient, metrics of a population's health may have little relevance to localized human activities, pollution, or regional prey availability. Likewise, if there is a low degree of dietary specialization among individuals, the habitat an individual is sampled in may not be a reliable indicator of its propensity for anthropogenic food or habitat use. While conservation physiology is an increasingly common field focused on assessing wildlife health (Madliger et al., 2020), knowledge of the relationships between habitat use, diet,

infections, and/or contaminant exposure on health greatly increase the utility of physiological monitoring (Cooke et al., 2021). GPS tracking data can provide key information about movement and habitat use patterns of a highly variable species and can provide a better understanding of the impacts anthropogenic activity may have on wildlife health.

The glaucous-winged gull (*Larus glaucescens*) has been identified as an effective biomonitor of long-term shifts in marine food-web and contaminant trends in the biologically rich, but highly disturbed coastal ecosystem of the Salish Sea (Blight et al., 2015; Davis et al., 2017). GWGUs are particularly reliant on British Columbia year-round, and previous tracking data has highlighted the use of the Salish Sea and landfills by Alaskan breeding GWGUs during the winter (Hatch et al., 2011). Although this species readily adapts to human dominated areas to forage and nest (Blight et al., 2019; Kroc, 2018b), their population has declined in southern British Columbia (Bower, 2009; Sullivan et al., 2002) concurrent with increasing human population density in this area (Statistics Canada, 2017). While GWGU declines may in part be related to decreases in high trophic level and marine prey in their diet (Blight et al., 2015), little is known about the health impacts of urban habitat use on this species. Further, our knowledge of individual variation in GWGU home range sizes or habitat use during winter is limited.

In this study, we used GPS devices to track the winter movements and habitat use of glaucous-winged gulls in relation to natural and human-dominated habitats, throughout an anthropogenically influenced coastal ecosystem. Broadly, we aimed to use tracking data to inform long-term wintering monitoring of GWGU diets, contaminant burdens, and physiological health (see Chapter 2), with GWGUs serving as a potential indicator of the impacts of urbanization on the health of the Salish Sea ecosystem. Specifically, our objectives were to 1) examine the degree of regional fidelity, site fidelity, and habitat use variability exhibited by GWGUs during winter, 2) characterize the timing and distance of movements from wintering grounds to breeding colonies, and 3) evaluate the inter-annual consistency of winter movements and habitat use.

1.3. Methods

1.3.1. Study area

The Salish Sea (49° 20' 10.4", -123° 50' 21.6") encompasses the protected waters of southern British Columbia (BC), including the Strait of Georgia and Strait of Juan de Fuca, as well as Puget Sound in Washington state. In January and February of 2020 and 2021, we sampled non-breeding, adult GWGUs throughout the Canadian portion of the Salish Sea (Figure 1.1). Anthropogenic influence varies throughout the Salish Sea, with varying levels of human population density, industrial activity, urbanization, agricultural land use, and more. These factors can impact the spatial and temporal availability of anthropogenic subsidies to gulls, as well as the nutritional quality (Blight et al., 2015) and exposure to contaminants in their diet (Chen et al., 2012; Elliott & Elliott, 2016; Ross et al., 2004). As resource availability and human activity can influence gull movements and behaviour, we attempted to sample gulls in various habitat types including landfills, 'urban' areas, and 'natural' areas throughout the Salish Sea. Landfills were sampled in the Lower Mainland, Greater Victoria, Southern Vancouver Island, and the Northern Salish Sea. Satellite imagery from the North American Land Change Monitoring System database was used to guide categorization of capture locations into 'urban' versus 'natural' habitat types (250 x 250 m resolution; North American Land Change Monitoring System, 2021). We also attempted to spatially distribute our sampling sites throughout a range of human population densities, guided by human population density (people/km²) census data, (Statistics Canada, 2017). Qualitatively, beaches near high human population densities, as well as city parks, were categorized as urban habitats. In contrast, natural habitats included beaches in areas with relatively lower human population densities and less industrial activity.

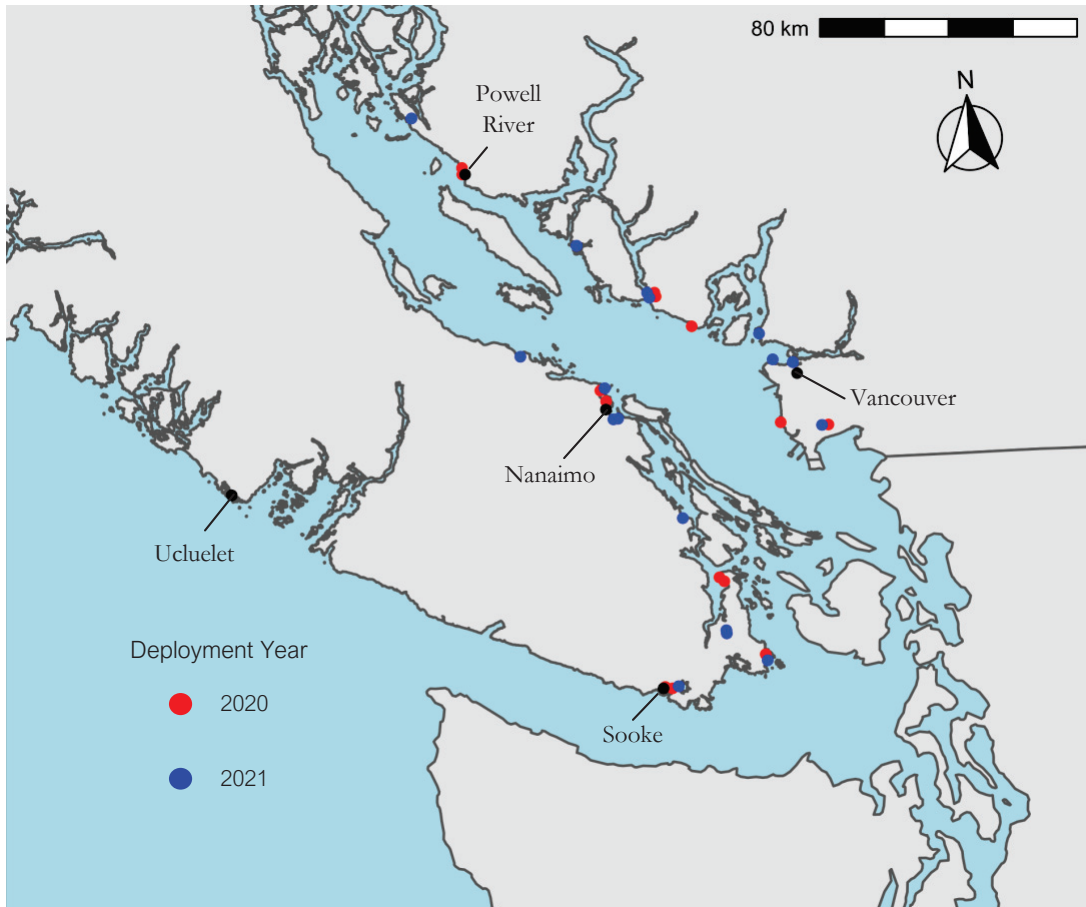


Figure 1.1. GPS tag deployment locations of glaucous-winged gulls throughout the Salish Sea in January and February of 2020 and 2021.

1.3.2. Data Collection

Research was conducted under Environment and Climate Change Canada (ECCC) Banding Permit #10667F, and ECCC Migratory Bird Sanctuary Permit #MM-BC-2020-0002. Animal use protocols were approved by ECCC’s Western and Northern Animal Care Committee (21MH03), as well as the Simon Fraser University Animal Care Committee (protocol no. 1318B-20). All personnel completed mandatory Animal Care training.

Adult gulls were live-captured, primarily using baited noose-mats (Liu et al., 2017), but occasionally with pneumatic CO² net guns when bait was not an effective attractant (Edwards & Gilchrist, 2011). All gulls were photographed, aged, banded, and morphometric measurements were collected, including mass (± 20 g) and tarsus length (± 0.01 mm). Using a non-heparinized lancet and capillary tube, approximately 10 μ L of

whole blood was collected from the tarsal vein. This was stored in 95% EtOH and assayed in the lab to determine gull sex using DNA sexing primers (Burg & Croxall, 2001; Dawson et al., 2016; Walsh et al., 2013).

Gulls were each fitted with one of two models of solar-powered tracking devices, either a 'Ecotone Kite-L' (17g) GPS-GPRS (global penetrating radar systems) tag or a 'Microwave 20-70' (25g) GPS-GSM (global system for mobile communications) tag. Devices were mounted on a thin neoprene foam pad and secured to each gull using a biodegradable Teflon leg-loop harness (Thaxter et al., 2014, 2016). Similar tracking devices have previously been used on other gull species (Clark et al., 2016; Juvaste et al., 2017) and did not exceed 3% of an individual's body weight (Phillips et al., 2003). One gull tagged in 2020 was re-captured in 2021 and showed minimal signs of feather wear from the device and harness. Location data was collected at variable time intervals and remotely transmitted through cellular tower networks. Microwave GPS-GSM tag duty cycles auto-adjust to preserve battery charge using an internally programmed algorithm, but throughout the winter study period, locations were collected approximately once per hour or, often, more frequently. Ecotone GPS-GPRS tags duty cycles were set at fixed intervals, but the rate of data collection was adjusted throughout the study period to preserve battery condition. During winter, most tags went through occasional multi-day periods when little or no data was collected due to low battery charge when conditions were not optimal (i.e. shorter day length and lack of sunny weather). All location data has been uploaded and stored in the Movebank Data Repository ("Glaucous-winged gulls (GWGU), in the Salish Sea, BC, Canada"; www.movebank.org).

1.3.3. Data Analysis

Location data was downloaded directly from a Movebank repository, using the *move* package (Kranstauber et al., 2021), and all analyses were conducted in *R* version 4.1.3 (R Core Team, 2022). Data was filtered to exclude implausible coordinates and include only locations fixed by 3 or more satellites.

Regional and site fidelity of winter ranges were assessed by visual inspection. Trips by some individuals outside of their typical winter range were noted, as were trips to Pacific herring hotspots during peak spawn throughout winter. Frequently visited habitat types were also investigated; in particular, the number of gulls using landfills,

heavy use of urban parks or other urban areas, as well as agricultural areas during winter were examined. Additionally, differences between day and nighttime habitat use by individuals were compared. Lastly, we examined the consistency of migration habits, winter and breeding home ranges, as well as winter site fidelity and habitat use by individuals across years.

On some individuals, tags transmitted location data for multiple years, but the rate and consistency of data collection declined over time due to reduction in battery function (Table 1.1). However, initial and subsequent years of data were used to characterize movements related to migration timing, stop-overs, winter home range region, site fidelity, and frequently used habitat types, as well as the consistency of these movements for individuals across years. We defined the winter period onset as the initial day of tag deployment for each individual and ending on the average date long-distance migrants left the Salish Sea (March 20th). For individuals with multiple years of tracking data, in subsequent years we defined winter as beginning on January 01 to make winter period examined comparable among years, though migrant GWGUs have typically returned to wintering grounds by November (Hayward & Verbeek, 2020).

Migratory individuals and the dates they began and ended their migration were identified by visual inspection. We defined the onset of migration as the date an individual left its main wintering or breeding range, even if it was for a short movement to access forage fish spawn before continuing longer distance travel. Termination of migration was determined by the date individuals first arrived at potential breeding sites (near Cordova, Alaska (AK) for long-distance migrants and near Cleland Island, BC for short-distance migrants). Total distance travelled was approximated as a straight trajectory from winter sites to Cordova, AK for long-distance migrants, and as a series of straight trajectories around Vancouver Island (south or north depending on individual's route) to west coast breeding locations. Distances travelled are an underestimate.

1.4. Results

In total, 29 GPS-GMS tags (n = 12 Ecotone and 17 Microwave tags) were deployed on 31 adult GWGUs throughout the Salish Sea in 2020 (n = 14) and 2021 (n = 17). Of these, two Ecotone tags used in 2020 were recovered in the field, refurbished, and re-deployed on new individuals in 2021. Four tags, which provided reliable data for

less than a day, were excluded from all analyses. Overall, 27 tags provided data for at least one winter period (from deployments in January or February until March 20th or later) and in some cases for two (n = 14) or three (n = 3) winters (Table 1.1).

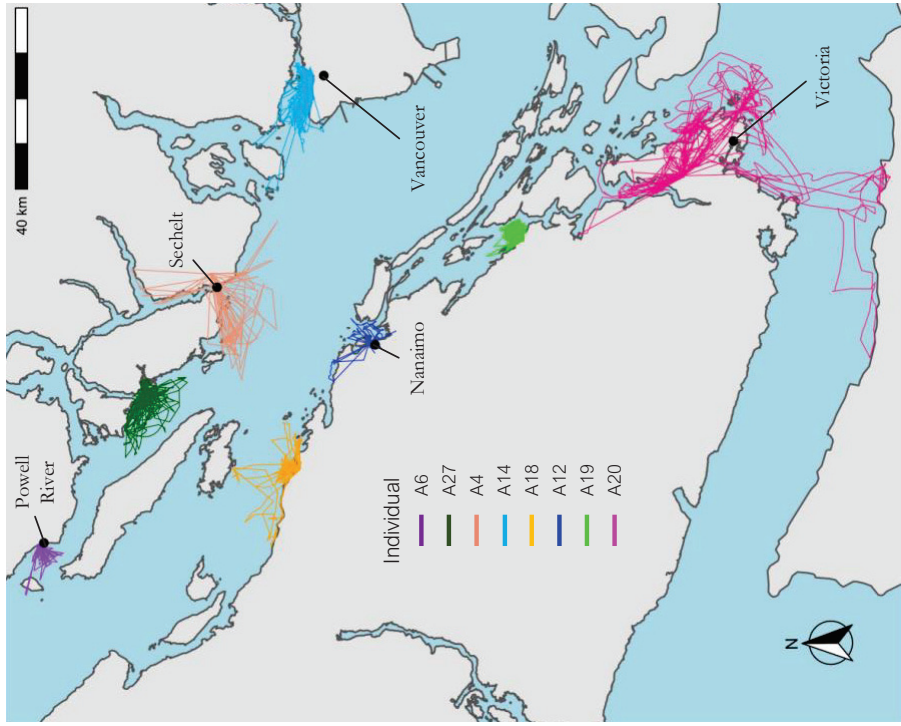
1.4.1. Winter movements within the Salish Sea

Winter home range and regional fidelity

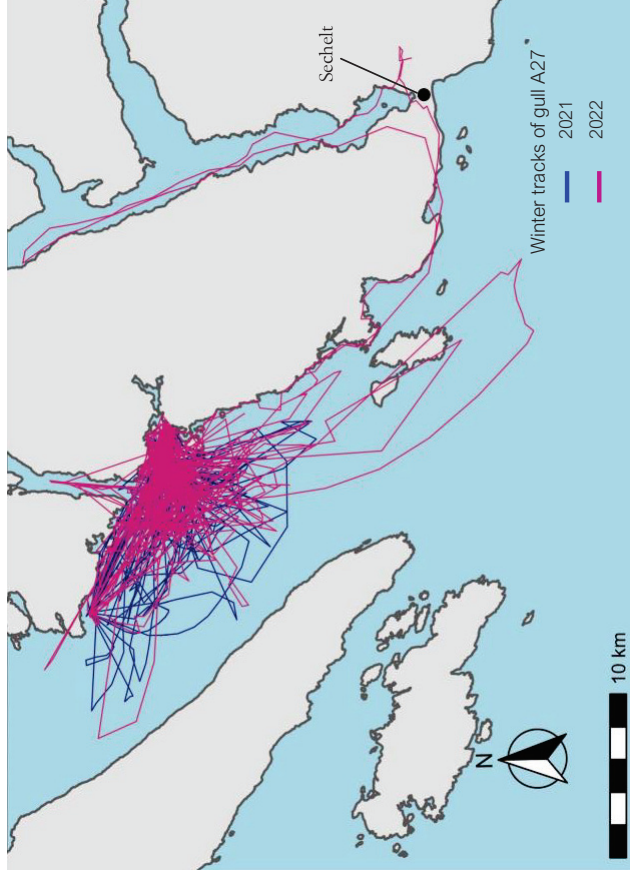
All glaucous-winged gulls remained within the Salish Sea throughout winter (from the date of tag deployment or January 01 – March 20th), exhibiting localized home ranges and regional site fidelity (Figure 1.2a). Additionally, winter ranges for individuals remained consistent across multiple years (Figure 1.2b).

Despite overall regional site fidelity exhibited by wintering GWGUs, and aside from trips to major Pacific herring spawn hot spots, ephemeral trips outside core home ranges were taken by some individuals during winter (25.9% of the population; n = 7). These trips were usually less than two days in duration to and from adjacent regions of the Salish Sea, involving flights along the coastline, across the Strait of Georgia, or across the Strait of Juan de Fuca. For instance, three individuals with home ranges in the greater Victoria region, and one from Nanaimo, briefly visited the Olympic Peninsula at least once. At least three gulls made one or more trips to landfills located outside of their core home range.

Generally, capture locations were indicative of the wintering region used by individuals (88.9%, n = 24), though there were a few exceptions (n = 3) which were mostly associated with the use of landfills outside of an individual's core home range. For instance, a long-distance migrant, gull A23, was captured at the Nanaimo Landfill, but was primarily based on the Sunshine Coast for the winter. However, it utilized both the Sechelt and Nanaimo landfills, though the latter less frequently, making several trips across the Strait of Georgia before its migration to AK. Similarly, gull A22 was captured at the Hartland Landfill, where it returned to several times during the winter, but was primarily based near Maple Bay on Vancouver Island. Lastly, gull A11, captured in Sooke Basin, utilized this area for several weeks after capture, but by early April it moved to nearby downtown Victoria where it remained for the rest of the winter, breeding season, and subsequent years.



a.



b.

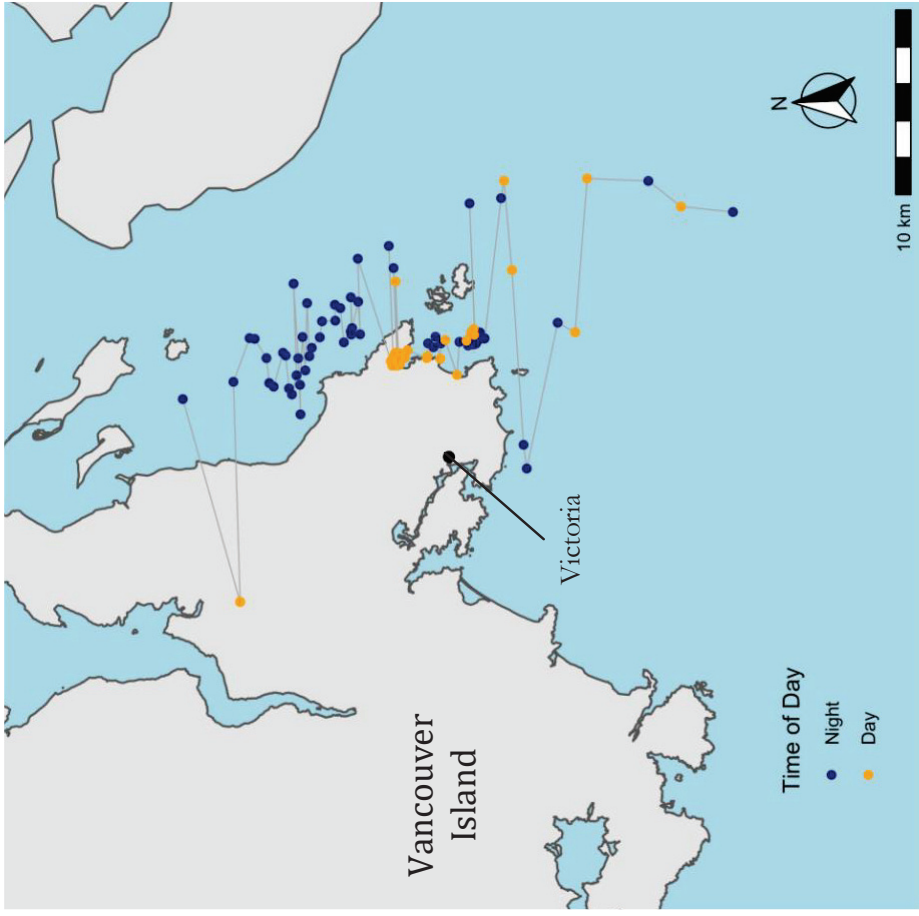
Figure 1.2. (a) Movements in a single winter period, for multiple individuals, demonstrating the regional fidelity exhibited by adult glaucous-winged gulls during winter. Winter movement tracks are displayed in a different colour for each individual. b) Individuals were also consistent in their use of particular wintering regions across years, as demonstrated by tracks for gull A27 in 2021 and 2022.

Site fidelity and habitat use

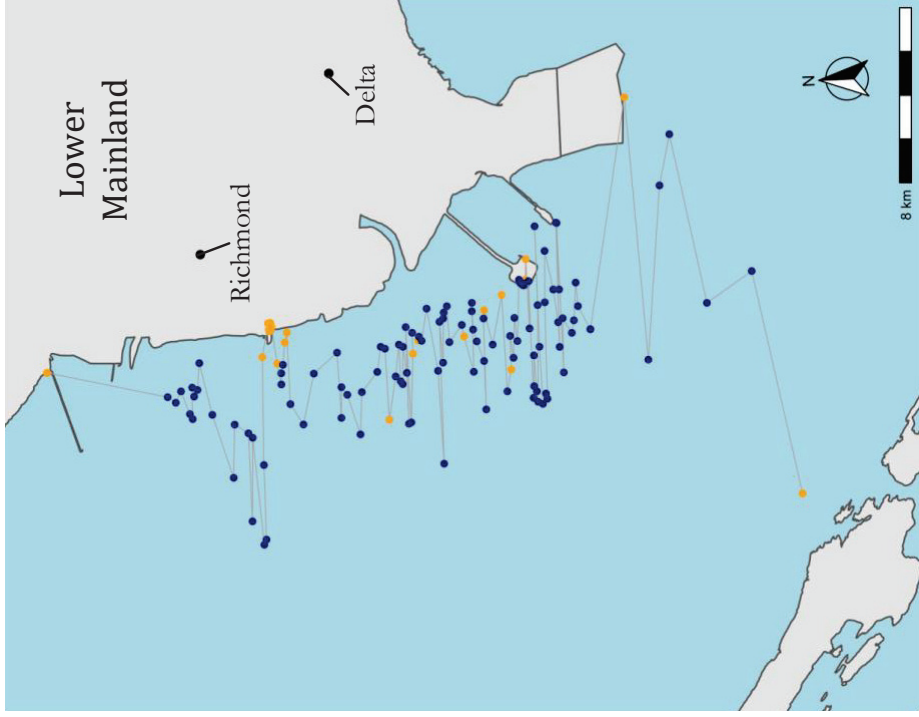
The affinity for specific sites within their home range varied among individuals. Although some displayed very high site fidelity (e.g. birds A9 and A1 habitually used the same urban waterfront parks; Figure 1.3a and 1.3b), most individuals roamed more widely and incorporated a large variety of sites and habitat types into their winter use areas (e.g. resident gull A8 and migrant gull A25; Figure 1.4a and 1.4b). The tendency for high or low site fidelity remained generally consistent by individuals in subsequent winters. Site fidelity and the degree of habitat use variability appeared to differ among individuals with home ranges in the same region. For instance, gulls A7 and A8 were both captured on the Saanich peninsula in the greater Victoria region, however, landfill use was only observed by gull A8, which utilized a wide variety of habitat types (Figure 1.4a) compared to high site fidelity and lower variety of habitat use by gull A7.

Landfills were regularly visited by glaucous-winged gulls. Of the seven adults captured at landfills, (25.9% of all gulls sampled), each returned multiple times during winter, with three visiting more than one landfill. Nearly half the birds captured at landfills ($n = 3$ of 7; 42.9%) underwent long-distance migrations to Alaska for the breeding season. Gulls captured in other habitat types also used landfills to varying degrees ($n = 9$), with five individuals using these sites frequently, and four apparently only visiting landfills once in a year. Overall, we observed landfill use at least once by 59.3% of gulls ($n = 16$ of 27) and recurrent use by at least 44.4% ($n = 12$) of gulls in this study.

Glaucous-winged gull habitat use varied markedly between day and night. During the day, gulls were primarily observed on shore and at land-based sites, and occasionally at sea. However, during night times, GWGUs were almost exclusively located on the water and occasionally on structures at marine industrial sites (e.g. on dock pilings, barges, etc.). Marine night locations were typically concentrated near frequently used day areas (Figure 1.3a and 1.3b).

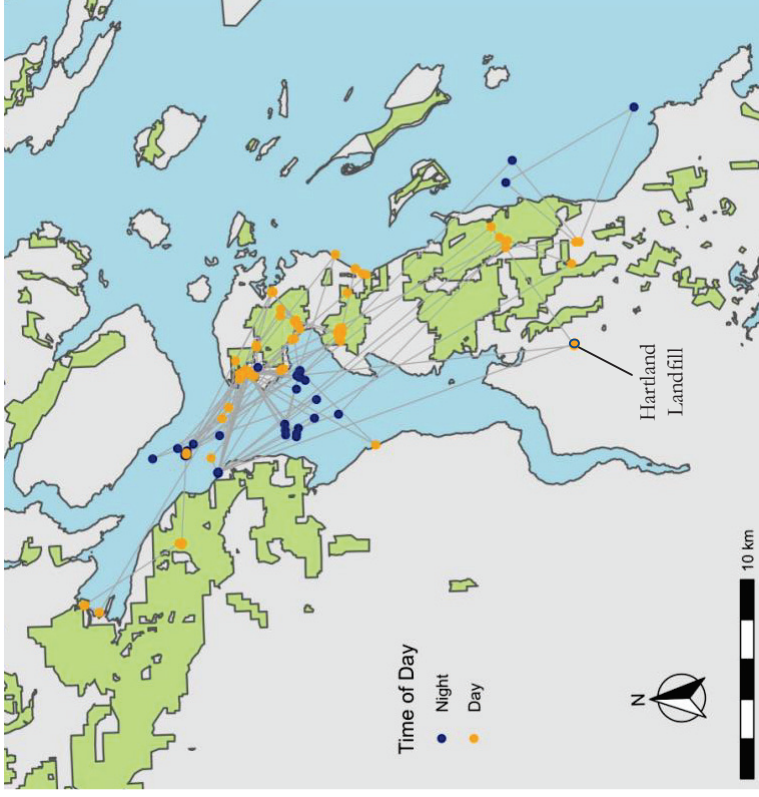


a.

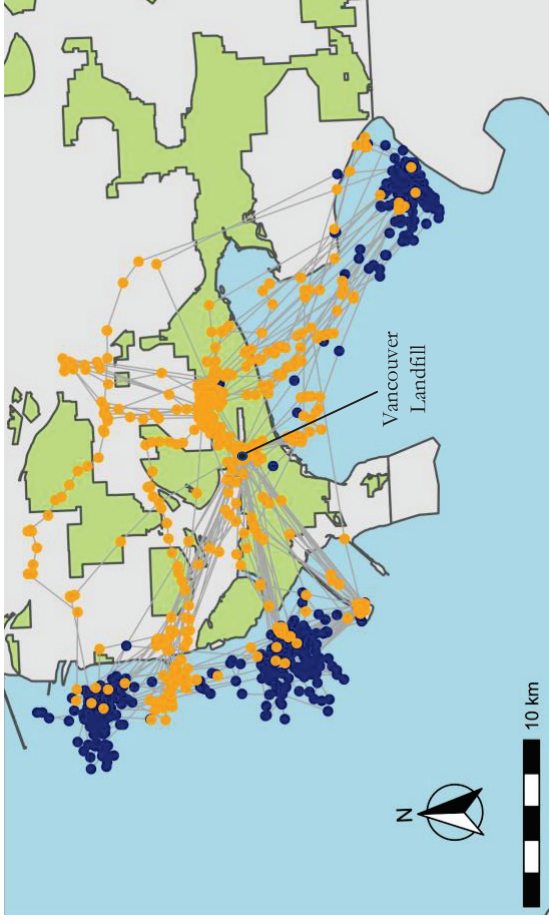


b.

Figure 1.3. Winter locations for gull A9 (a) and A1 (b) in 2020. Both individuals exhibited high site fidelity during winter with most daytime locations (yellow) at one main site each: Cadboro Bay Beach for gull A9 and Garry Point Park for gull A1. For all tagged glaucous-winged gulls in our study, daytime locations were primarily on land (yellow), while nearly all nighttime observations (blue) were on the water.



a.



b.

Figure 1.4. Winter locations for gull A8 (a) and A25 (b) in 2020. Both individuals utilized multiple habitat types and sites within their home ranges, including landfills. Low site fidelity and variable habitat use was typical for most gulls, in contrast the high site fidelity displayed by gulls A9 and A1 (Figure 1.3). Note: green areas denote agricultural lands.

Pacific herring spawn

Pacific herring (*Clupea pallasii*) are considered a keystone species within the Salish Sea ecosystem, and the annual spawning periods of this migratory forage fish attract many species of marine mammals and seabirds throughout the eastern Pacific. Contemporary hot spots for herring spawn in the Salish Sea primarily occur between Nanaimo and Campbell River on the east coast of Vancouver Island. At minimum, 37% (n = 10 of 27) of tagged GWGUs travelled to this area at least once during peak herring spawn, which occurs between late February and early April. At least 75% (n = 9 of 12) of gulls with home ranges in the northern Salish Sea (i.e. individuals from the Sunshine Coast and on Vancouver Island between Nanaimo and Campbell River) were observed at hot spots, indicating regional proximity as one driver of use. Resident gulls typically spent <1 – 2 days at herring hot spots before returning to their usual home range and some made recurrent trips to this area. Glaucous-winged gulls that migrated to Alaska for the breeding season also foraged in herring hot spots for several days enroute to Alaska. Though not all GWGUs conspicuously travelled to this area, gulls may be accessing herring without leaving their home range as adult Pacific herring migrate through the Salish Sea and remnant populations return to spawn outside of hot spots (Grinnell, 2021). Short-distance migrants may have also utilized herring spawn outside of the Salish Sea on the west coast of Vancouver Island prior to breeding. Additionally, our estimates of herring use are likely underestimated as tags with low battery charge collect data less frequently and may not have captured all trips to hotspots.

1.4.2. Movements out of the Salish Sea

We found that 22.2% of tagged GWGUs migrated out of the Salish Sea during our study (n = 6 of 27), including three individuals that migrated to Alaska (AK) for the breeding season, and three that travelled to the west coast of Vancouver Island prior to (n = 2), or during the breeding season (n = 1). Of these, data was collected for multiple years for one long distance migrant (to AK) and two short distance migrants (to the west coast of Vancouver Island), all of which displayed wintering region fidelity upon their return from migration. The other 77.8% of GWGUs in our study remained resident within the Salish Sea throughout all years observed (n = 21). Year-round residents selected breeding sites within or very close to their wintering grounds. Additionally, residents

tracked for more than one year consistently utilized the same breeding and wintering areas annually (n = 14).

Long distance migrants

Long-distance migrants travelled for an average of 32 ± 3 days and $> 1,800$ kms from their respective wintering areas in the Salish Sea to breeding grounds near Cordova, AK. Migration was observed during both day and night, though primarily during daylight hours (Figure 1.7). On average in 2020 and 2021, long-distance migrants departed their wintering areas around March 8th, and the Salish Sea around March 21st (Table 1.2). After leaving wintering sites, but prior to departing the Salish Sea, AK migrants spent 8 -15 days in areas with abundant Pacific herring (*Clupea pallasii*) spawn activity, primarily between the towns of Parksville and Campbell River on the east coast of Vancouver Island. The Nass River, a key eulachon (*Thaleichthys pacificus*) spawning area in the spring, was an initial stop-over site for two of the three individuals (A2 and A25) with visits lasting 5 - 15 days. Two individuals (A23 and A25) also utilized the nearby waters between Annette and Duke Island south of Ketchikan, AK for 2 - 6 days. Other multi-day stop over sites for one or more individuals included south of Port Hardy, BC, north of Price Island, BC, and several coastal sites in Alaska including along the perimeter of Baranof Island and the deltas of the Alsek, Stikine, and Dangerous rivers.

All long-distance migrants arrived at breeding areas near Cordova, AK by early to mid-April. However, no specific breeding site was apparent for gull A23 as its GPS tag failed shortly after arrival in AK (Table 1.1 and 1.2). The remaining two individuals began southbound migration between September 5-7th (Table 1.2). Gull A2's tag ceased transmission just north of Vancouver Island, after 25 days of travel (Table 1.2) and gull A25 proceeded to its former wintering grounds in the Lower Mainland region, arriving in late October, 52 days after departure from AK (Table 1.2). Gull A25 has completed two successful migrations to AK during our study, displaying winter and breeding site fidelity, as well as repeated use of stop over sites during south and northbound migrations (Figure 1.5).

Short-distance migrants

Three individuals migrated from the Salish Sea to the west coast of Vancouver Island. Gull A26, which over-wintered in the Sooke Basin, appeared to breed at Cleland

Island near Tofino, and frequently used the Sydney Harbour area for two consecutive breeding seasons (Figure 1.6a). Another individual, gull A17, flew north from Nanaimo along the coastline to northern Vancouver Island in late March. It proceeded to transit between Brooks Peninsula and Nootka Sound several times and finally to the Broken Group Islands near Ucluelet. Gull A17's tag ceased transmission in early April, before an established breeding site was apparent (Table 1.3). The third short distance migrant, gull A7, appeared to be a year-round resident of the Salish Sea from the time it was tagged in winter 2020 until the start of the 2021 breeding season, contrary to the consistency in inter-annual movements displayed by other migratory and resident GWGUs. Gull A7 left the Saanich Peninsula on May 21st, 2021 and travelled extensively between the west coast of Vancouver Island and the central coast of BC near Price Island. It did not appear to breed in any location in 2021, and by early July, it promptly returned to the Saanich Peninsula where it has been otherwise resident year-round in 2020 and 2022 (Figure 1.6b). All three short distance migrants visited Nootka Sound, and two of three (gulls A17 and A7) spent time at Brooks Peninsula. In contrast to AK migrants, west coast breeders reached their destination within approximately one day of travel (Table 1.3).

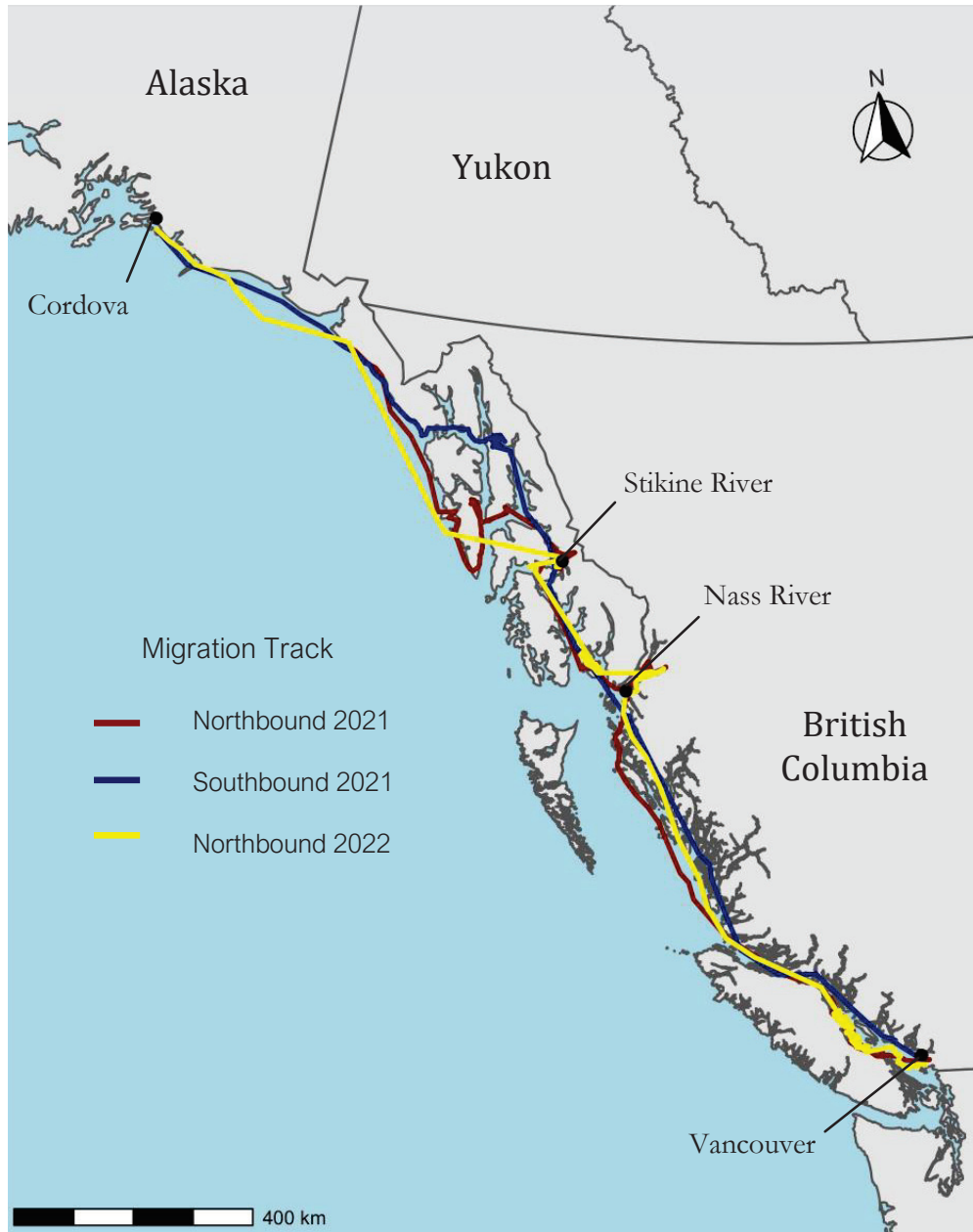
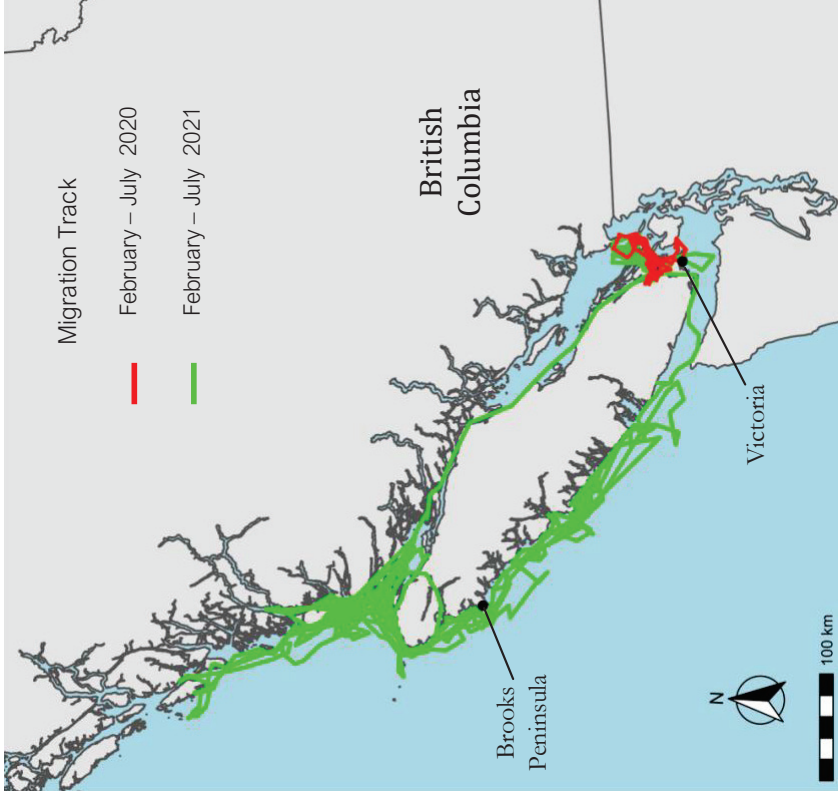
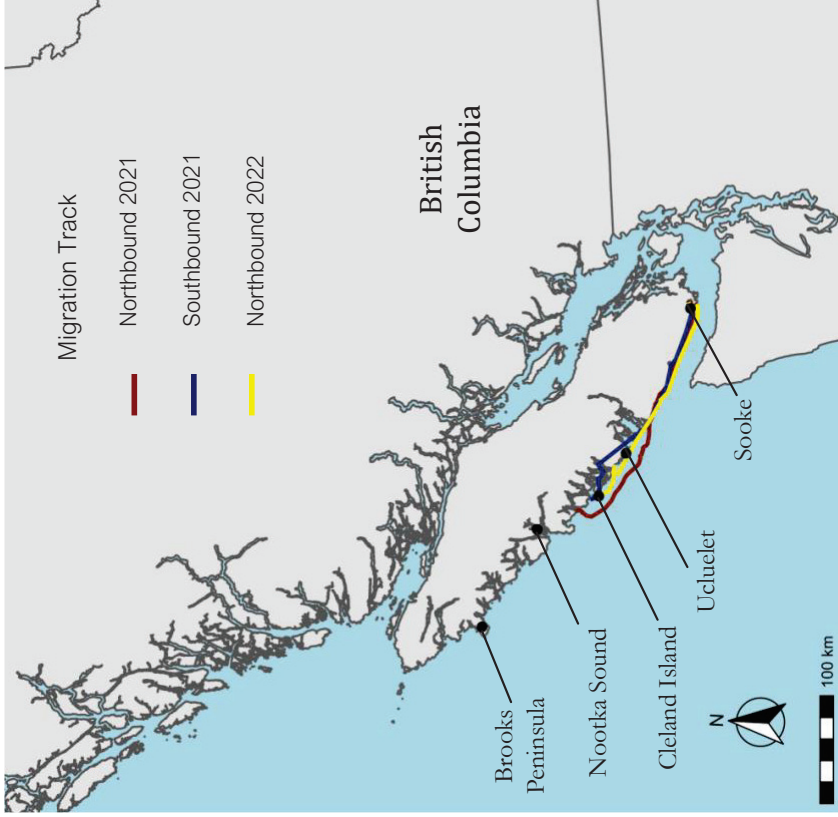


Figure 1.5. Migration tracks for gull A25 to and from over-wintering grounds in the Lower Mainland of BC and breeding grounds near Cordova, AK. The Nass River near Prince Rupert, BC and surrounding Annette and Duke Islands south of Ketchikan were utilized as stop over sites for 6 – 15 days each, as well as multiple days at the Stikine River near Wrangell, AK each during northbound migrations in 2020 and 2021. Migration was observed day and night (Figure 1.7).



a.



b.

Figure 1.6. (a) Gull A26 displayed consistent inter-annual migration patterns, traveling from its wintering grounds near Sooke, BC to its breeding grounds on Cleland Island, where it returned in 2021 and 2022. (b) In contrast, the movements of gull A7 transitioning to the breeding season were highly varied between 2020 and 2021. Although gull A7 remained resident near Victoria, BC year-round in 2020 and winter of 2021, in summer of 2021 it travelled extensively around the west coast of Vancouver Island and the Central Coast of BC before returning to its previous range where it appears to have remained throughout winter 2022.

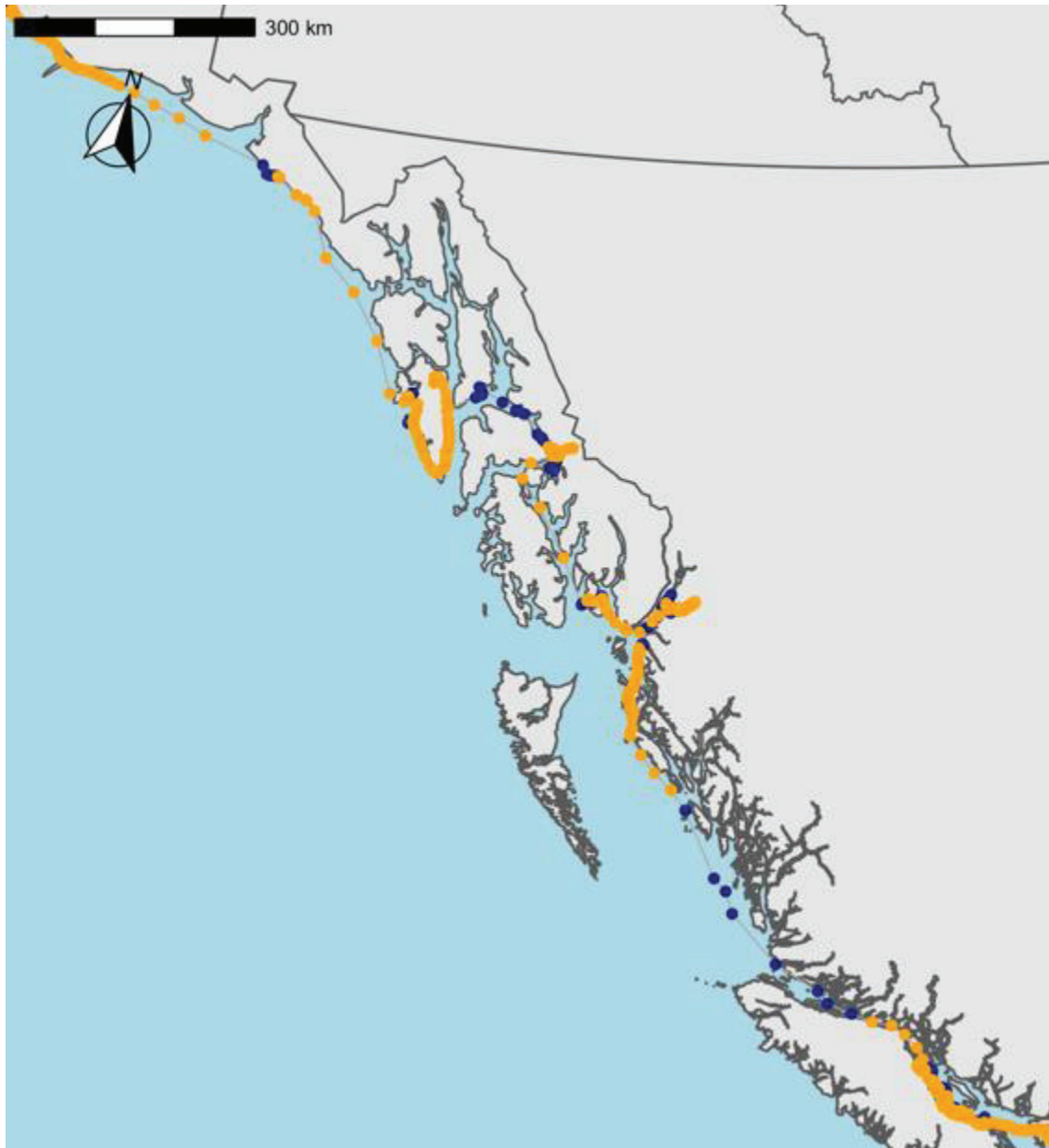


Figure 1.7. Migration occurred during both night and day, but with the majority of travel completed during daylight hours as exhibited by the migration to breeding grounds by gull A25 in spring of 2021.

1.5. Discussion

In this study, we used GPS tracking devices to characterize the winter movements and habitat use of adult glaucous-winged gulls throughout varying levels of anthropogenic activity in the Salish Sea of British Columbia, Canada. Our goal was to inform the interpretation of physiological assessment of health in gulls as described in

Chapter 2. Broadly, we found that GWGUs exhibit strong regional site fidelity in winter home ranges for both residents and migrants across years, with evidence of individual variation in wintering site fidelity and habitat use. We also identified marked temporal variation in habitat use by GWGUs, found mostly in land-based habitat during the day, but almost exclusively on the water at night. Lastly, we expand upon previous GPS tracking studies characterizing GWGU migration patterns and highlight the significance of forage fish spawning sites and landfills, especially for gulls which make long-distance migrations to breed in Alaska, but also for year-round residents.

The regional site fidelity displayed by wintering gulls in our study aligns with previous findings of high natal site and breeding territory fidelity by GWGUs in the Salish Sea (Hayward & Verbeek, 2020; Kroc, 2018a). Like (Hatch et al., 2011), we observed consistency in breeding site use by individuals which migrated to Alaska for the breeding period. Other studies have shown GWGU foraging efficiency increases with age (Hayward & Verbeek, 2020). Presumably familiarity with a territory over time is advantageous in a long-lived seabird like the GWGU, though their infrequent excursions outside of core home ranges also concur with the opportunistic nature of this species.

Likewise, capture sites were within the core winter range for most gulls, indicating a strong fidelity to the regions within which they were sampled. In the context of long-term monitoring (e.g. of diet, contaminant exposure, or physiological condition) this regional fidelity may allow changes in GWGU behaviour and health to be tracked in relation to localized environmental changes, including those stemming from anthropogenic activity. However, due to a portion of the wintering population breeding outside of the Salish Sea, only biological samples that reflect recent status, such as blood or plasma, should be collected for winter monitoring.

Winter site fidelity and habitat use appears to differ among individuals, ranging from more “specialized” gulls observed at only a few sites to more generalist gulls which used a wide variety of sites and habitat types. This individual variation did not appear related to region of capture or specific habitats, considering differences were observed among individuals occupying overlapping home ranges. However, more in depth analyses should explore how the likelihood of using certain habitats relates to their proximity, availability, and abundance within an individual’s core home range.

Very high site fidelity was uncommon within our study population, but individuals utilizing this strategy typically occupied small winter territories based at urban waterfront parks. As these sites all have frequent human presence, intertidal prey, and grassy fields harbouring earthworms, they likely provide consistent access to a variety of foods within a small area. So, while habitat use appears to be very specialized for some gulls, more investigation is needed to determine whether this translates into foraging specializations, or if these individuals are incorporating a wide variety of prey items into their diets. In other gulls, foraging specializations have been identified and the degree of dietary specialization has been linked to reproductive success. For instance, in herring gulls, those specializing on either marine prey or anthropogenic food sources had higher reproductive success than those exhibiting mixed resource use (O'Hanlon & Nager, 2018). In a changing and increasingly human impacted environment, flexibility is likely an advantage for GWGUs, but incorporating more anthropogenic prey appears to have negative reproductive consequences in the Salish Sea compared with GWGUs on the west coast which consume more marine prey (Davis et al., 2015). As potential habitat and foraging specializations can lead to different levels of contaminant exposure (Peterson et al., 2017; Zorrozua, Monperrus, et al., 2020), further investigation of this aspect of GWGU movement ecology would benefit the interpretation of health monitoring (see Chapter 2) and the overall utility of GWGUs as a biomonitor.

Interestingly, our findings indicate that in contrast to more land-based habitat use during the day, GWGUs are found almost exclusively roosting on the water at night. This aligns with other findings that although GWGUs are commonly seen foraging, socializing, and loafing during the day, only limited foraging is observed on shore at night when low tides occur during winter (Ward, 1973). Roosting on the water presumably reduces the risk of predation by nocturnal, land-based mammalian predators. Although, the possibility of gulls foraging on zooplankton, which make diurnal migrations to the water surface at night (Sato et al., 2013), could be explored. We also found that while long-distant migrants primarily travelled during the day, some migration occurred at night. This has not previously been documented for GWGUs (Hayward & Verbeek, 2020), highlighting the opportunity to further investigate nighttime activities in this species.

Compared with other times of year, food resources are constrained for GWGUs wintering in the northeastern Pacific. During winter, daylight (i.e. when most foraging

occurs) is reduced to approximately eight hours per day at the solstice and the lowest tides occur at night, potentially limiting intertidal foraging opportunities. In addition, multi-decadal spatial and temporal declines have been documented for Pacific herring and other forage fish in the Salish Sea due to anthropogenic activity (Therriault et al., 2009). Additionally, reduced human traffic to beaches and parks during winter may also reduce access to anthropogenic food sources. These constraints may in turn increase reliance on landfills, which are a stable food-source year-round, despite the associated risks including predation by bald eagles (Elliott et al., 2006) and hazards such as trauma from heavy machinery, entanglement, or poisoning (Elliott et al., 2006).

Travel to access herring spawn by long and short distance migrants, as well as year-round resident gulls highlights the continued importance of access to forage fish for GWGUs (Marston et al., 2002). Ephemeral bait balls, or spawns of herring and other forage fish may explain some of the brief trips taken by GWGUs outside of their core home ranges during winter. Moreover, the frequency and importance of these excursions are likely underestimated in our study due to their short duration and infrequent data collected by some tags when battery charge was low (days with no data collection or as few as 1-4 points per day). Forage fish were also clearly of importance in fuelling energy intensive journeys by long-distance migrants, which timed the onset of migration with use of peak herring spawn activity in the Salish Sea, and subsequent multi-day stop-overs enroute (for e.g. at the Nass River, coinciding with eulachon spawn).

As with forage fish reliance, landfill use was widespread among wintering GWGUs, and the connection was particularly strong for long-distance migrants as has been noted previously (Hatch et al., 2011). Landfills are a unique source of anthropogenic food subsidies and are especially attractive to opportunistic omnivores like gulls. Landfills without gull-deterrence programs can offer high-efficiency energy gain (Patenaude-Monette et al., 2014) and likely provide increased food availability during the constrained winter periods (Duhem et al., 2008; Gyimesi et al., 2016; Real et al., 2017; Weiser & Powell, 2010). Although few studies have examined the impact of winter landfill use on gull population sizes or health, for some species, landfill subsidies may alleviate seasonal resource constraints (Zorroza, Aldalur, et al., 2020) and contribute to increased over-winter survival (Duhem et al., 2008). However, greater reliance on anthropogenic subsidies has also been associated with reduced breeding

success (Anderson et al., 2019; Blight, 2011; O’Hanlon et al., 2017) and greater oxidative stress (Laranjeiro et al., 2020) in some gulls.

Our data suggest that glaucous-winged gulls use landfills year-round in the Salish Sea, but studies in previous decades have demonstrated an influx of GWGUs at landfills during winter (Ward, 1973). Contemporarily, GWGUs still flock to landfills throughout the Salish Sea in large numbers, despite the implementation of effective gull-deterrence programs at most landfills, the diversion of much organic waste through municipal composting programs (*Organic Waste Diversion: CleanBC Organics Infrastructure and Collection Program*, n.d.) and the risk of mortality or injury present at these sites (Elliott et al., 2006; Lindborg et al., 2012). Schmidt et al. (1993) found that during the breeding season, GWGUs presence at landfills increased in the evenings, but was dependent on tide height, suggesting landfills are more attractive when the availability of other resources is limited.

All long-distance migrants in our study were captured at landfills. Perhaps despite the risks and challenges associated with landfill foraging, these sites still offer the high efficiency energy gain necessary for fuelling long-distance migrations due to highly concentrated food availability compared to most natural habitats. Although migrants exhibited regional wintering site fidelity in our study, and others (Hatch et al., 2011), it is possible that year-round residents have better territorial claim to habitat types which may be more easily defended or require more specialization for efficient foraging (e.g. hunting earthworms in a park or digging for clams on the beach), leading migrants to incorporate more landfill use where feeding occurs in a frenzy and feeding grounds are not easily defended. However, even though our analyses of habitat use were a preliminary overview, specialization on landfill use by migrants was not apparent, as gulls were still found to utilize a wide variety of habitat types. Further analyses should explore how much time GWGUs spent foraging at landfills, as opposed to loafing or socializing, which also attract gulls to these sites (Belant et al., 1993; Hebert et al., 1999; Ward, 1973).

Despite resource constraints during winter, the Salish Sea continues to be an important area for GWGUs, retaining a high proportion of year-round residents while also supporting an influx of overwintering migrants (Hayward & Verbeek, 2020; Ward, 1973). However, a variety of risks are posed to this species through further human

development, increased reliance on anthropogenic food sources, and degradation of marine environments. In particular, the threat of an oil spill will likely be significant and difficult to manage for this species during winter. Although GWGUs are dispersed across land and shore-based locations during day, they consistently congregate to roost on the water at night, which can span over 12 hours in winter. Impacts to wintering grounds in the Salish Sea will likely have localized effects on regional breeding populations, due to high regional fidelity by year-round residents, but also may impact breeding colonies across a wide geographic area due to weak migratory connectivity displayed by this species (Hatch et al., 2011). Although various efforts have been successful in reducing access to food at landfills, these sites are still sought by gulls indicating their role in buffering winter foraging constraints and long-term degradation of forage fish availability. However, landfills may pose significant risks to GWGUs where anecdotally, three of four tags that failed within approximately one day of deployment were on gulls captured at landfills, indicating possibly mortality. Gull landfill use can also pose risks to humans, especially use by long-distance migrants which have the potential to spread harmful pathogens, such as anti-microbial resistant bacteria, across wide geographic distances (Ahlstrom et al., 2021; Franklin et al., 2020; Zeballos-Gross et al., 2021).

In the context of long-term monitoring (see Chapter 2), and use of GWGUs as a biomonitor, high regional site fidelity should allow us to detect changes over time in response to localized impacts, whether natural or due to anthropogenic causes. However, due to variation among individuals in habitat use, it is important to concurrently measure diet and contaminant exposure along with health, which may help explain individual variation in health. Better understanding these relationships will enable physiological monitoring to be more effectively used for conservation of this species and provide insight into the impacts of human activity to GWGU health.

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1.7. Supplemental Information

Table 1.1. Summary of GPS tag deployments on adult glaucous-winged gulls captured throughout the British Columbian portion of the Salish Sea in January and February of 2020 and 2021 (n = 31). Gulls were fitted with one of two types of tags: 'Ecotone Kite-L' (17g) GPS-GPRS (global penetrating radar systems) tag or a 'Microwave 20-70' (25g) GPS-GSM (global system for mobile communications) tag.

Bird ID	Band ID	Sex	Body mass (g)	Device (g)	Load (%)	Site	Latitude	Longitude	Deployed	Last date of transmission	Winters with contact	Final status
A1	1547-09513	U	1310	17	1.3	Garry Point (Vancouver)	49.1250709	-123.19261	23-Jan-20	10-Aug-21	2	Indeterminate
A2	1547-09514	U	1100	17	1.55	Vancouver Landfill (Vancouver)	49.1000644	-123.00532	24-Jan-20	10-Oct-20	1	Indeterminate, possibly battery failure
A3	1547-09515	F	1000	17	1.7	Sechelt Landfill (Sunshine Coast)	49.4884939	-123.7124	27-Jan-20	29-Jan-20	None	Tag Failure
A4	1547-09516	F	875	17	1.94	Sechelt Landfill (Sunshine Coast)	49.4884939	-123.7124	27-Jan-20	13-Apr-22	3	Currently operating
A5	1547-09875	F	975	17	1.74	Chaster Creek (Sunshine Coast)	49.3901204	-123.55652	28-Jan-20	13-May-20	1	Mortality
A6	1547-09877	F	900	17	1.89	Willingdon Beach Park (Powell River)	49.8470063	-124.53265	29-Jan-20	11-Jun-21	1	Indeterminate
A7	1216-01024	F	850	17	2	Tseycum Beach (Saanich)	48.6645711	-123.45449	18-Feb-20	20-Mar-22	2	Currently operating
A8	1216-01026	M	1150	17	1.48	Chalet Beach (Saanich)	48.6878842	-123.4745	19-Feb-20	09-Apr-22	3	Currently operating
A9	1216-01040	F	925	17	1.84	Cadboro-Gyro Park (Victoria)	48.4596693	-123.29443	25-Feb-20	12-Apr-22	3	Currently operating
A10	1216-01043	F	950	17	1.79	Wright Road Beach (Sooke)	48.3653348	-123.73101	26-Feb-20	05-Aug-20	1	Indeterminate
A11	1216-01045	F	1125	17	1.51	Billings Spit (Sooke)	48.3777386	-123.69151	26-Feb-20	27-Feb-20	None	Tag Failure
A12	1216-00405	F	900	25	2.78	Queen Elizabeth Promenade (Nanaimo)	49.1726818	-123.94144	27-Feb-20	26-Aug-20	2	Indeterminate
A13	1216-00407	F	945	25	2.65	Departure Bay Beach (Nanaimo)	49.205696	-123.96919	27-Feb-20	14-Nov-20	2	Indeterminate

A14	2406-09309	M	1155	25	2.16	Spanish Banks Beach East (Vancouver)	49.2757897	-123.21244	07-Jan-21	20-Jul-21	1	Indeterminate
A15	2406-09317	F	940	25	2.66	Sunset Beach Park (Vancouver)	49.2781767	-123.13655	14-Jan-21	12-Apr-22	2	Currently operating
A16	1216-00416	M	975	25	2.56	Cattle Point (Oak Bay)	48.4386347	-123.29254	14-Jan-21	12-Apr-22	2	Currently operating
A17	1216-00429	M	1295	25	1.93	Wheatcroft Park (Nanaimo)	49.2270228	-123.95208	18-Jan-21	13-Apr-21	1	Indeterminate
A18	1216-00433	F	1095	25	2.28	Parksville Community Park (Parksville)	49.3279618	-124.30794	19-Jan-21	08-Aug-21	1	Indeterminate
A19	1216-00443	M	1115	25	2.24	Crofton Ferry Terminal (Crofton)	48.8651189	-123.6404	21-Jan-21	05-May-21	1	Indeterminate
A20	1216-00360	F	1025	25	2.44	Hartland Landfill (Victoria)	48.5376926	-123.4663	22-Jan-21	10-Sep-21	2	Indeterminate
A21	2406-09331	F	950	25	2.63	Horseshoe Bay (West Vancouver)	49.3753536	-123.27548	22-Jan-21	12-Apr-22	2	Currently operating
A22	1216-00361	M	1265	25	1.98	Hartland Landfill (Victoria)	48.5376926	-123.4663	22-Jan-21	06-Jun-21	1	Indeterminate
A23	1216-00362	F	1055	25	2.37	Nanaimo Landfill (Nanaimo)	49.1195214	-123.90345	26-Jan-21	04-May-21	1	Indeterminate
A24	1216-00364	F	945	25	2.65	Nanaimo Landfill (Nanaimo)	49.1195214	-123.90345	26-Jan-21	31-Jan-21	None	Indeterminate
A25	2406-09333	M	1265	25	1.98	Vancouver Landfill (Delta)	49.0984203	-123.02115	26-Jan-21	12-Apr-22	2	Currently operating
A26	1216-00372	M	1065	25	2.35	Goodridge Road (Sooke)	48.3882741	-123.66203	27-Jan-21	12-Apr-22	2	Currently operating
A27	2406-09340	F	965	25	2.59	Ivines Landing (Sunshine Coast)	49.6327494	-124.05738	28-Jan-21	12-Apr-22	2	Currently operating
A28	2406-09355	F	955	25	2.62	Lund Marina (Lund, Upper Sunshine Coast)	49.9812867	-124.76233	09-Feb-21	12-Apr-22	2	Currently operating
A29	1547-09880	M	1130	17	1.5	Willingdon Beach Park (Powell River)	49.8469851	-124.53258	10-Feb-21	13-Apr-22	2	Currently operating
A30	2406-09368	F	915	17	1.86	Salish Soils Inc Compost Facility (Sunshine Coast)	49.4819176	-123.74729	17-Feb-21	13-Apr-22	2	Currently operating
A31	1547-54050	U	1135	17	1.5	Salish Soils Inc Compost Facility (Sunshine Coast)	49.4819176	-123.74729	17-Feb-21	17-Feb-21	None	Mortality

Table 1.2. Summary of long-distance migrations to and from wintering grounds in the Salish Sea and breeding grounds in Alaska.

Bird ID	Year	Departed wintering grounds	Departed Salish Sea	Herring spawn (d)	Breeding grounds arrival	Migration duration (d)	Stopovers (d)	Breeding grounds departure	Wintering grounds arrival	Migration duration (d)	Stopovers (d)	Notes
A23	1 of 1	2021-03-13	2021-03-24	12	2021-04-13	31	13	Tag failure	NA	NA	NA	Northbound stopovers near Port Hardy, north of Price Island, and south of Keitchikan.
A2	1 of 1	2020-03-09	2020-03-23	15	2020-04-05	27	7	2020-09-05	Incomplete - tag failure	NA	NA	Northbound stops at Nass River, near Sitka Island, and the Alsek River estuary.
A25	1 of 2	2021-03-11	2021-03-19	9	2021-04-15	34	23	2021-09-07	2021-10-29	52	46	Northbound stops at Nass River (~2 weeks), south of Keitchikan (6 days), the Sitkine River estuary, and Baranof Island. Southbound travelled two days to Juneau and stayed for 46 day stopover, then six days of direct travel to Salish Sea.
A25	2 of 2	2022-03-01	2022-03-14	14	2022-04-04	34	19	Has not yet occurred	NA	NA	NA	Northbound stops at same location for similar durations except last stopover was at Dangerous River Delta instead of Baranof Island.

Table 1.3. Summary of short distance migrations exhibited by adult glaucous-winged gulls prior to or during the breeding season.

Bird ID	Year	Departed wintering grounds	Departed Salish Sea	Herring spawn (d)	Breeding grounds arrival	Migration duration (d)	Stopovers (d)	Breeding grounds departure	Wintering grounds arrival	Migration duration (d)	Notes
A26	1 of 2	2021-03-25	2021-04-09	Herring use indeterminate	2021-04-09	~ 1	0	2021-09-14	2021-09-15	~ 1	NA
A26	2 of 2	2022-03-12	2022-03-12	Herring use indeterminate	2022-03-13	~ 1	0	Has not yet occurred	NA	NA	NA
A17	1 of 1	2021-03-18	2021-03-20	~1	Indeterminate	~ 16	Indeterminate	Tag failure	NA	NA	Individual was mobile from 2021-03-21 to 2021-04-05 with frequent trips between Price Island and Brooks Peninsula.
A7	1 of 3	NA	NA	None observed	NA	NA	NA	NA	NA	NA	Did not migrate
A7	2 of 3	2021-05-21	2021-05-21	Herring use indeterminate	2021-07-03	1	0	NA – no breeding site observed	NA	NA	Individual was mobile by 2021-05-31 with frequent trips between the central coast and west coast of VI throughout June.
A7	3 of 3	NA	NA	None observed	NA	NA	NA	NA	NA	NA	Did not migrate

Chapter 2. Physiological health of wintering glaucous-winged gulls

2.1. Abstract

Gulls (Laridae) use natural and urban environments and are useful ‘biomonitors’ of coastal ecosystem health. I studied physiological health (from blood samples) in glaucous-winged gulls (*Larus glaucescens*; GWGU) during winter in the Salish Sea, a region undergoing rapid anthropogenic change. There was marked individual variation in, and covariation among, physiological traits (glucose, triglycerides, hemoglobin, hematocrit, and oxidative state). However, region and habitat type where birds were captured did not predict variation in health biomarkers, even though these included varying levels of urban development and anthropogenic activity, and despite strong regional fidelity exhibited by this species (Chapter 1). This study establishes reference values for health biomarkers which can be used for long-term monitoring of future anthropogenic impacts in the Salish Sea.

2.2. Introduction

During a time of rapid anthropogenically-driven environmental change (Dirzo et al., 2014), marine ecosystems are under increasing stress from the impacts of coastal development (Bishop et al., 2017), habitat degradation (Gibson et al., 2007), over-fishing (Jackson et al., 2001), invasive species (Molnar et al., 2008), ocean acidification (Cornwall & Eddy, 2015), climate change (Henson et al., 2017), and pollution (Shahidul Islam & Tanaka, 2004). As such, understanding coastal ecosystem health is of vital importance (Tett et al., 2013). Some of the very species that are most impacted by anthropogenic change also act as useful ‘biomonitors,’ providing insight into the state of ecosystems and emerging shifts that may be occurring (Mallory et al., 2010; Piatt et al., 2007). In this context, seabirds are ideal biomonitors, sensitively reflecting changes in the marine environment through changes in their diet, habitat use, population trends, reproductive output, and overall health (Moore & Kuletz, 2019).

Seabirds, which typically forage at a high trophic level in the marine food web, are susceptible to bioaccumulation of toxins and can provide insight into environmental

contaminant trends. For instance, seabirds have been studied for long-term tracking of mercury pollution in the arctic (Bond et al., 2015; Provencher et al., 2014), halogenated hydrocarbons in the Great Lakes (Hebert et al., 1999), and a variety of other contaminants worldwide (e.g. Mallory & Braune, 2012; Pérez et al., 2008; Viñas et al., 2020), which can play a role in seabird (Bustnes et al., 2015; Koivula & Eeva, 2010), human (Alm et al., 2018; Burger et al., 2008), and ecosystem health (Driscoll et al., 2013; Smits & Fernie, 2013). In particular, gulls (Family Laridae) are often used as indicators of marine and urban environmental health (Davis et al., 2017; Laranjeiro et al., 2020; Thorstensen et al., 2021; Zorrozuza, Monperrus, et al., 2020) as they respond to anthropogenically-modified habitats by utilizing both marine and terrestrial food subsidies (N. Isaksson et al., 2016; O'Hanlon et al., 2020), and also utilize both natural and human-built structures for roosting and nesting (Blight et al., 2019; Kroc, 2018).

Long-term monitoring of gulls has revealed changes in marine food webs and pollution in the environment over time (Blight et al., 2015a; K. H. Elliott & Elliott, 2016; Hebert et al., 1999). As generalist foragers, gulls are able to adapt to anthropogenic influence by foraging in landfills, agricultural land, and urban areas (Bécares et al., 2015; Isaksson et al., 2016; Juvaste et al., 2017). Although access to anthropogenic subsidies can release gull populations from food constraints and increase over-winter survival (Zorrozuza, Aldalur, et al., 2020; Duhem et al., 2008), in some cases it is also associated with negative health and reproductive consequences at both the individual and population level (Anderson et al., 2019; Lopes et al., 2022). In this context, conservation physiology (Madliger et al., 2020), provides useful tools that can be utilized to evaluate underlying indicators of health in individuals and wildlife populations. Establishing reference values and conducting long-term physiological monitoring can provide insight into health issues before pathology is apparent (Dietz et al., 2019), which helps to identify underlying mechanisms of population declines (Cooke et al., 2021), and increases the scope of environmental information gained from seabirds (Mallory et al., 2010). Combined with studies of diet, contaminant loads, and population trends, physiological monitoring can be used to assess potential environmental stressors related to poor nutrition, toxins, or behaviour (Birnie-Gauvin et al., 2017; Dietz et al., 2019; Hegseth et al., 2014; Laranjeiro et al., 2020; Sagerup et al., 2009; Wayland et al., 2010). In gulls, physiological monitoring has linked foraging at landfills and urban areas with high exposure to halogenated flame retardants (Sorais et al., 2020), depressed levels of

omega-3 fatty acids (Lopes et al., 2022), greater oxidative stress (Laranjeiro et al., 2020), and both better (O'Hanlon et al., 2017; Ronconi et al., 2014) and worse body condition (Auman et al., 2008; Steigerwald et al., 2015). Conversely, the greater reliance on terrestrial and lower trophic level prey items by urban foraging gulls reduces their exposure to mercury (Clatterbuck et al., 2021; Peterson et al., 2017; Ramos et al., 2013; Thorne et al., 2021; Zorrozua, Monperrus, et al., 2020) and other heavy metals (Laranjeiro et al., 2020; Ramos et al., 2013). Although physiology can provide detailed insight into species and ecosystem level health, this tool is often overlooked and results in a lack of biomarker reference values for most species (Mallory et al., 2010). Additionally, studies of physiological status of non-breeding birds are even less frequent, despite the obvious importance of health throughout the annual cycle (Garcia et al., 2010; Mallory et al., 2015; Merklings et al., 2017; Minias, 2015; Reid, 1987).

The Salish Sea, on the northeastern edge of the Pacific Ocean, encompasses the Strait of Georgia, the Strait of Juan de Fuca, British Columbia (BC) and Puget Sound, Washington (WA). This biologically productive area is a globally significant location for breeding and wintering birds (Crewe et al., 2012; Gaydos et al., 2015a; *IBA Site Listing*, n.d.). Harbouring numerous human population centres, including three large metropolises (Vancouver and Victoria, BC and Seattle, WA), the Salish Sea is strongly, and increasingly, influenced by human activity (Crewe et al., 2012; Halpern et al., 2007). In the marine environment, industrial fishing pressure, habitat degradation, and the recovery of marine mammal populations have led to forage fish declines (Schweigert et al., 2010; Therriault et al., 2009), while legacy contaminants are an ongoing concern for both wildlife and humans (Chen et al., 2012; Elliott & Elliott, 2016; Ross et al., 2004). Other local pollution threats include oil spills (Gaydos et al., 2015a), derelict fishing gear (Good et al., 2009), vessel disturbance, and the continued expansion of industrial development in estuarine habitat (Gaydos et al., 2015b). Currently, Environment and Climate Change Canada (ECCC) has a mandate through the Ocean's Protection Plan to assess threats to wildlife posed by new and proposed industrial projects (Canada, 2018) including the Trans-Mountain Pipeline expansion which will increase crude oil tanker traffic in the Salish Sea by approximately seven-fold (Port of Vancouver, 2015; Short, 2015; Trans Mountain, 2017). Canada also has a regional stewardship responsibility to glaucous-winged gulls (*Larus glaucescens*), which rely heavily on the Salish Sea region for breeding and overwintering (Environment Canada, 2013). As such, it is a research

priority of ECCC to study the wintering habitat use and contaminant exposure of glaucous-winged gulls in the Salish Sea.

In this study, we assessed variation in physiological health, sex ratio, and body mass of glaucous-winged gulls (GWGUs) wintering in the Salish Sea region of BC. Multiple physiological biomarkers were measured as indicators of health, including nutritional state (glucose and plasma triglycerides), aerobic capacity (hemoglobin, hematocrit), and oxidative state (total antioxidants and reactive oxygen metabolites). We then examined whether each of these physiological biomarkers varied among regions or habitat types throughout the Salish Sea. Additionally, we compared birds in the Salish Sea to a small group of gulls sampled on the West Coast of Vancouver Island. Finally, we tested whether the overall health of individuals, using principal components analysis to generate indices incorporating all six measured biomarkers, varied regionally or among habitat types of capture sites.

2.3. Methods

2.3.1. Study area

The Salish Sea (49° 20' 10.4", -123° 50' 21.6") encompasses the protected waters of southern British Columbia, including the Strait of Georgia and Strait of Juan de Fuca, as well as Puget Sound in Washington state. In January and February of 2020 and 2021, we sampled adult GWGUs throughout the Canadian portion of the Salish Sea (Figure 2.1). Though most GWGUs are resident here year-round (Chapter 1), sampling during this period ensured that migrant gulls, which typically reach non-breeding grounds by November (Hayward & Verbeek, 2020), had also over-wintered here for at least two months. We attempted to sample evenly between the following regions: 'Lower Mainland', 'Greater Victoria', 'Southern Vancouver Island', and the 'Northern Salish Sea.' Regions were categorized primarily by geographic proximity, but generally had similar beach substrate types and levels of anthropogenic influence throughout a given region (Figures 2.2 and 2.3).

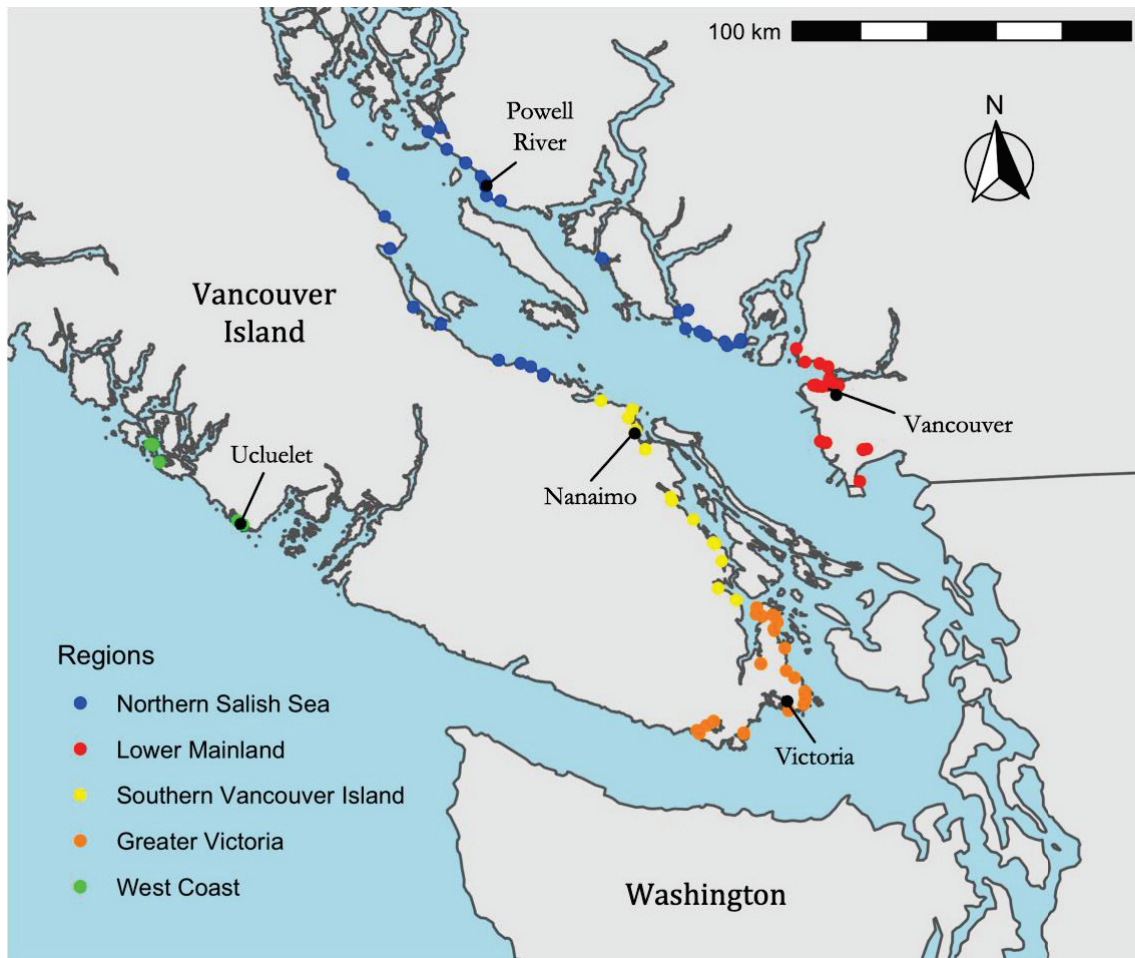


Figure 2.1. Map of glaucous-winged gull capture locations by regions in the Salish Sea and west coast of Vancouver Island in 2020 and 2021.

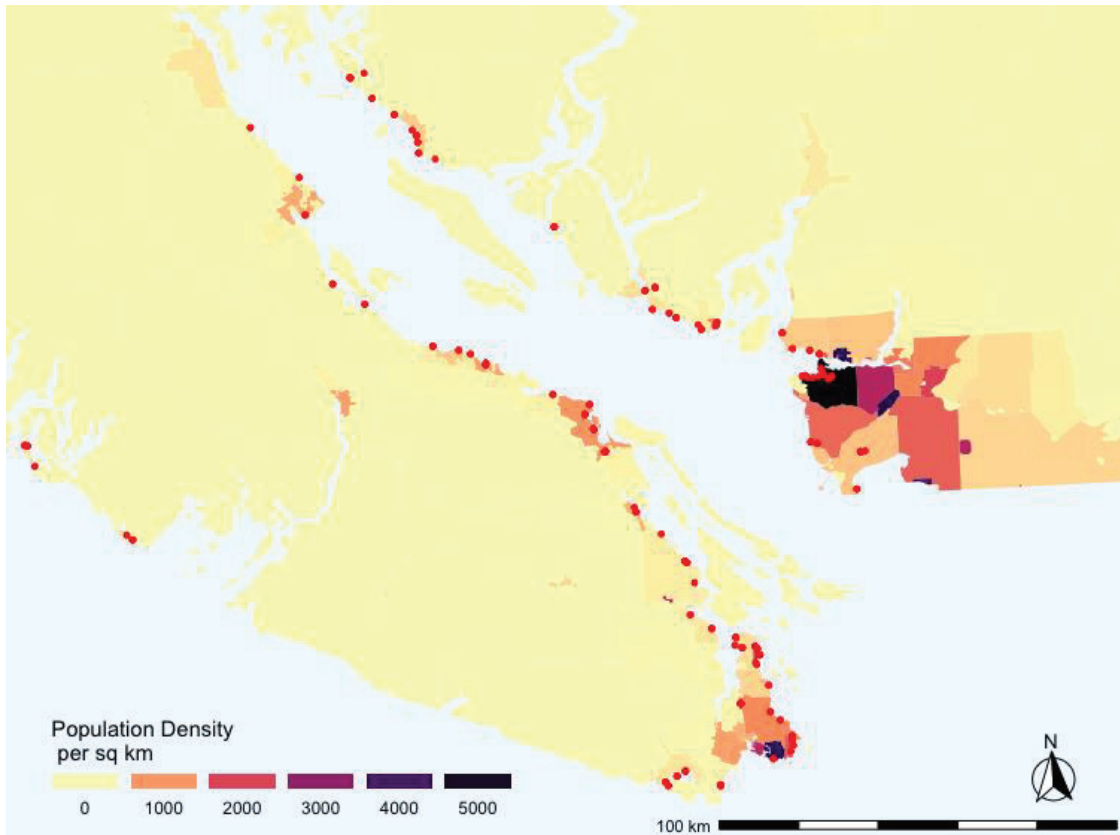


Figure 2.2. Map of human population density/ km^2 within the Salish Sea of British Columbia, Canada. Densities are based on 2016 census data and calculated within each census subdivision (Statistics Canada, 2017). Red points are capture locations of adult glaucous-winged gulls in 2020 and 2021.

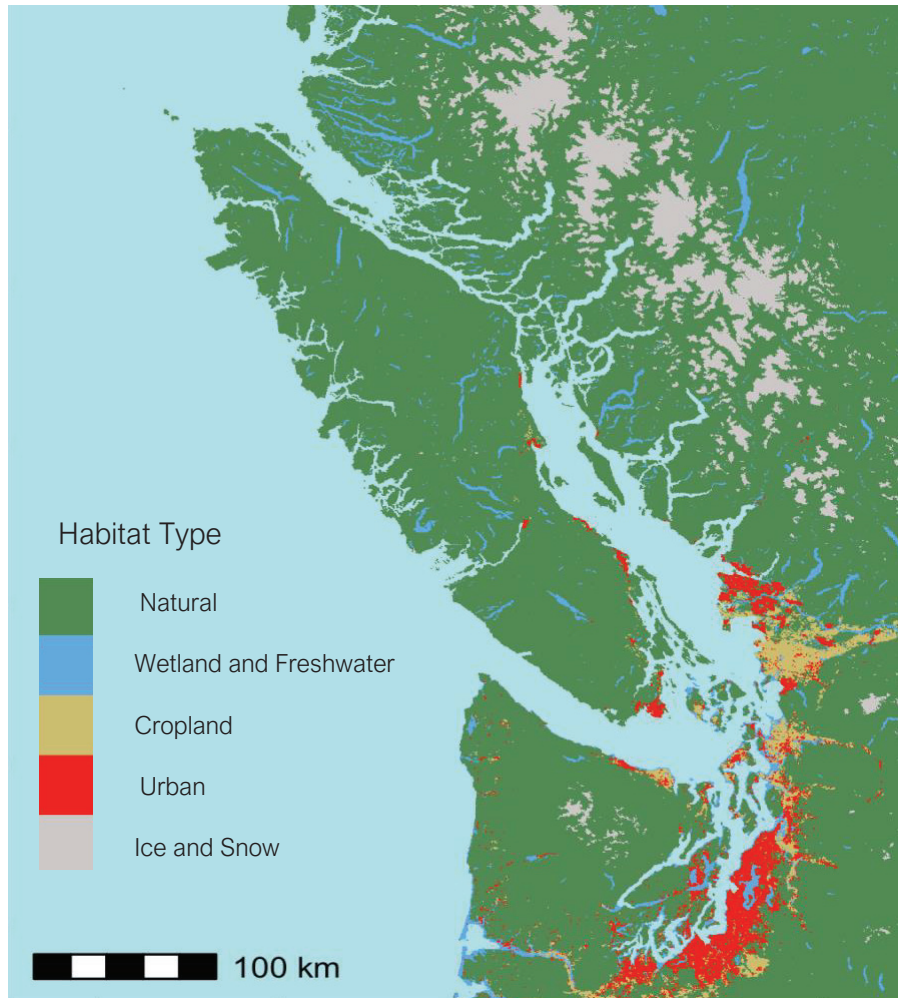


Figure 2.3. Landcover types within the Salish Sea, British Columbia, Canada, displayed at 250 m resolution (North American Land Change Monitoring System, 2021).

Within each region, we attempted to sample evenly among various habitat types including landfills, ‘urban,’ and ‘natural’ areas (Table 2.1). However, the levels of human population density (Figure 2.2), urbanization, and other types of land use varied among regions (Figure 2.3), and thus, urban habitat types are over-represented in some regions (Table 2.1). Urban areas are comprised of beaches near high human population densities, as well as city parks. Natural habitats included beaches in areas with considerably lower human population densities, and less industrial activity. Landfills were sampled in the Lower Mainland, Greater Victoria, Southern Vancouver Island, and the Northern Salish Sea. Satellite imagery from the North American Land Change Monitoring System database was used to guide categorization of capture locations into ‘urban’ versus ‘natural’ habitat types (250 x 250 m resolution, North American Land Change Monitoring System, 2021) while human population density (people/km²) was obtained using census data, (Statistics Canada, 2017).

Additionally, 10 gulls were sampled on the west coast of Vancouver Island near the small towns of Ucluelet and Tofino for comparison with Salish Sea birds. Previous research has demonstrated that GWGUs on the west coast of Vancouver Island have a diet that is much richer in marine fish (Davis et al., 2015; Vermeer, 1982), with lower exposure to some contaminants than birds from the Salish Sea (Chen et al., 2012; Roscales et al., 2016), which could potentially elicit differences in physiology.

Table 2.1. Distribution of glaucous-winged gull capture location habitat types within each sampling region. Habitat types of capture locations were not differentiated for the gulls caught in the outgroup sampling area, the West Coast of Vancouver Island (n = 10). All values reported are sample sizes.

Region	Landfill	Urban	Natural	West Coast	Total
Lower Mainland	8	26	0	0	34
Greater Victoria	10	36	9	0	55
Southern Vancouver Island	7	24	5	0	36
Northern Salish Sea	8	37	23	0	68
West Coast	0	0	0	10	10

2.3.2. Data Collection

Research was conducted under Environment and Climate Change Canada (ECCC) Banding Permit #10667F, and ECCC Migratory Bird Sanctuary Permit #MM-BC-2020-0002. Animal use protocols were approved by ECCC's Western and Northern Animal Care Committee (21MH03), as well as the Simon Fraser University Animal Care Committee (protocol no. 1318B-20). All personnel completed mandatory Animal Care training.

Gulls were live-captured primarily using baited noose-mats (Liu et al., 2017), but occasionally pneumatic CO₂ net guns were used when bait was not an effective attractant (Edwards & Gilchrist, 2011). All gulls were photographed, aged, banded, and morphometric measurements including mass (± 20 g) and tarsus length (± 0.01 mm), were collected. Using a 27.5-gauge heparinized needle and syringe, we collected no more than 6 mL of blood (<1% of body weight) from each adult gull from the brachial vein of one wing (Sheldon et al., 2008). Using a non-heparinized lancet and capillary tube, approximately 20 μ L of whole blood was collected from the tarsal vein, then half was stored in 95% EtOH to determine gull sex, and the rest was used to determine glucose levels (mmol/L) in the field using a handheld glucose meter (Accu-check Aviva; Roche, Basel, Switzerland). Handling time was calculated for each individual and defined as number of minutes from capture time until blood collection was finished. To examine aerobic capacity, 5 μ L of fresh, whole blood was added to 1.25 mL of Drabkin's reagent (D5941 Sigma-Aldrich Canada, Oakville, Ontario, Canada) for estimation of hemoglobin and two capillary tubes were immediately filled with fresh whole blood, stored at 4°C for up to 6 hours before centrifuging for three minutes at 13,000 g, to determine hematocrit (Microspin 24; Vulcon Technologies, Grandview, Missouri, USA). The remaining blood was stored in a vacutainer at 4°C for up to 6 hours before it was centrifuged for 10 mins at 5000 rpm. Plasma (non-delipidated) was collected and stored at -20°C for up to 1 month and then at -80°C until assayed.

2.3.3. Laboratory analyses

Plasma triglyceride levels (mmol L⁻¹) were analyzed with a colorimetric assay according to the manufacturer's instructions (Sigma-Aldrich Co.) (Fowler & Williams, 2017). Hematocrit was measured using digital calipers (± 0.01 mm) and determined as a

percentage of packed red cell volume to total column height (plasma plus packed red cell volume; Williams et al., 2004). Hemoglobin was measured using the cyanomethemoglobin method (Drabkin & Austin, 1932) modified for use with a microplate spectrophotometer and absorbance read at 540 nm (Wagner et al., 2008). Total antioxidant titres (OXY; $\mu\text{mol HClO mL}^{-1}$) and reactive oxygen metabolites (dROMs; $\text{mg H}_2\text{O}_2 \text{ dL}^{-1}$) in the plasma were measured using OXY and dROMs kits, respectively, from Diacron International (Grosseto, Italy). OXY absorbances were read at 490 nm, and dROMs at 546 nm, using protocols modified after Guindre-Parker et al. (2013) and Casagrande et al. (2012), respectively. All assays were run using 96-well plates and a microplate spectrophotometer (BioTek Powerwave 340; BioTek Instruments, Winooski, Vermont). Inter-assay variation was 4.11% (triglycerides), 2.20% (hemoglobin), 9.41% (OXY), 10.57% (dROMs), and intra-assay variation was 6.74% (triglycerides), 1.60% (hemoglobin), 4.27% (OXY), and 6.69% (dROMs).

To determine gull sex, DNA was extracted from blood stored in 95% EtOH using a modified Chelex protocol (Burg & Croxall, 2001; Walsh et al., 2013). Individuals were sexed using the Z43BF/Z43BR Primer Pair (Dawson et al., 2016); the forward primer modified with M13 to allow incorporation of fluorescent marker to run on Licor gel. All PCR reactions were conducted in 10 μL reactions with 1 μL of genomic DNA. PCR cocktails contained 2.0 μL ClearFlexi Buffer 5x (Promega), 2.5 mM MgCl_2 , 200 μM dNTP, 1 μM each primer, 0.05 μM M13 primer, 0.5 units GoTaq (Promega). We used the following Thermocycler Conditions: 1 cycle of 30 seconds at 94°C; 35 cycles of 30 seconds at 94°C, and 45 sec at 55°C, and 45 seconds at 72°C, with a final extension for 5 minutes at 72°C, and 5 sec at 4°C. All PCR products were run on a 6% acrylamide gel. All gels included known positives (one male and one female) to maintain consistency across gels.

2.3.4. Data analysis

For quality control, samples with an intra-assay CV > 10% for replicates run in triplicate (hemoglobin and OXY), or CV > 12% if run in duplicate (triglycerides and dROMs), were re-assayed on a subsequent plate if no obvious outlier could be removed. A single mean value was calculated for each sample among all replicates once any obvious outliers were removed. We also investigated the relationship between plasma

colour and dROMs values, as colouration can potentially influence absorbance readings (Costantini, 2016). However, we did not observe issues with plasma colour when absorbances were read at 546 nm (Casagrande et al., 2020). For all physiological biomarkers measured, sample distributions were examined for normality and whether values were biologically plausible based on reference values for other gulls (Doussang et al., 2015; Laranjeiro et al., 2020; Minias, 2015; Newman et al., 1997). Implausible values were removed for hemoglobin ($n = 6$; > 24 g/dL) and OXY ($n = 1$; < 110 $\mu\text{mol HClO/mL}$). Log transformations were used for triglycerides, glucose, and dROMs (Fowler & Williams, 2017).

Covariation of sex, mass, and physiological traits

All analyses were performed using *R version 4.1.3* (R Core Team, 2022), with significance determined using an alpha level of 0.05. Pearson's correlation coefficients were used to examine pairwise relationships between the six physiological biomarkers measured, and to test the potential effect of handling time on each trait.

Glaucous-winged gulls are sexually dimorphic in mass (Hayward & Verbeek, 2020) and individual variation in body size and behavioural differences between sexes can impact physiological measurements in gulls (Doussang et al., 2015; Merklings et al., 2017; Minias, 2015; Newman et al., 1997). To address potential bias due to sex in our data, we first determined whether the ratio of females to males sampled was significantly different, a) among capture years and b) between years, using the Chi-squared test. We also tested whether mass significantly varied with gull sex using ANOVA.

Using linear mixed effects models, we determined whether significant variation in any of the six biomarkers measured was explained by a) sex, b) mass, c) sex + mass, or d) sex*mass. Free fatty acids in plasma can impact dROMs assay results and may need to be controlled for (Pérez-Rodríguez et al., 2015), so we additionally tested whether triglycerides, or any combination of triglycerides, sex, and mass explained significant variation in dROMs measurements. All models were run with year as a random effect, except for hemoglobin which was only measured in 2021. We used the Akaike Information Criterion for small sample sizes (AICc) to determine the model of best fit. For a given trait, if the model with the lowest AICc score included sex as a significant effect, sex was included as an interaction term with region or habitat in future models, whereas

mass as a significant effect was treated as a covariate only. If neither mass nor sex were significant, no covariate or interaction term was included.

Examining regional and habitat related variation in health

Next, we assessed whether each trait varied significantly by a) region or b) habitat type at capture. ANOVA and post-hoc Tukey tests for pairwise comparisons were performed using *lme4* (Bates et al., 2015). Year of capture was included as a random effect for all models, excluding hemoglobin which was only measured in 2021, to control for potential environmental variation between capture years. Covariates (mass, and/or triglycerides) and interaction terms (sex*region or sex*habitat, and sex*mass) determined previously by model selection were included as needed. Least-squares means were calculated using the *emmeans* package in *R Studio* (Lenth, 2022).

Principal components analysis (PCA) was used to examine the pattern of correlations and distributions of GWGU physiological biomarkers, and to provide indices of 'health' for gulls wintering in the Salish Sea. Additionally, PCA scores for individuals were used to compare physiological health among regions and habitat types of capture locations. Specifically, we tested whether individual scores from the first principal component (PC1) or the second principal component (PC2) varied significantly among region or habitat type using ANOVA and post-hoc Tukey tests for pairwise comparisons.

2.4. Results

A total of 202 adult glaucous-winged gulls were sampled in 2020 (n = 53) and 2021 (n = 149). Morphological traits including mass (g; n = 200) and tarsus length (mm; n = 199) were measured, in addition to triglycerides (mmol L⁻¹), glucose (mmol L⁻¹), hemoglobin (g dL⁻¹), hematocrit (packed cell volume %), OXY (μmol HClO mL⁻¹), and dROMs levels (mg H₂O₂ dL⁻¹; Table 2.2).

Table 2.2. Sample size, mean \pm standard deviation, and range for various body size and physiological biomarkers measured in glaucous-winged gulls sampled throughout the Salish Sea and the west coast of Vancouver Island, British Columbia. All values are untransformed.

Indicator of health:	Physiological Trait:	<i>n</i> =	Mean \pm Standard Deviation	Range
"Nutritional state"	Free triglycerides (g/mmolL)	156	1.23 \pm 0.82	0.28 – 5.42
	Glucose (g/mmolL)	152	14.9 \pm 3.46	9.3 – 29.1
"Aerobic capacity"	Hemoglobin (g/dL)	126	15.91 \pm 2.49	9.96 – 23.0
	Hematocrit (%)	151	46.4 \pm 4.4	31.8 - 58
"Oxidative status"	OXY (μ mol HClO/mL)	139	242 \pm 34	158 - 320
	dROMs (mg H ₂ O ₂ /dL)	151	2.38 \pm 1.51	0.43 – 10.3
"Body Size"	Mass	200	1022 \pm 142	775 - 1435
	Tarsus	202	68.8 \pm 3.7	59.9 – 78.1
	Mass/Tarsus "condition"	199	14.8 \pm 1.6	11.4 – 20.0

2.4.1. Covariation of sex, body mass, and physiological traits

Mass and all physiological biomarkers were independent of handling time, i.e. the time between initial capture and blood sampling (Table 2.3; $P > 0.05$ in all cases). Hematocrit and glucose were negatively correlated (Table 2.3; $r = -0.287$, $P < 0.001$). Several traits were positively correlated including triglycerides and glucose (Table 2.3; $r = 0.206$, $P = 0.01$), hemoglobin and hematocrit (Table 2.3; $r = 0.292$, $P < 0.001$), OXY and dROMs (Table 2.3; $r = 0.198$, $P = 0.02$), hematocrit and dROMs (Table 2.2; $r = 0.282$, $P < 0.001$), and triglycerides and dROMs (Table 2.3; $r = 0.246$, $P = 0.002$).

Table 2.3. Pearson’s correlation coefficients (r) for comparisons between all physiological traits, and between traits and handling time. Traits and variables measured include: mass (g), hemoglobin (g/dL), hematocrit (packed cell volume %), triglycerides (log(mmol L⁻¹)), glucose (log(mmol L⁻¹)), ‘OXY’ = total antioxidant titres (µmol HClO mL⁻¹), ‘dROMs’ = reactive oxygen metabolites (log(mg H₂O₂ dL⁻¹)), and handling time measured as minutes from time of capture until blood sample was collected.

Comparison		
	<i>r</i>	P value
Triglycerides vs Glucose	0.206	0.01
Triglycerides vs Hemoglobin	0.032	0.72
Triglycerides vs Hematocrit	-0.027	0.75
Triglycerides vs OXY	0.129	0.13
Triglycerides vs dROMs	0.246	0.002
Glucose vs OXY	-0.049	0.58
Glucose vs dROMs	-0.012	0.88
Hemoglobin vs Glucose	-0.004	0.97
Hemoglobin vs Hematocrit	0.292	<0.001
Hemoglobin vs OXY	0.064	0.51
Hemoglobin vs dROMs	0.064	0.49
Hematocrit vs Glucose	-0.287	<0.001
Hematocrit vs OXY	0.099	0.26
Hematocrit vs dROMs	0.282	<0.001
OXY vs dROMs	0.198	0.022
Handling time vs Hemoglobin	-0.111	0.32
Handling time vs Hematocrit	0.170	0.11
Handling time vs Triglycerides	0.037	0.73
Handling time vs Glucose	0.186	0.09
Handling time vs OXY	0.021	0.86
Handling time vs dROMs	0.047	0.67

Note: Significance was measured at the 95% confidence level and significant results are denoted in bold.

Sex was determined for 196 out of 202 birds sampled. The sex ratio of adult gulls captured in our study was significantly skewed ($X^2 = 28.6$; $DF = 1$; $P < 0.001$), with more females (69.0%; $n = 136$) sampled than males (30.1%, $n = 61$). The sex ratio also differed significantly between capture years ($X^2 = 9.57$; $DF = 1$; $P < 0.002$) with females representing 86.3% of birds captured in 2020 ($n = 44$ of 51), and 63.0% in 2021 ($n = 92$ of 146). This pattern appeared consistent among most regions and habitat type categories, but significant differences in sex-ratios between categories were not tested

due to small sample sizes among some region and habitat categories when divided by sex (Figure 2.5a and b; Tables 2.3 and 2.4). Body mass varied significantly by sex ($F_{1,192} = 207.4$; $P < 0.001$) with males (1173 ± 123 g) weighing more on average than females (951 ± 86 g; Figure 2.6a).

Table 2.4. Least square means and standard error for physiological traits by region of capture.

Trait	Region									
	West Coast		Northern Salish Sea		Southern Vancouver Island		Greater Victoria		Lower Mainland	
	Mean \pm SE	n =	Mean \pm SE	n =	Mean \pm SE	n =	Mean \pm SE	n =	Mean \pm SE	n =
Mass	1068 \pm 38	9	1048 \pm 19	67	1099 \pm 22	33	1049 \pm 15	53	1072 \pm 22	32
Triglycerides	-0.04 \pm 0.10	9	-0.03 \pm 0.06	53	0.02 \pm 0.06	29	0.02 \pm 0.04	38	-0.02 \pm 0.07	25
Glucose	1.2 \pm 0.1	9	1.2 \pm 0.1	52	1.2 \pm 0.1	30	1.2 \pm 0.1	36	1.2 \pm 0.1	24
Hemoglobin (region*sex + sex*mass)	15.3 \pm 0.9	9	15.2 \pm 0.5	41	14.4 \pm 0.6	23	14.6 \pm 0.6	25	15.6 \pm 0.6	23
Hematocrit	43.7 \pm 2.7	9	43.6 \pm 2.4	50	45.6 \pm 2.5	30	46.9 \pm 2.4	36	42.9 \pm 2.5	25
OXY	239 \pm 14	9	234 \pm 8	44	243 \pm 9	26	254 \pm 6	34	240 \pm 10	26
dROMs	0.34 \pm 0.08	10	0.28 \pm 0.05	50	0.37 \pm 0.06	30	0.41 \pm 0.05	38	0.30 \pm 0.07	23

Note: Triglycerides, glucose, and dROMs were log transformed. Models for all traits were run with year as a random effect, except for hemoglobin which was only sampled in 2020. Models for mass, triglycerides, hemoglobin, and dROMs included sex as an interaction term. Sex*mass was included as an additional interaction term for hemoglobin.

Table 2.5. Least squares means and standard error for physiological traits by habitat type of capture.

Trait	Habitat Type							
	West Coast		Natural		Urban		Landfill	
	Mean \pm SE	n =	Mean \pm SE	n =	Mean \pm SE	n =	Mean \pm SE	n =
Mass	1068 \pm 38	9	1022 \pm 23	36	1061 \pm 12	118	1103 \pm 23	31
Triglycerides	-0.037 \pm 0.099	9	0.021 \pm 0.062	34	-0.024 \pm 0.041	90	0.029 \pm 0.073	21
Glucose	1.16 \pm 0.06	9	1.20 \pm 0.05	30	1.20 \pm 0.05	90	1.20 \pm 0.5	22
Hemoglobin (habitat*sex + sex*mass)	15.9 \pm 0.9	9	15.8 \pm 0.7	24	14.8 \pm 0.4	68	16.5 \pm 0.7	20
Hematocrit	44.3 \pm 2.3	9	44.9 \pm 1.9	31	45.4 \pm 1.8	89	44.7 \pm 2.0	21
OXY	239 \pm 14	9	238 \pm 8	26	246 \pm 6	84	231 \pm 10	20
dROMs	0.349 \pm 0.09	10	0.369 \pm 0.06	31	0.347 \pm 0.06	88	0.270 \pm 0.07	22

Note: Triglycerides, glucose, and dROMs were log transformed. Models for all traits were run with year as a random effect, except for hemoglobin which was only sampled in 2020. Models for Mass, triglycerides, hemoglobin, and dROMs included sex as an interaction term. Sex*mass was included as an additional interaction term for hemoglobin.

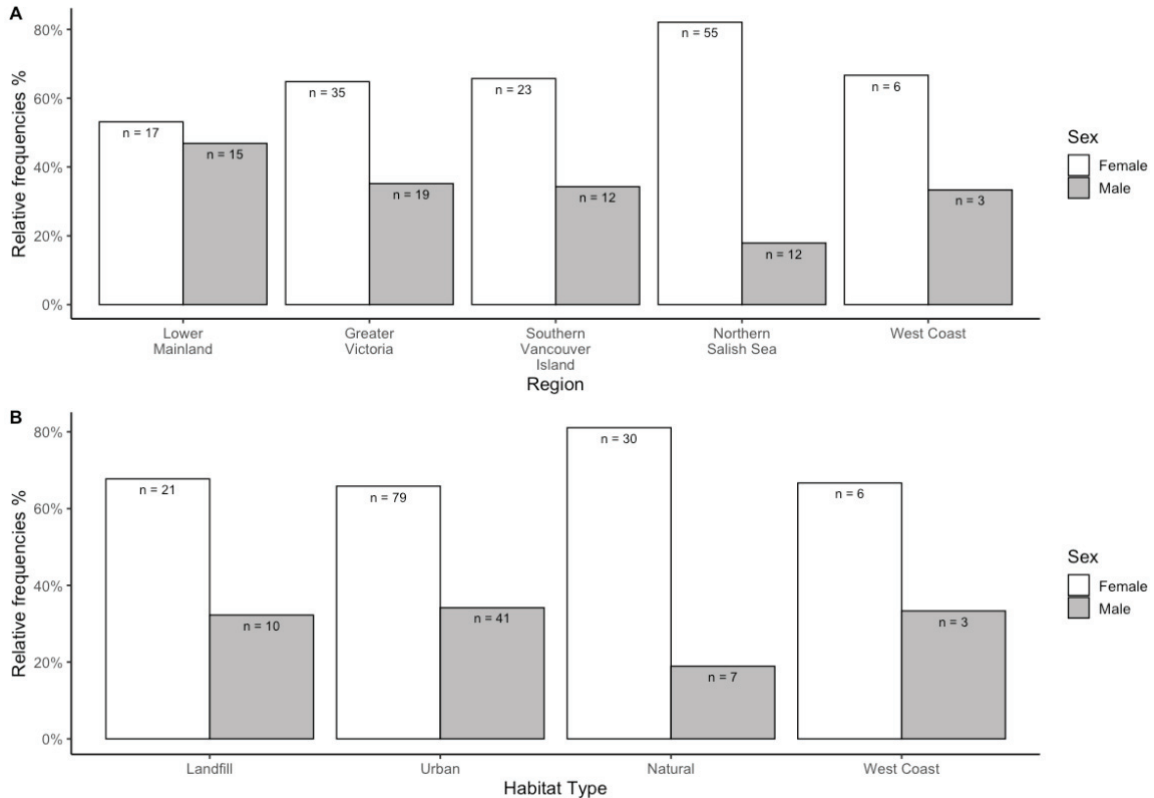


Figure 2.4. Relative frequency (%) of adult female and male glaucous-winged gulls captured throughout regions (A) and habitat types (B) of the study area in 2020 and 2021. Count total is stated at the top of each bar.

Body mass and plasma triglycerides both varied significantly with sex (Figure 2.6 a and c, respectively; Table 2.6) and thus we controlled for the variation due to sex by including it as an interaction term in ANOVAs testing variation of mass or triglycerides by region or habitat (Table 2.6). Likewise, hemoglobin varied significantly by sex (Figure 2.6 b). Additionally, it was the only biomarker which varied significantly with mass ($F_{1,119} = 5.00$; $P = 0.03$), and the best model for testing variation in hemoglobin included sex, mass, and a significant interaction between sex and mass (Table 2.6). dROMs levels varied significantly by sex (Figure 2.6 d), as well as triglycerides levels (Table 2.6), but the dROMs model with the lowest AICs score included only triglycerides as a covariate in ANOVAs testing the variation of dROMs levels by region or habitat type of capture locations (Table 2.6).

Table 2.6. Fixed effects from the best fit linear mixed effects models chosen by Akaike Information Criterion for small sample sizes (AICc).

Trait	Fixed effect	Estimate	DF	P value
Mass	Sex	222.10	192	<0.001
Triglycerides	Sex	-0.156	149	<0.001
Hemoglobin	Sex	-14.988	3,114	0.007
Hemoglobin	Mass	-0.009	3,114	0.04
Hemoglobin	Sex*Mass	0.0134	3,114	0.01
dROMs	Triglycerides	0.2253	141	0.003
Glucose	(Sex)	0.0133	143	0.39
Hematocrit	(Sex)	-0.879	143	0.24
OXY	(Sex)	-4.056	134	0.51

Note: Significance was measured at the 95% confidence level and significant results are denoted in bold. Year was included as a random effect in all models except hemoglobin which was only measured in 2021. Only significant fixed effects were included as terms in subsequent models testing variation of gull mass and physiological biomarkers by a) region or b) habitat. Significant effects of mass were included as covariates whereas sex was included as an interaction term with region or habitat. Glucose, hematocrit, and OXY did not have any significant fixed effects.

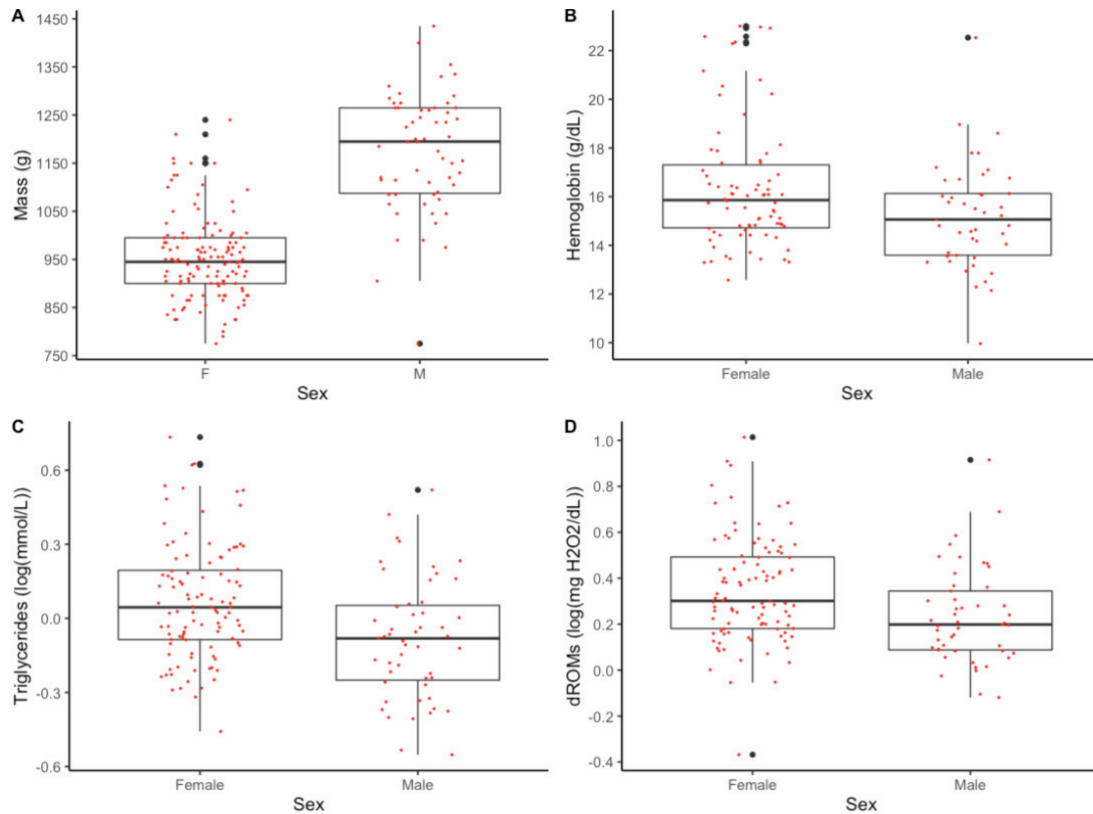


Figure 2.5. Morphological and physiological traits that differed significantly by sex of adult glaucous-winged gulls. Triglycerides and dROMs values were log transformed.

2.4.2. Variation in body mass and physiological traits by region

Body mass ($F_{4,184} = 1.46$; $P = 0.21$), hemoglobin ($F_4 = 1.0$; $P = 0.4$), triglycerides ($F_{4,144} = 0.3$; $P = 0.88$), glucose ($F_{4,146} = 1.3$; $P = 0.27$), and OXY levels ($F_{4,134} = 1.7$; $P = 0.16$) did not differ significantly between regions. However, hematocrit (packed cell volume; PCV%) varied significantly with region ($F_{4,142} = 5.4$; $P < 0.001$). Birds from the Greater Victoria region had significantly higher PCV% than birds captured in the Northern Salish Sea (Table 2.4; $P = 0.002$) and Lower Mainland (Table 2.4; $P = 0.002$). Additionally, birds from Southern Vancouver Island had nearly significantly higher PCV% than birds from the Lower Mainland (Table 2.4; $P = 0.08$). No other pairwise comparisons among regions were significant (Table 2.4; $P > 0.17$ in all cases). There was a marginally significant effect of region on dROMs ($F_{4,143} = 2.09$; $P = 0.085$) with birds in the Northern Salish Sea having nearly significantly lower dROMs levels than birds captured in the Greater Victoria region (Table 2.4; $P = 0.08$). All other pairwise

comparisons of dROMs among regions were non-significant (Table 2.4; $P > 0.4$ in all cases).

Principal components analysis was conducted on a subset of data, including only individuals for which all six physiological biomarkers were measured ($n = 94$). PC1 explained 27.0% of the variation among the physiological variables, and PC2 explained 24.3% of total variation. PC1 was positively influenced by dROMs, hematocrit, OXY, hemoglobin, and weakly by triglycerides, while glucose had a similarly weak but negative influence (Table 2.7). Conversely, PC2 was positively influenced by triglycerides, glucose, OXY, and dROMs, but negatively by hematocrit and hemoglobin (Table 2.7). Principal component scores of individuals for PC1, presumed to represent good overall health, did not differ significantly by region ($F_4 = 1.75$; $P = 0.15$). Similarly, PC2, a putative measure of nutritional status, did not vary significantly by region (Figure 2.6; $F_4 = 1.0$; $P = 0.4$).

Table 2.7. Loading scores for each variable determined by principal components analysis. PC = principal component.

Biomarker	PC1	PC2
Triglycerides	0.177	0.655
Glucose	-0.177	0.432
Hemoglobin	0.438	-0.199
Hematocrit	0.501	-0.436
OXY	0.393	0.302
dROMs	0.583	0.253

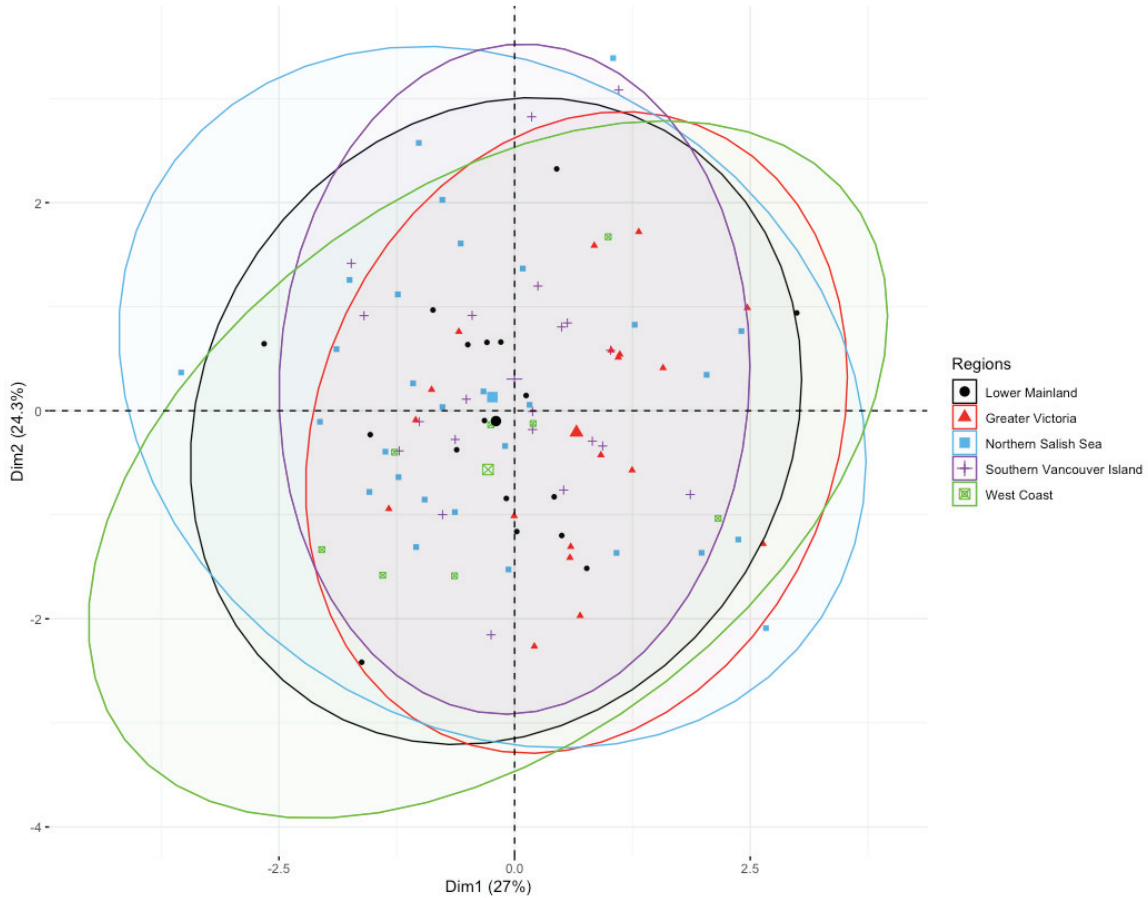


Figure 2.6. Biplot of principal components analysis (PCA) results for individual glaucous-winged gulls from each capture region. Individual positions on the plot are based on PCA conducted on six physiological measurements of health including: triglycerides, glucose, hematocrit, hemoglobin, OXY, and dROMs levels. Triglycerides, glucose, and dROMs values were log transformed before PCA was conducted. Dim1 = principal component 1; Dim2 = principal component 2. Circles represent 95% concentration ellipses.

2.4.3. Variation in body mass and physiological traits by habitat type

Body mass differed significantly with habitat type of capture location ($F_{3,186} = 2.80$; $P = 0.04$) after controlling for covariation in mass due to sex and the interaction between sex and habitat type. Birds captured in natural habitat areas had lower body mass (Table 2.5) than those captured at landfills (Table 2.5; $P = 0.03$), but no other pairwise comparisons among habitat types were significant ($P > 0.2$ in all cases).

Hemoglobin levels also varied significantly with the habitat type of capture locations ($F_3 = 3.68$; $P = 0.01$), with the largest, marginally non-significant, differences in hemoglobin levels between urban and landfill habitats (Table 2.5; $P = 0.05$). All other pairwise comparisons were non-significant ($P > 0.5$ in all cases). No other physiological traits varied significantly with habitat type: triglycerides ($F_{3,146} = 0.40$; $P = 0.75$), glucose ($F_{3,147} = 0.96$; $P = 0.41$), PCV% ($F_{3,143} = 0.33$; $P = 0.8$), OXY ($F_{3,1345} = 1.1$; $P = 0.34$), and dROMs ($F_{3,144} = 0.93$; $P = 0.42$). Similarly, principal components one (PC1; $F_3 = 1.0$; $P = 0.6$) and two (PC2; $F_3 = 0.68$; $P = 0.6$) did not differ significantly by habitat (Figure 2.7).

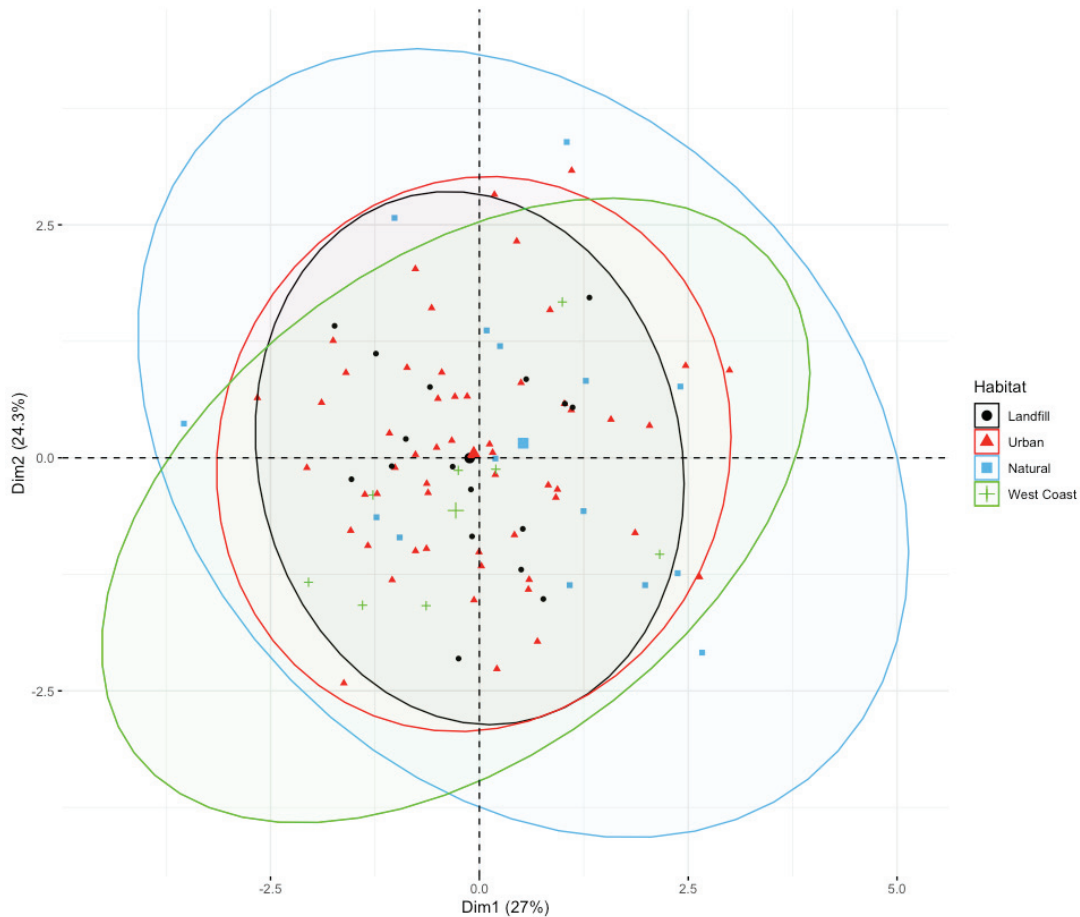


Figure 2.7. Biplot of principal components analysis (PCA) results for individual glaucous-winged gulls from each habitat type of capture. Individual positions on the plot are based on PCA conducted on six physiological measurements of health including: triglycerides, glucose, hematocrit, hemoglobin, OXY, and dROMs levels. Triglycerides, glucose, and dROMs values were log transformed before PCA was conducted. Dim1 = principal component 1; Dim2 = principal component 2. Circles represent 95% concentration ellipses.

2.5. Discussion

In this study, we used a physiological approach to assess the health of a declining generalist seabird species, the glaucous-winged gull, wintering in the biologically important coastal Salish Sea ecosystem. We established reference values for six health biomarkers, providing a foundation for long-term physiological monitoring in relation to future anthropogenic impacts in this region. There were positive correlations between plasma triglycerides and glucose levels, and hemoglobin and hematocrit, indicating similarity in biomarkers representing nutritional and aerobic status, respectively. Additionally, a positive relationship between antioxidant capacity (OXY) and reactive oxygen metabolites (dROMs) suggests most individuals were capable of counteracting oxidative damage by upregulating antioxidant levels and achieving oxidative balance. Sex was an important covariate for body mass, plasma triglycerides, hemoglobin, and dROMs (when triglyceride levels were not accounted for). Nevertheless, despite marked individual variation in, and covariation among, physiological traits, regions and habitat types where birds were captured did not predict variation in health biomarkers even though these were characterized by varying levels of urban development and anthropogenic activity. However, the general consistency in biomarker ranges for GWGUs throughout the Salish Sea and west coast of Vancouver Island should allow future monitoring efforts to assess the impacts of potentially localized threats to health, such as oil spills, in comparison with GWGUs from unaffected regions. Our analysis of an overall “index of health,” using principal components analysis, similarly did not reveal clustering of individuals with differing health by region or habitat type of capture sites. However, variation in overall health indices among individuals was best explained in PC1 by strong and positive factor loadings by biomarkers representing aerobic capacity (hemoglobin and hematocrit) and oxidative status (OXY and dROMs), and in PC2 by strong, positive loadings of nutrition and energy status biomarkers (triglycerides and glucose) and negative loadings by aerobic capacity biomarkers. Although GWGUs are quite faithful to a regional area throughout winter, habitat specialization does not appear to be widespread in the population (Chapter 1). The generalist nature of this species, as well as the large geographic coverage and widespread anthropogenic activity among our region categories, may limit the utility of ‘capture region’ or ‘habitat type’ to provide explanations for variation in GWGU biomarkers and overall health. However, future investigations into relationships between

diet, contaminant loads, parasitic infections, and physiological health could provide insight into individual variation in GWGU health.

Informed by widespread and large sample sizes in our study, we found biomarkers measured in non-breeding glaucous-winged gulls were mainly consistent with published ranges for GWGUs (Hughes et al., 1993; Mahoney & Jehl, 1985; Newman et al., 1997) and other gull species (e.g. Costantini et al., 2019; D'Amico et al., 2018; Doussang et al., 2015; Fox et al., 2007; Garcia et al., 2010; Indykiewicz et al., 2019; Kim et al., 2013; Laranjeiro et al., 2020; Leclaire et al., 2015; Lopes et al., 2022; Marteinson & Verreault, 2020; Minias et al., 2019; Mostaghni et al., 2005; Pekarik et al., 2016; Rubolini et al., 2006; Saino et al., 2011; Totzke et al., 1999; Valle et al., 2020). However, plasma triglycerides levels were relatively low in our population (1.23 ± 0.82 mmol/l) compared with breeding GWGUs in Alaska (9.1 ± 9.9 mmol/L; Newman et al., 1997) and other adult gulls (Fox et al., 2007; Garcia et al., 2010; Indykiewicz et al., 2019). Likewise, glucose levels in our study (14.9 ± 3.46 mmol/l) were lower than in breeding GWGUs (17.8 ± 3.3 mmol/l; Newman et al., 1997). This may suggest that non-breeding GWGUs are experiencing less nutritional stress or lower energy expenditure compared to breeding birds (Minias et al., 2019; Totzke et al., 1999), or that these physiological markers are upregulated in breeding GWGUs, for example due to reproduction itself such as the presence of lipid-rich yolk precursors during egg-laying (Williams, 2012).

There were weak to moderate positive relationships between the six different physiological traits corresponding to nutritional/energy state, aerobic capacity, and oxidative status (Table 2.7). As hemoglobin is the oxygen-carrying protein found in red blood cells, and hematocrit measures the volume of red blood cells, higher levels of both imply higher aerobic capacity and ability to maintain higher workloads, although very high hematocrit levels may also represent dehydration (Williams, 2012). Likewise, metabolites such as glucose and triglycerides can provide insight into the short- and medium-term nutritional state of an individual, respectively, and can be indicative of recent dietary intake or food consumption (Morales et al., 2020). Although higher plasma triglyceride levels can indicate fattening, i.e. excess energy availability, (Lyons et al., 2008; Williams et al., 1999), high glucose levels can be an indication of nutritional stress or high energy expenditure, and therefore poorer health (Minias et al., 2019). Additionally, low hematocrit levels have been linked with increased glucose mobilization

(Yap et al., 2018), which may explain the negative relationship we found between glucose and hematocrit. However, we found a positive correlation between dROMs levels and hematocrit. This suggests high hematocrit may not be a positive indicator of health in GWGUs. The negative correlation between hematocrit and glucose might be better interpreted as birds with high hematocrit having less available energy. Finally, poor diet, contaminant burdens, infections, and physical exercise can lead to oxidative damage in birds (Isaksson, 2015), but the positive relationship between OXY and dROMs suggests most GWGUs sampled were able to avoid oxidative damage by upregulating their antioxidant capacity to achieve oxidative balance (Monaghan et al., 2009). However, there may still be costs to producing more antioxidants to overcome oxidative damage (Saino et al., 2011).

There was a profound female-biased sex ratio (69.0%) among all birds sampled in our study and this appeared generally consistent throughout all regions and habitat types. There is little other information available on sex-ratios in this partially migratory species, especially during the non-breeding period (Hayward & Verbeek, 2020). The sex-ratio could reflect more females in the adult population (higher male mortality), or a higher proportion of females utilizing the Salish Sea for overwintering (with males wintering at other locations). However, we cannot rule out the possibility that our capture methods or sites (i.e. land-based only) may have biased the sex ratio in our sampling.

As found in Alaskan breeding GWGUs (Newman et al., 1997), non-breeding individuals displayed sexual dimorphism in mass, with males weighing more than females. We found hemoglobin levels were higher in female GWGUs, in contrast to studies of other gulls where either no difference was reported between sexes (Doussang et al., 2015; Indykiewicz et al., 2017) or where hemoglobin was higher in males (Minias et al., 2019). Females also had higher plasma triglyceride levels than males, as found in breeding GWGUs and other gulls (Garcia et al., 2010; Indykiewicz et al., 2017; Newman et al., 1997), and higher dROMs levels, which have not previously been seen in gulls (Leclaire et al., 2015; Saino et al., 2011). Although we observed higher dROMs in females, a meta-analysis by Costantini (2018) found similar resistance to oxidative stress in birds regardless of sex. Nevertheless, it is important that any future physiological monitoring of health in this, and perhaps other, species should take sex into account.

Regional differences in anthropogenic activity and degree of urbanization can impact gull resource use, through both prey quality and availability (e.g. Duhem et al., 2008; Furst et al., 2018; O'Hanlon et al., 2017; O'Hanlon & Nager, 2018), and exposure to contaminants (e.g. Martenson & Verreault, 2020; Peterson et al., 2017; Roscales et al., 2016; Sorais et al., 2020; Thorne et al., 2021; Thorstensen et al., 2021; Vicente et al., 2012; Viñas et al., 2020). Like other gulls, GWGUs are generalist foragers that utilize a variety of urban and natural habitats (Davis et al., 2015; Hatch et al., 2011; Ward, 1973). The Salish Sea is considered a highly disturbed ecosystem and experiences greater levels of anthropogenic activity than on the west coast of Vancouver Island. Regional differences in diet suggest that relying more heavily on non-marine or low trophic-level prey corresponds with lower reproductive success (Blight, 2011; Blight et al., 2015), as well as differing exposure to contaminants (Chen et al., 2012; Gebbink et al., 2011), compared with GWGUs on the west coast of Vancouver Island (Davis et al., 2015, 2017; Vermeer, 1982; Ward, 1973). Despite this, we did not find strong differences in physiological variation among Salish Sea regions or the west coast. However, the small sample size ($n = 9$) and proximity to human activity at west coast capture sites may confound our results somewhat. Hematocrit levels varied among regions, with individuals sampled in the Greater Victoria region having higher hematocrit than individuals in the Northern Salish Sea or Lower Mainland. Though marginally non-significant, birds caught in the Greater Victoria area also had higher dROMs levels compared with birds in the Northern Salish Sea. Anemia (i.e. low hematocrit) has been linked with oil exposure in seabirds (Scoville & Doherty, 2017) and some parasitic infections (Fair et al., 2007; Minias, 2015), while high hematocrit can also indicate poor health due to dehydration (Williams, 2012). However, it has been suggested that hemoglobin is a more reliable indicator of condition than hematocrit alone (Minias, 2015), and we did not find any significant differences in hemoglobin levels in relation to region of capture.

In contrast, hemoglobin was the only biomarker that varied significantly among habitat types (after accounting for other covariates). Though only marginally non-significant, gulls sampled at landfills had higher hemoglobin levels on average than those from urban areas. We also found that body mass was higher in individuals sampled at landfills than natural areas. Higher hemoglobin and body mass suggest landfill use may be associated with better condition in non-breeding GWGUs. Landfill

use has been associated with population growth (Duhem et al., 2008), higher body condition (Auman et al., 2008; Steigerwald et al., 2015), higher reproductive output (Weiser & Powell, 2010) and reduced foraging effort (Langley et al., 2021) in some gull species. However, in other studies positive reproductive outcomes are more strongly related to diets high in marine prey (Bennett et al., 2017; O'Hanlon et al., 2017; Real et al., 2017). Landfill and highly urban foraging has also been linked to both higher (Lopes et al., 2021; Seif et al., 2018; Sorais et al., 2020) and lower (Peterson et al., 2017; Thorne et al., 2021) exposure to various contaminants, exposure to antimicrobial resistant bacteria (Ahlstrom et al., 2019; Alm et al., 2018), and potentially lower winter survival (Anderson et al., 2019). Additionally, diets reliant on human foods have been reported to affect some aspects of gull physiology, with potential adverse effects (Marteinson & Verreault, 2020). For instance, yellow-legged gulls in highly urban environments had poorer nutritional quality with lower levels of omega 3's in their plasma (Lopes et al., 2022), which can cause inflammation induced increases in oxidative stress (C. Isaksson, 2015).

Previous studies have suggested that winter landfill use is important to migratory GWGUs (Hatch et al., 2011; Ward, 1973) and Ward (1973) speculated that access to landfills in the Salish Sea may increase adult survival during this period. Conversely though, he found that in the breeding season, chick fledging success increased with high trophic level marine prey in the diet (Ward, 1973). In contrast to the 1970s, contemporary waste management practices in the Salish Sea now divert much food waste to composting facilities. Additionally, three out of the four landfills we sampled use falconry and other effective methods to deter gulls from the active face of landfills, resulting in reduced foraging opportunities at these sites compared with previous decades. Belant et al. (1993) found that when higher quality food was available elsewhere, herring gulls were mainly engaged in socializing and loafing at landfills, as opposed to foraging. It is possible that wintering GWGUs gain better condition from landfill use, through increased resource access and reduced foraging effort. However, potential individual specialization has not yet been investigated in this species and capturing a gull at a landfill does not necessarily signify its previous foraging habits. Alternatively, landfills may be sought by GWGUs with better condition primarily for social reasons. Linking dietary information (e.g. using stable isotope analysis) and information

on contaminants with physiological biomarkers, as well as increased knowledge of GWGU habitat use (via radio-telemetry studies), will better resolve these questions.

The goal of our use of principal components analysis was to provide an overall index of health (Fowler & Williams, 2017; Travers et al., 2010) by assessing covariation among all six physiological biomarkers measured. The first principal component explained 27% of the variation among overall health in individuals and was driven positively by aerobic capacity and oxidative status biomarkers. Hematocrit and hemoglobin have been reported to increase with workload (Yap et al., 2019) and increased physical exercise can lead to the production of more reactive oxygen species which would require greater antioxidant production to mitigate oxidative stress (C. Isaksson, 2015). Conversely, our second principal component was driven positively by nutritional biomarkers, which were inversely related to aerobic capacity biomarkers. This may indicate that birds with higher nutrition and energy state biomarker levels are not expending as much energy to forage and thus do not need higher aerobic capacity or that high glucose and triglyceride levels are not conducive to high aerobic capacity. However, hematocrit may not be a positive indicator of health at extremes of the range which complicates interpretation. Overall, the first two principal components only explained ~50% of the variation in the physiological biomarkers we measured. However, the PCA results mirror our findings using univariate analysis, as individuals with similar overall health indices did not cluster by region or habitat type, suggesting that due to the generalist habitat use observed in this species (Chapter 1), broad regions or habitat types based on capture sites are not useful in explaining individual variation in gull health in Salish Sea.

Our study provides a foundation for long-term physiological monitoring of health of a key indicator species, glaucous-winged gulls, for which Canada has regional stewardship responsibility, and within the Salish Sea, a biologically important coastal ecosystem that will be increasingly impacted by human activity. Our physiological reference values provide a baseline to compare with future responses to environmental stressors in this species and should enable the rapid detection of emerging threats to glaucous-winged gull health and population status. Further work combining this physiological approach to health with analyses incorporating diet (from stable isotope analysis), contaminant load, infection, and habitat use information (from GPS tag studies) will help tease apart the complex environmental and behavioural factors driving

variation in health and better understand the impacts of human activities in the Salish Sea Ecosystem.

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