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Une analyse comparative des paramètres physiologiques de santé entre les différentes classes d'âge chez un oiseau marin jouant le rôle de sentinelle

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A COMPARATIVE ANALYSIS OF PHYSIOLOGICAL HEALTH PARAMETERS ACROSS AGE CLASSES IN A SENTINEL SEABIRD WINTERING ALONG THE CANADIAN WEST COAST

EXERCISE THESIS

to obtain the title of
VETERINARY DOCTOR

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the Paul Sabatier University of Toulouse*

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TABLE OF CONTENTS

List of Figure	7
List of Table.....	8
List of Acronyms... ..	9
Résumé du manuscrit en Français	10
INTRODUCTION	13
1 MATERIALS AND METHODS	18
1.1 Study Area.....	18
1.2 Study population and standard field procedures for capture and sampling GWGU .	18
1.2.1 Data collection	18
1.2.2 Age determination.....	19
1.3 Bird health assessment	19
1.3.1 Aerobic capacity	19
1.3.2 Oxidative status	20
1.3.3 Data quality control	20
1.4 Statistical analyses and choice of variables	20
1.4.1 Covariation of handling time, size-corrected body mass, and physiological traits	21
1.4.2 Examining age related variation in health	22
2 RESULTS	24
2.1 Covariation of handling time, size-corrected body mass, and physiological traits ...	24
2.2 Examining age related variation in health and body measurement.....	26
3 DISCUSSION	30
3.1 Covariation of handling time, size-corrected body mass, and physiological traits ...	30
3.2 Examining age related variation in health and body measurement.....	31
4 PERSPECTIVES	33
REFERENCES	34
APPENDIX	45

LIST OF FIGURES

Figure 1: Number of individuals captured by group of age in each region.	22
Figure 2: Scatter plot of glucose that differed significantly by handling time. Glucose values were log transformed.	26
Figure 3: Morphological and physiological traits that differed significantly by age of Glaucous-winged gulls.	27
Figure 4: Biplot of principal components analysis (PCA) results for individual glaucous-winged gulls from each age group. Individual positions on the plot are based on PCA conducted on five physiological measurements of health including: glucose, hematocrit, hemoglobin, OXY, and d-ROMs levels.	29
Figure 5: Variable correlation plots of components analysis (PCA) results for individual glaucous-winged gulls from each age group. Showing the relationship between each five physiological measurements of health including: glucose (gluclog), hematocrit (xhct), hemoglobin (hb), OXY, and d-ROMs (dromlog) levels.	29

LIST OF TABLES

Table 1: Number of individuals captured by group of age in each region.....	22
Table 2: Sample size, mean \pm standard deviation, and range for body size and physiological traits measured in GWGU sampled throughout coastal British Columbia.	24
Table 3: Fixed effects from the best fit linear mixed effects models chosen by Akaike Information Criterion for small sample sizes (AICc).....	25
Table 4: Pearson correlation coefficients (r) for comparisons between all physiological traits and with handling time and size-corrected body mass. Traits and variables measured were size-corrected body mass, hemoglobin (g/dL), hematocrit (% PCV), glucose (log(mmol L-1)), "OXY" = total antioxidant titers (μ mol HClO mL-1), "dROMs" = reactive oxygen metabolites (log(mg H2O2 dL-1)), and handling time (min). ...	25
Table 5: Least squares means and standard error for physiological traits by Age of GWGU captured.	28
Table 6: Coordinate score for each variable determined by principal components analysis. ..	28

LIST OF ACRONYMS

AIC	Akaike's Information Criterion
AICc	Akaike's Information Criterion corrected for small sample sizes
ANOVA	Analysis of Variance
B.C.	British Columbia
CV	Coefficient of variation (%)
dROMs	Reactive oxygen metabolites
ECCC	Environment and Climate Change Canada
GWGU	Glaucous-winged gull
Hb	Hemoglobin
Ht	Hematocrit
OXY	Total antioxidant titres
PC1	First principal component
PC2	Second principal component
PCA	Principal components analysis
PCV	Packed cell volume (%)
POCT	Point-of-care testing
ROS	Reactive oxygen species
SFU	Simon Fraser University
TMX	Trans Mountain pipeline
USA	United States of America
WC	'West Coast' of Vancouver Island

RESUME DU MANUSCRIT EN FRANCAIS

La mer des Salish abrite des écosystèmes écologiquement riches, d'importance mondiale pour de nombreuses espèces hivernantes et reproductrices (Crewe et al., 2012). En hiver, par exemple, le littoral devient un sanctuaire où les oiseaux marins migrateurs se nourrissent et se reposent (Gaydos & Pearson, 2011). Cette région est également importante pour les espèces culturellement emblématiques, telles que l'Orque résident du sud (Hanson et al., 2021).

Toutefois, cette côte maritime est soumise à une pression croissante en raison de l'activité humaine. Au sein de cette région réside notamment le port de Vancouver, qui est le plus grand port du Canada (Cargo and Terminals, 2015). Le gouvernement canadien est conscient que ces pressions ont déjà un impact sur la santé des écosystèmes ainsi que sur les populations humaines et animales en raison de la perte d'habitat (Reise, 2005; Yan et al., 2021), de la pollution (Cabral et al., 2019), de la modification des paysages (Bulleri & Chapman, 2010) et du changement climatique (Harley et al., 2006).

Cependant, d'autres pressions anthropiques sont attendues dans cette région, avec notamment le projet d'extension de l'oléoduc Trans Mountain (TMX) qui a débuté en 2019. Cela multipliera par sept environ le trafic de pétroliers dans la mer des Salish (Jonasson et al., 2019; Short, s. d.; Trans Mountain - Expansion Project, s. d.).

La conservation de la mer des Salish est primordiale, néanmoins les efforts de gestion des écosystèmes marins nécessitent l'amélioration des cadres prédictifs (Bernhardt & Leslie, 2013; Harley et al., 2006).

Tout d'abord, à cet égard, les espèces sentinelles sont de bons modèles pour le suivi des changements environnementaux. Elles sont en mesure de fournir des données sur l'exposition à ces perturbateurs et les effets sur la santé, car elles sont elles-mêmes sensibles à ces perturbations (Beeby, 2001; Moore & Kuletz, 2019; O'Brien et al., s. d.; Valli, 2001). Ainsi, le "Salish Sea Gull Project" a été mis en place pour surveiller la santé de ces écosystèmes côtiers, utilisant le Goéland à ailes grises (*Larus glaucescens*, GWGU) comme indicateur. En effet, ce Goéland est l'espèce de *Laridae* la plus abondante dans la mer des Salish, et est présent toute l'année. Cette espèce est généralistes et occupe les habitats côtiers dans les zones urbaines et naturelles.

Par ailleurs, pour mieux prévoir ces changements, la physiologie de la conservation fournit des outils utiles pour évaluer les indicateurs sous-jacents de la santé chez les individus et les populations d'animaux sauvages et détecter des anomalies précocement. Cependant, on sait peu de choses sur les valeurs de base des paramètres physiologique en lien avec la santé des GWGU

pendant la période d'hivernage. Nos connaissances sont encore moins développées en ce qui concerne l'influence de l'âge sur ces paramètres. Or, ces connaissances semblent importantes pour le suivi à long terme et la conservation de cette population.

Ce projet, cherche alors à établir des intervalles de référence des paramètres physiologiques de la population de GWGU, de nos jours, avant la mise en service des nouveaux projets industriels susceptibles d'impacter la santé des écosystèmes dans les années futures. Ainsi, à l'avenir, lors du suivi à long terme, nous pourrions nous servir de ces intervalles de référence pour évaluer la modification de l'état de santé subclinique de la population des GWGU. En effet, cette surveillance nous permettra de détecter des anomalies, associées à des processus pathologiques potentiels, avant même que les signes cliniques ne soient visibles (Cooke et al., 2021). À terme, cela contribuera à détecter plus précocement l'impact de ces nouveaux facteurs de stress sur la santé des populations et des écosystèmes côtiers de la Colombie-Britannique.

Afin d'avoir des données d'autant plus précises pour cette surveillance, nous nous sommes demandé, dans cette étude, si la variation des paramètres physiologiques individuels pouvait être liée à l'âge. En étudiant notamment les paramètres physiologiques tel que l'état nutritionnel, l'état aérobie, l'état oxydatif et également la condition physique. Cette question, nous permettra de déterminer s'il faut établir un intervalle de référence distinct pour certaines classes d'âge, afin d'obtenir une détection plus sensible et précoce de la modification de l'état de santé subclinique de la population de cette sentinelle.

Notre interprétation des résultats est que ce sont surtout les individus de premier hiver, c'est à dire les juvéniles, qui présentent un état aérobie et oxydatif plus faible que les autres groupes d'âge. Cette variation semble montrer que les juvéniles ont une santé subclinique, concernant les paramètres physiologiques, moins bonne que les subadultes et les adultes. Ces résultats semblent cohérents avec la littérature scientifique. En effet, cela peut être dû au fait qu'ils sont encore en train d'apprendre à se nourrir, et qu'ils sont plus souvent obligés de se contenter de nourriture humaine, plus facile à obtenir (Greig et al., 1983). Ils semblent également avoir un système immunitaire plus vulnérable et sont plus sensibles aux maladies et au parasitisme, qui peuvent être très coûteux et entraîner des taux de mortalité élevés chez les jeunes (Sol et al., 2003).

L'inclusion de l'échantillonnage des GWGU du premier hiver dans le protocole de surveillance de la santé de la mer des Salish semble donc être d'un intérêt majeur. En effet, dans un contexte d'utilisation de sentinelles, ces jeunes oiseaux semblent être plus vulnérables que les autres classes d'âge, ce qui les rendrait plus sensibles aux perturbations. Ils sont donc susceptibles d'être les premiers à montrer des signes physiologiques de l'impact d'un changement

défavorable. Au cours des trois premières années de cette étude, seule les paramètres de physiologie en lien avec la santé des GWGU adultes ont été évaluée. Cependant, au vu de nos résultats, il serait intéressant d'établir des intervalles de référence pour les paramètres physiologiques des juvéniles également. Ceci est en accord avec l'objectif du programme "Salish Sea Gull Project", qui cherche à effectuer un suivi de la santé des écosystèmes côtiers de la Colombie-Britannique, afin de détecter les perturbations liées aux facteurs de stress environnementaux à un stade précoce.

INTRODUCTION

Coastal oceans are home to a multitude of living marine resources and are highly productive ecosystems. However, the rapid environmental changes driven by various natural and anthropogenic pressure require the development of a new set of tools to monitor changes in ecosystem dynamics. In this context, seabirds are increasingly important sentinels for monitoring the health of these ecosystems and enhancing management plans. Indeed, coastal environments are increasingly challenged by the development of human activities (e.g., fishing, commercial maritime traffic, or leisure activities) (Ansari & Matondkar, 2014; Halpern et al., 2008), the modification of the coastal and maritime landscape (Bulleri & Chapman, 2010), climate change (Harley et al., 2006), the loss of natural habitats (Reise, 2005; Yan et al., 2021), as well as maritime pollution (Cabral et al., 2019). The accumulation of such pressures and their interactions has a major impact on the equilibrium and resilience of coastal ecosystems (Reise, 2005; Yan et al., 2021), an effect exacerbated by the sharp decline in species diversity (Worm et al., 2006). As a result, the economic and social systems that depend upon marine environments can be severely undermined by the depletion of resources and their quality (Paul, 2004; Pontavice et al., 2020; Shahidul Islam & Tanaka, 2004). Efforts to manage and conserve marine ecosystems require improvements in predictive frameworks, i.e. better forecasting of changes to better understand the impacts they may have on populations, and integrating the latter's capacity to adapt. (Bernhardt & Leslie, 2013; Harley et al., 2006).

In this respect, sentinel or key indicator species are good models for monitoring of environmental change. They are able to provide integrated data on exposure and health effects, as they are sensitive to these disturbances themselves (Beeby, 2001; Moore & Kuletz, 2019; O'Brien et al., s. d.; Valli, 2001).

Seabirds are an integral ecological component of coastal and marine ecosystems, feeding at a high trophic level and interacting extensively with the food web over a wide geographical area (Sergio et al., 2008). In addition, they are generally long-lived, ubiquitous, and exposed to all threats affecting the oceans, making them particularly useful indicator species for monitoring changes in ecosystem health (Mallory et al., 2010; Cairns, s. d.; Piatt & Sydeman, 2007; Hipfner, 2008). In particular, seabirds have often been used to monitor pollution by contaminants, since they bioaccumulate contaminants with age (Burger & Gochfeld, 2004; Provencher et al., 2014). For example, when plastic pollution in the oceans has been investigated, seabirds have also been used to assess the level of plastic pollution in a given geographical area by examining the debris used by seabirds to build their nests (Lato et al., 2021; Votier et al., 2011), or by assessing plastic ingestion during necropsy (Avery-Gomm et

al., 2018; Linnebjerg et al., 2021). Seabirds have also frequently been studied to monitor other marine resources (Cairns, s. d.; Iverson et al., 2007) and to raise concerns about overfishing, in order to protect important marine areas (Maxwell & Morgan, 2013). Seabirds are also important indicators of ecosystem health and equilibrium in the context of climate change (Wolf et al., 2010). Thus, long-term studies monitoring seabird populations' demography, health, or reproductive success provide an index of marine ecosystem responses to perturbation.

Canada's Pacific West Coast is one of those coastal and marine landscapes whose waters are highly productive and biologically diverse, but which face increasing human-induced pressures and challenges. The Salish Sea and the Pacific North Coast are areas with significant ecological values that set them apart. The Salish Sea is a protected body of water located in southern British Columbia (BC). It stretches from the Strait of Georgia to Puget Sound and the Strait of Juan de Fuca, where it meets the Pacific Ocean and serves as a natural border between Canada and the US. (Appendix 1.). The Pacific North Coast area extends from the Canada-U.S. border of Alaska in the north, including Haida Gwaii archipelago, to northwest Vancouver Island (Brooks Peninsula) and Quadra Island in the south (Appendix 2.). Canada's west coast waters are home to a diverse range of marine life, making them a crucial habitat for wildlife. This productive area is a globally significant location for breeding and wintering birds (Crewe et al., 2012). Particularly in winter, the shores become a sanctuary for migratory seabirds to feed and rest, including Marbled Murrelet (*Brachyramphus marmoratus*), Western Grebe (*Aechmophorus occidentalis*), and a diversity of gulls (Gaydos & Pearson, 2011). These waters are rich and present strategic points of migration also for marine species such as killer whales, some of which have become resident (Hanson et al., 2021), and Pacific salmon (Quinn & Dittman, 1990). The Canadian government is aware that these pressures are already impacting the west coast of British Columbia and that the health of Canada's Pacific waters is deteriorating, as are their resources and their ability to provide other essential services to humans and other forms of life (Ban et al., 2010; Laranjeiro et al., 2020). Indeed, within this region lies the Port of Vancouver, Canada's largest and North America's most diversified port (Cargo and Terminals, 2015). Activities carried out in the port are likely to affect the Salish sea health. However, further anthropogenic changes are expected in this region with the Trans Mountain (TMX) pipeline expansion project. The expansion of the TMX network is represented by the construction of a new twin pipeline to the one built in 1953. Construction of this new pipeline began in late 2019 and is scheduled for completion in 2024. This pipeline runs from Edmonton, Alberta, to the Westridge marine terminal and Chevron refinery, both in Burnaby, British Columbia (Canada, 2022)(Appendix 3.). This expansion will increase the pipeline's capacity from 300,000 to 890,000 barrels per day (Canada, 2022), which will increase tanker

traffic in the Salish Sea by around sevenfold (Jonasson et al., 2019; Short, s. d.; Trans Mountain - Expansion Project, s. d.) and may have a huge impact on the health of BC's maritime coasts. Currently, Environment and Climate Change Canada (ECCC) has a mandate, as part of the Oceans Protection Plan, to assess the threats posed to wildlife by new and proposed industrial projects (Public Statements on Strategic Environmental Assessments, 2011). The Government of Canada is undertaking the development and implementation, with industry support, of a seabird monitoring and protection program for the Salish Sea. This program is called “Salish Sea Gull Project” and started in winter 2020. This program is led by Dr. Mark Hipfner (ECCC) in collaboration with Dr. Tony Williams (Centre for Wildlife Ecology, Simon Fraser University), Dr. Theresa Burg (University of Lethbridge), and researchers from ECCC’s Science and Technology Branch. This Program has been made to measure the survival rates, movements, habitats, diets, and contaminant levels of gulls across the Salish Sea.

The purpose of the current project is to study the long-term impacts of these new anthropogenic pressures, notably the TMX pipeline, and monitor changes in the health of coastal ecosystems in British Columbia. To assess the health of a marine ecosystem under heavy anthropogenic pressure, gulls (*Laridae* family) are often used as sentinels (Davis et al., 2017; Laranjeiro et al., 2020). In fact, in habitats heavily modified by humans, gulls use marine and terrestrial food subsidies (Isaksson, 2015; Davis et al., 2017), and also use natural and human-built structures for roosting and nesting (Blight et al., 2019). The most abundant gull on the British Columbia coast is Glaucous-winged Gulls (*Larus glaucescens*), which is a year-round resident. Canada also has a regional stewardship responsibility to Glaucous-winged Gulls (GWGU), which rely heavily on the BC region for breeding and overwintering (Blight et al., 2015). As such, it is a research priority of ECCC to assess on a long-term basis the health of this species in the Salish Sea and Pacific North Coast.

Long-term monitoring of gulls has revealed that, as generalist predators, they use human food resources (Whitehorne & Connolly, 2013). This has enabled them to overcome the constraint of food limitation during winter, but this behavior can also be associated with a decline in certain health and fitness parameters (Choy et al., 2022; Davis et al., 2017). In this regard, conservation physiology provides useful tools to assess the parameters involved in individual and population health. Thus, monitoring the long-term physiological health parameters of the GWGU population would enable us to better understand and predict how they are affected by new stressors. This will enable us to highlight abnormal health parameters and thus potential pathology even before symptoms are visible (Cooke et al., 2021). For this, establishing reference ranges is a critical point. Although physiology can provide detailed information on the health of species and ecosystems, this tool is often neglected, resulting in a

lack of biomarker reference values for most species (Mallory et al., 2010). Furthermore, studies on the physiological status of non-breeding birds during winter 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). And finally, we know even less about the influence of age on their physiological health. However, this knowledge seems important for the long-term monitoring and conservation of this population.

Since winter 2020, this project has been seeking to establish reference ranges for the physiological parameters of the overwintering GWGU population. This will enable us to establish a baseline of *Laridae* physiological health, nowadays, before the launch of new industrial projects likely to impact ecosystem health in future years, such as TMX.

In the future, we will be able to use these reference ranges of physiological health to study whether these new anthropogenic pressures are having an impact on the subclinical health of these sentinel birds, and thus whether there is a decline in ecosystem health. Furthermore, during the first three years of this study, only the physiological health of adult GWGU was assessed. In order to obtain even more reliable data for this monitoring, we wondered in this study whether any variation in individual physiological parameters might be age-related. This question will enable us to determine whether physiological health differs between age groups, and whether it would be useful to establish distinct reference ranges for certain age classes, to obtain more sensitive and earlier detection of changes in the subclinical health status of this sentinel population.

A range of physiological biomarkers has been proposed as indices of individual health. Oxidative stress can be defined as the imbalance between oxidant and antioxidant molecules, in favor of the oxidants, that causes aging and disease (Sies et al., 2017). In another long-lived seabird, exposure to oxidative damage appears to increase with age, but only in old age, when a high investment in reproduction at the expense of defense is foreseeable. These data also showed that oxidative damage levels are predictive of colony attendance in all age classes: juveniles fledged with a high oxidative damage exposure index were less likely to be seen in the breeding colony again later (Herborn et al., 2016). A better understanding of how variation in oxidative damage and antioxidant capacity is related to age in GWGU, should help uncover whether Reactive Oxygen Species (ROS) induced oxidative damage is a significant cost to free-living animals and has a responsibility in aging (Selman et al., 2012). Aerobic capacity, which contributes to endurance and performance, is a physiological trait vital to the body's health and fitness. Aerobic capacity depends on the blood's ability to carry oxygen, which is determined by hemoglobin concentration and blood flow velocity (Calbet et al., 2006). These properties are

reflected by hematocrit or packed cell volume (PCV) which is a measurement of the proportion of blood that is made up of erythrocytes. Hemoglobin and blood viscosity increase linearly and exponentially, respectively, with hematocrit (Hedrick et al., 1986). For long-lived seabirds, hematocrit decreases with aging and juveniles have lower hematocrit value (Elliott et al., 2015). Similarly, hematocrit decreases with age in pigeons (Prinzinger & Misovic, 2010). As hematocrit is often used as an index of body condition (Murphy, 2010), the interpretation would be that older birds are in poorer body condition (Elliott et al., 2015). Plasma glucose is another physiological trait of interest in wild birds. High plasma concentrations of glucose and triglycerides generally correspond to recent energy intake. These different metabolites could therefore be used to determine a bird's recent nutritional status. (Alonso-Alvarez & Ferrer, 2001; Morales et al., 2020). With glucose, we could determine whether there is an age difference in recent nutritional intake, but we should be cautious as plasma glucose levels are also related to acute stress (Ramage-Healey & Romero, 2001).

In this study, we assessed variation in physiological health, and body condition (mass, size-corrected mass) of Glaucous-winged Gulls wintering in the BC coast, and provide GWGU health reference range. Specifically (1) multiple physiological biomarkers were measured as indicators of subclinical health, including nutritional state (glucose), aerobic capacity (hemoglobin, hematocrit), and oxidative state (total antioxidants and reactive oxygen metabolites). We then examined whether each of these physiological biomarkers varied among age classes, using a univariate analysis, and (2) we tested whether the overall health of individuals, assessed using principal components analysis to generate a single index incorporating all five physiological measured biomarkers, varied with age classes, i.e. a multivariate approach. To this end, we classify individuals into five age groups: first-winter, second-winter, third-winter, fourth-winter, and full adults (4+ years). Based on the scientific literature, even though scarce, we hypothesize that oxidative status should not vary between sub-adults, and oxidative damage would be more likely in adults. For aerobic status, we expect to find lower values in first-winter birds and similar values between sub-adults and adults. For plasma glucose levels, we expect first-years to have lower levels than the rest of the age group, as they are still in the process of learning to feed themselves. As for body condition, we expect this parameter to increase with age. Finally, since the "full adults" age group includes both young adults and aging adults, who show poorer health according to the literature. We hypothesize that for overall health, it would be the third and fourth winter age group that would show the best overall health. Having a more efficient behavior than the first and second year to eat more natural food (Wunderle, 1991) and less damage on health due to aging.

1 MATERIALS AND METHODS

1.1 Study Area

During the winter of 2023, from January 5 to March 3, a total of $n=184$ adult Glaucous-winged gulls of different age classes were sampled throughout the Canadian portion of the Salish Sea (Appendix 1.), the West Coast of Vancouver Island (Tofino) and the Pacific North Coast (Appendix 2.). These study sites hold a significant ecological value that sets them apart but are also subject to high anthropogenic pressures.

We attempted to sample the following regions consistently:

- From the Salish Sea: “Lower Mainland”, “Greater Victoria”, and “Southern Vancouver Island”, “Northern Salish Sea”,
- From the West Coast of Vancouver Island: “West Coast”,
- From the Pacific North Coast: “Northern Vancouver Island”, “Northern British Columbia Coast”, and “Haida Gwaii”.

Regions were classified primarily on the basis of geographic proximity, but beach substrate types and levels of anthropogenic influence were generally similar within a given region.

1.2 Study population and standard field procedures for capture and sampling GWGU

1.2.1 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 (23MH03). All personnel completed mandatory Animal Care training.

Gulls were live-captured using baited noose-mats (Liu et al., 2017). All gulls were photographed, aged, banded, and morphometric measurements including mass (± 20 g) and tarsus length (± 0.01 mm), were collected. Using a 27 gauge, 13mm heparinized needle and a syringe, we collected no more than 6 mL of blood ($<1\%$ of body weight) from each gull from the brachial vein, with a maximum of 3 mL collected per 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 for gull genetics analysis to determine gull sex, and the rest was used to determine glucose levels (mmol/L) in the field using a Point-of-care testing glucometer (Accu-check Aviva; Roche, Basel, Switzerland). Handling time was calculated for each individual and defined as the 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 (see below). Then, two heparinized 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 was collected and stored at -20°C until analysis.

1.2.2 Age determination

We determined the age of each individual by observing their physical characteristics that distinguish them within their age group (Olsen et al., s. d.).

First-winter (age 1) are greyish-brown with plumage pattern, with black bill and dirty pink legs. Body and wings are concolorous. Second-winter (age 2) are quite similar to first-winter, but bill starts to develop a pale base and tip. The feathers are still greyish-brown and the coverts even have a fainter pattern, often with uniform grey median coverts. Third-winter (age 3) has a mixture of greyish-brown and adult plumage, with grey upperparts and broad white edges to tertials and secondaries. They have a bicolored, fleshy black-tipped bill. Four-winter (age 4) has almost entirely adult plumage but one can still find some feathers a little brown, in particular, visible at the level of the very white tail in an adult. And despite being very similar to adult plumage they often have a very extensive dark subterminal marking on the yellow bill. We can also find a more extensive brownish diluted hood often penetrating onto breast-sides. Full adult winter (age 4+) has pink legs, uppersides pale grey with very broad tertial and secondary tips, creating a complete white line, and their bill in winter often has a dark subterminal marking.

1.3 Bird health assessment

1.3.1 Aerobic capacity

Hematocrit, is the percent of whole blood volume that is comprised of red blood cells and was expressed as the volume of the part of the capillary occupied by red blood cells per total volume of blood in the capillary (Williams et al., 2004). To measure it we were using digital calipers (± 0.01 mm). Because blood was collected in two capillary tubes for almost all individuals, we could calculate the within-individual repeatability of our hematological

variables; these were found to be high (r^2 : 0.795, $P < 0.001$). We always calculated mean hematocrit with the two measurements.

Hemoglobin (Hb; g/dl) 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).

1.3.2 Oxidative status

Total antioxidant titres (OXY; $\mu\text{mol HClO mL}^{-1}$) and reactive oxygen metabolites (d-ROMs; $\text{mg H}_2\text{O}_2 \text{ dL}^{-1}$) in the plasma were measured using OXY and d-ROMs kits, respectively, from Diacron International (Grosseto, Italy). OXY absorbances were read at 490 nm, and d-ROMs 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).

1.3.3 Data quality control

Inter-assay variation was 1.48% (hemoglobin; $n=187$ triplicates), 9.68% (OXY; $n=192$ triplicates) and 4.33% (d-ROMs; $n=190$ duplicates). Intra-assay variation was 1.48% (hemoglobin; $n=10$ triplicates), 6.29% (OXY; $n=10$ triplicates) and 4.60% (d-ROMs; $n=5$ duplicates). To control for the quality of our analyses, the inter- and intra-assay coefficients of variability (CV) were calculated from duplicate or triplicate samples and analyses were repeated, for samples with too high intra-assay CV, if no clear outliers could be eliminated by an error during the analysis. Indeed, these analyses were repeated for intra-assay $\text{CV} > 12\%$ if they were performed in duplicate (d-ROMs), or having an intra-assay $\text{CV} > 10\%$ for replicates performed in triplicate (hemoglobin and OXY). If after these re-analyses, CV intra-assays were still too high for some samples, these data were discarded from the dataset. This was the case for OXY ($n=2$, intra-assays $>10\%$) and for d-ROM ($n=5$, intra-assays $>12\%$). For each sample, once the outliers have been eliminated, a mean value of the replicates is calculated for each sample.

1.4 Statistical analyses and choice of variables

Results obtained were statistically analyzed using R version 4.2.1 (2022-06-23 ucrt) and we performed Shapiro-Wilk test to check the normality of distribution of variables. Normality was considered when $P > 0.05$. D-ROM and Glucose values did not conform to this assumption of normality, so they were converted by log-transformation before being analyzed (Fowler &

Williams, 2017; Sugiura et al., 2016; Vágási et al., 2020). All measured physiological biomarkers were also examined for outliers. These values could be due to errors during laboratory manipulations or could be related to the identification of subclinically abnormal individuals. These outliers were then compared with reference values for other *Laridae* to determine whether they were biologically plausible, i.e. potentially representing true individual biological variation (Averbeck, 1992; Doussang et al., 2015; García et al., 2023; Newman et al., 1997), or whether they belonged to subclinically abnormal individuals who might have a pathology that would skew the establishment of reference values. The values were removed for hemoglobin (n=6; >22 g/dL), hematocrit (n=2; <35 PCV%) and d-ROMs (n=3, >9 mg H2O2 dL-1).

1.4.1 Covariation of handling time, size-corrected body mass, and physiological traits

To test the pairwise relationship between the five measured physiological biomarkers and examine the potential effect of handling time and size-corrected body mass on each trait, Pearson correlation coefficient analysis was applied. Handling time is an important factor that could affect physiological condition due to the acute stress it can produce (Carere & Vanoers, 2004; Cohen et al., 2007; Ramage-Healey & Romero, 2001; Vágási et al., 2020). Size-corrected body mass also represents an important health factor reflecting the relative physical condition and fitness of individuals, regarding energy reserves. Thus, the body mass was corrected by an indicator of structural size, the tarsal length. (Jiménez-Peñuela et al., 2019; Owen1 & Cook1, 1977). Then we used four separate general linear mixed models using the “lme4” R package (Bates et al., 2014) with each of the physiological traits as a dependent variable in each model and with 1) handling time, 2) Size-corrected body mass, 3) handling time + Size-corrected body mass or 4) handling time * Size-corrected body mass as a fixed effect (independent variables). All models were run with the region as a random effect to control for potential non-independence in the data that could result from environmental variation between sampling sites. In addition, it was found in a previous study during this project that region and habitat type as independent variables did not significantly explain variation in these five physiological biomarkers (Hall, s. d.). Moreover, due to small sample sizes among some region when divided by age it was not possible to consider it as a fixed effect. (Figure 1, Table 1).

To compare the support of these four hypotheses explaining the variation in physiological traits and find the best-fitting model for each biomarker, we used Akaike's Information Criterion for small sample sizes (AICc). For each trait, if one of these fixed effects had a significant effect, at an alpha level of 0.05, on the variation in that trait then that fixed effect was included as a covariate in the model studying the impact of Age on the variation in each biomarker.

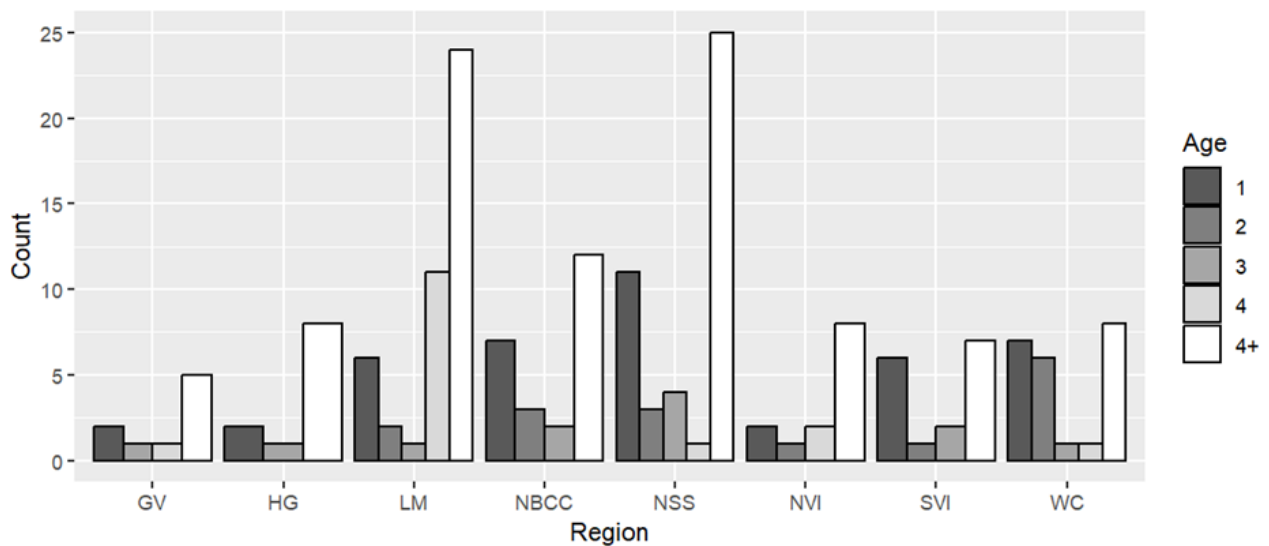


Figure 1: Number of individuals captured by group of age in each region.

Note: Total number of first-winter ($n=43$), second-winter ($n=16$), third-winter ($n=12$), fourth-winter ($n=16$) and full adults ($n=97$) Glaucous-winged gulls.

Table 1: Number of individuals captured by group of age in each region.

AGE	REGION								Total
	West coast of Vancouver Island	Salish sea				Pacific North Coast			
	West Coast (WC)	Northern Salish Sea (NSS)	Southern Vancouver Island (SVI)	Greater Victoria (GV)	Lower Mainland (LM)	Northern Vancouver Island (NVI)	Northern British Columbia coast (NBCC)	Haida Gwaii (HG)	
	n	n	n	n	n	n	n	n	
1	7	11	6	2	6	2	7	2	43
2	6	3	1	0	2	1	3	0	16
3	1	4	2	1	1	0	2	1	12
4	1	1	0	1	11	2	0	0	16
4+	8	25	7	5	24	8	12	8	97
All ages	23	44	16	9	44	13	24	11	184

1.4.2 Examining age related variation in health

As our main interest was to investigate the impact of age on variation in physiological traits, we first determined if the sampled age ratio was significantly different in our sample using the Chi-square test. This was done to address potential age bias in our data. We also tested whether body mass or size-corrected body mass varied significantly by gull age groups using analysis of variance (ANOVA) and Tukey's post hoc tests.

Then, we assessed whether each trait varied significantly across age groups. ANOVAs and Tukey post hoc tests for pairwise comparisons at the 95% confidence level were performed

using lme4 (Bates et al., 2014). For these models, age was the primary independent variable and covariates previously determined by model selection were included as appropriate. Only handling time was significant for glucose, so the other biomarkers did not have a covariate in their model to determine if age had an impact on the variation in physiological health. And the capture region was included as a random effect in all models. To determine pairwise comparisons between age groups Least squares means were calculated using the emmeans package in R Studio (Lenth, 2022).

Principal component analysis (PCA) was used to examine correlations and distributions of GWGU physiological characteristics, and to test if a single, multivariate index of "health" was related to age for gulls wintering on the British Columbia coast.

In addition, PCA scores for individuals were used to compare physiological health among ages. Specifically, we tested whether individual Principal Component 1 (PC1) or Principal Component 2 (PC2) scores varied significantly between ages using ANOVA and Tukey's post-hoc tests for pairwise comparisons.

2 RESULTS

A total of 184 adult Glaucous-winged gulls were sampled during the winter of 2023. Morphological traits include size-corrected body mass using mass (g) versus tarsus length (mm) measurements, glucose (mmol L⁻¹), hemoglobin (g dL⁻¹), hematocrit (packed cell volume %), OXY (μmol HClO mL⁻¹), and d-ROMs (mg H₂O₂ dL⁻¹) levels (Table 2).

Table 2: Sample size, mean ± standard deviation, and range for body size and physiological traits measured in GWGU sampled throughout coastal British Columbia.

Note: All values are untransformed.

Indicator of health:	Physiological Trait:	n =	Mean ± Standard Deviation	Range
“Nutritional state”	Glucose (mmol/L)	170	16.58 ± 4.58	8.8-33.1
“Aerobic capacity”	Hemoglobin (g/dL)	173	14.65 ± 2.27	7.02-20.93
	Hematocrit (%)	168	46.14 ± 3.20	36.51-54.45
“Oxidative status”	OXY (μmol HClO /mL)	167	236.97 ± 27.51	173.1-310.8
	d-ROM [Reactive oxygen metabolites] (mg H ₂ O ₂ /dL)	163	2.16 ± 1.23	0.50-8.35
“Body measurement”	Tarsus (mm)	184	68.96 ± 3.79	58.9-80.5
	Mass (g)	183	997.43 ± 130.1	760-1410
	“Size corrected body mass”: Mass/Tarsus	183	14.45 ± 1.5	11.48-19.03

2.1 Covariation of handling time, size-corrected body mass, and physiological traits

Correlation matrix analysis revealed that handling time had a significant correlation only with measured blood glucose (Table 4; $r = 0.218$, $P = 0.004$). This result was confirmed by running a linear mixed model to determine which covariates to include in each model. Indeed, we found that only handling time was present in the top-ranked models with AICs for glucose (Figure 2; Table 3). This was to take into account the impact of handling time which may cause an increase in glucose due to acute stress. (Vágási et al., 2020). For the other biomarkers, we did not include covariates in the models because none of the four hypotheses explained the variation in biomarker measurement (Table 3).

Comparing among physiological traits in a correlation matrix, glucose was weakly negatively correlated with OXY values (Table 4.; $r = -0.197$, $P = 0.013$). In contrast, several other physiological traits were positively correlated, including hemoglobin and hematocrit (Table 4; $r=0.533$, $P<0.05$) and also d-ROMs and OXY (Table 4.; $r=0.184$, $P=0.020$). In addition, there was a trend for a positive correlation between hematocrit and OXY (Table 4; $r=0.150$, $P=0.060$).

Table 3: Fixed effects from the best fit linear mixed effects models chosen by Akaike Information Criterion for small sample sizes (AICc).

Trait	Fixed effect (with the lower AICc)	Estimate	DF	P value
Hemoglobin	Size-corrected body mass	0.036	169	0.753
Log(d-ROM)	Size-corrected body mass	0.243	159	0.795
Log (Glucose)	Handling time	0.002	166	0.005
Hematocrit	Size-corrected body mass	0.251	164	0.126
OXY	Size-corrected body mass	0.644	163	0.648

Table 4: Pearson correlation coefficients (r) for comparisons between all physiological traits and with handling time and size-corrected body mass. Traits and variables measured were size-corrected body mass, hemoglobin (g/dL), hematocrit (% PCV), glucose (log(mmol L⁻¹)), "OXY" = total antioxidant titers (μmol HClO mL⁻¹), "dROMs" = reactive oxygen metabolites (log(mg H₂O₂ dL⁻¹), and handling time (min).

Note: Significance was measured at the 95% confidence level and significant results are denoted in bold. Glucose and d-ROM were log transformed.

comparison	r	p-value
Body mass vs handling time	-0.064	0.397
Body mass vs hematocrit	0.008	0.920
Body mass vs glucose	-0.116	0.131
Body mass vs d-ROM	0.018	0.815
Body mass vs OXY	0.015	0.850
Body mass vs hemoglobin	-0.043	0.572
size-corrected body mass vs handling time	-0.050	0.506
size-corrected body mass vs hematocrit	0.120	0.120
size-corrected body mass vs glucose	-0.093	0.225
size-corrected body mass vs d-ROM	0.040	0.612
size-corrected body mass vs OXY	0.035	0.654
size-corrected body mass vs hemoglobin	0.026	0.733
handling time vs hematocrit	0.051	0.516
handling time vs glucose	0.218	0.004
handling time vs d-ROM	-0.142	0.071
handling time vs OXY	<i>-0.140</i>	<i>0.074</i>
handling time vs hemoglobin	0.0912	0.235
hematocrit vs glucose	-0.075	0.351
hematocrit vs d-ROM	0.052	0.521
hematocrit vs OXY	0.150	0.060
hematocrit vs hemoglobin	0.533	0.000
glucose vs d-ROM	-0.039	0.630
glucose vs OXY	-0.200	0.012
glucose vs hemoglobin	0.102	0.197
d-ROM vs OXY	0.184	0.020
d-ROM vs hemoglobin	0.019	0.818
OXY vs hemoglobin	0.0325	0.683

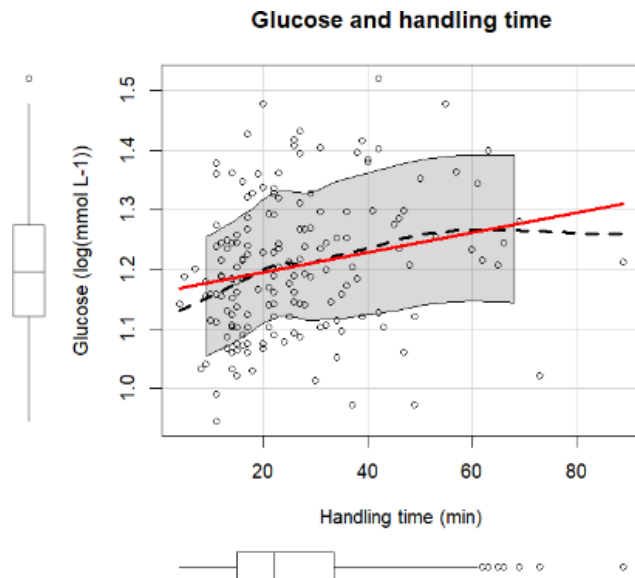


Figure 2: Scatter plot of glucose that differed significantly by handling time. Glucose values were log transformed.

Note: We can see the mean smoothing curve (black dotted line), the smoothing curves of the confidence intervals (black lines) and a regression obtained by the least squares (red line).

2.2 Examining age related variation in health and body measurement

We found that size-independent body mass varied significantly with age ($F_{4,176} = 2.656$; $P = 0.035$), with first-winter GWGU having significantly lower size-corrected body mass than full adult birds captured (Figure 3.a; Table 5; $P = 0.023$). However, body mass did not differ significantly between ages ($F_{4,178} = 1.236$; $P = 0.297$).

Among physiological traits, hematocrit (packed cell volume; PCV%) varied significantly with age ($F_{4,163} = 2.442$; $P = 0.048$), with first-winter GWGU having significantly lower PCV% than second-winter birds captured (Figure 3.b; Table 5; $P = 0.032$). D-ROMs also varied significantly with age ($F_{4,158} = 2.940$; $P = 0.022$), with first-winter GWGU having significantly higher log transformed d-ROM values than full adult birds (4+ years) captured (Figure 3.c; Table 5; $P = 0.021$). In addition, there was a marginally significant effect of age on hemoglobin ($F_{4,168} = 2.229$; $P = 0.067$) with first-winter GWGU having slightly lower hemoglobin values than full adult (4+) birds captured (Table 5; $P = 0.062$). All other pairwise comparisons of hemoglobin among age were non-significant ($P > 0.270$ in all cases). Glucose ($F_{4,162} = 1.958$; $P = 0.103$), and OXY levels ($F_{4,162} = 1.72$; $P = 0.148$), did not differ significantly between ages and no other pairwise comparisons among age were significant ($P > 0.05$ in all cases).

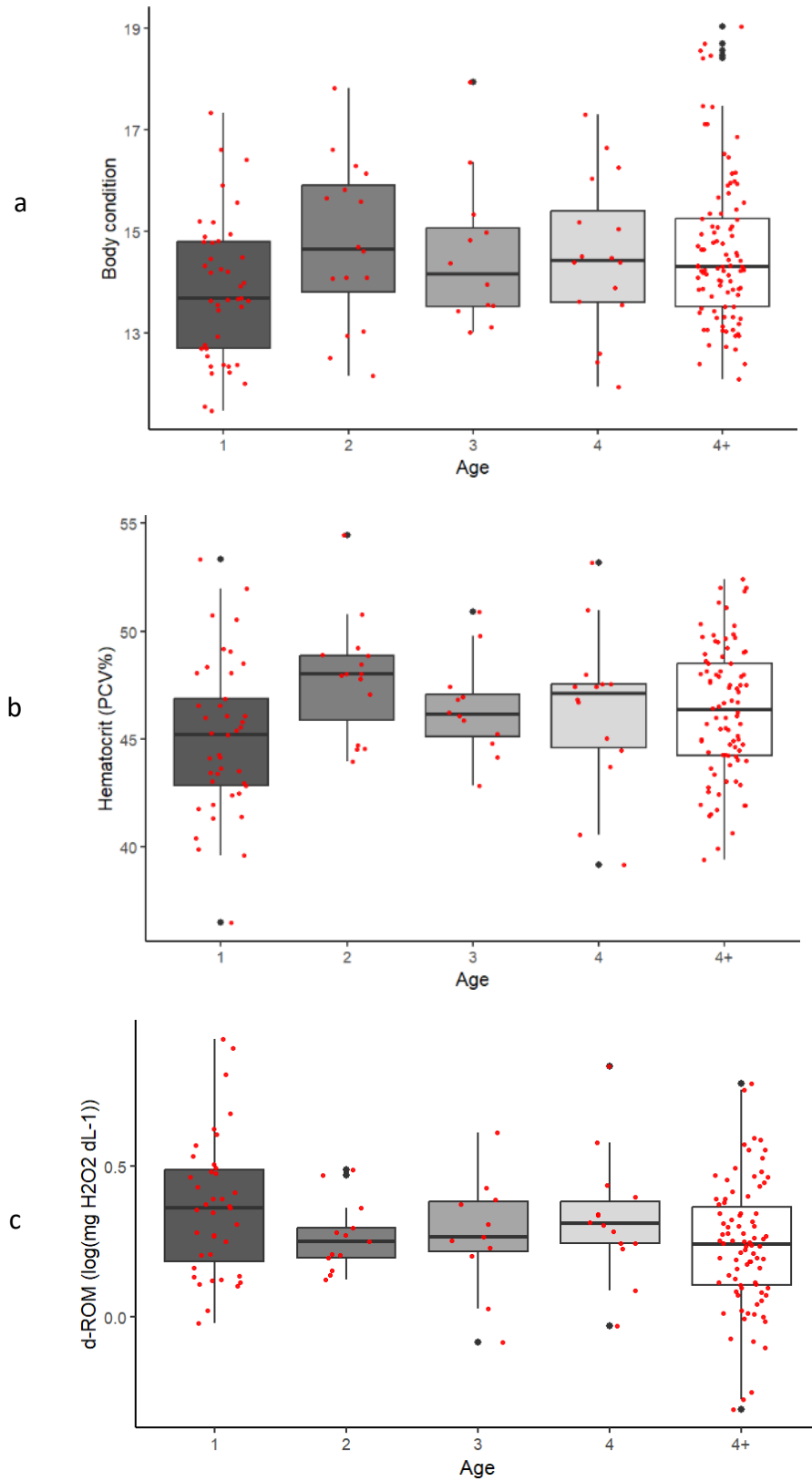


Figure 3: Morphological and physiological traits that differed significantly by age of Glaucous-winged gulls.
Note: D-ROMs values were log transformed.

Table 5: Least squares means and standard error for physiological traits by Age of GWGU captured.

Physiological Trait	Age									
	1		2		3		4		4+	
	Mean ± SE	n	Mean ± SE	n	Mean ± SE	n	Mean ± SE	n	Mean ± SE	n
Glucose (log(g/mmoL))	1.18 ± 0.11	38	1.17 ± 0.09	15	1.25 ± 0.12	12	1.17 ± 0.12	13	1.22 ± 0.11	90
Hemoglobin (g/dL)	13.76 ± 2.65	40	15.13 ± 2.12	15	15.20 ± 2.06	12	14.56 ± 1.32	16	14.90 ± 2.19	90
Hematocrit (%)	45.03 ± 3.54	41	47.82 ± 2.73	15	46.43 ± 2.24	12	46.32 ± 3.65	14	46.30 ± 3.02	86
OXY (µmol HClO /mL)	242.26 ± 27.30	41	226.20 ± 15.46	14	223.95 ± 20.29	11	242.81 ± 30.28	14	236.93 ± 28.84	87
d-ROM (log(mg H2O2/dL))	0.37 ± 0.23	39	0.27 ± 0.12	13	0.27 ± 0.19	11	0.33 ± 0.20	14	0.24 ± 0.21	86
Size-corrected body mass: Mass(g) /Tarsus(mm)	13.85 ± 1.39	42	14.76 ± 1.62	16	14.54 ± 1.47	12	14.52 ± 1.53	16	14.63 ± 1.49	97

Principal components analysis was conducted on a subset of data, including only individuals for which all five physiological biomarkers were measured (n = 137). PC1 explained 31.8% of the variation among the physiological variables, and PC2 explained 25% of total variation (figure 4). PC1 was strongly negatively influenced by hemoglobin and hematocrit, likely reflecting an “aerobic capacity” axis, and PC2 was negatively influenced by glucose and positively influenced by OXY and d-ROM (Table 6, Fig. 5) likely reflecting and “oxidative status” axis. Principal component scores of individuals for PC1, did not differ significantly by age (F4,132 = 1.188; P = 0.319). However, PC2, a presumed measure of oxidative status, did vary significantly by age (F4,132 = 2.949; P = 0.023). With first year GWGU having significantly different PC2 than third year birds captured (P = 0.0312).

Table 6: Coordinate score for each variable determined by principal components analysis.

Note : PC = principal component.

Biomarker	PC1	PC2
Glucose (log(g/mmoL))	-0.105	-0.685
Hemoglobin (g/dL)	-0.859	-0.222
Hematocrit (%)	-0.852	-0.034
OXY (µmol HClO /mL)	-0.289	0.678
d-ROM (log(mg H2O2/dL))	-0.184	0.520

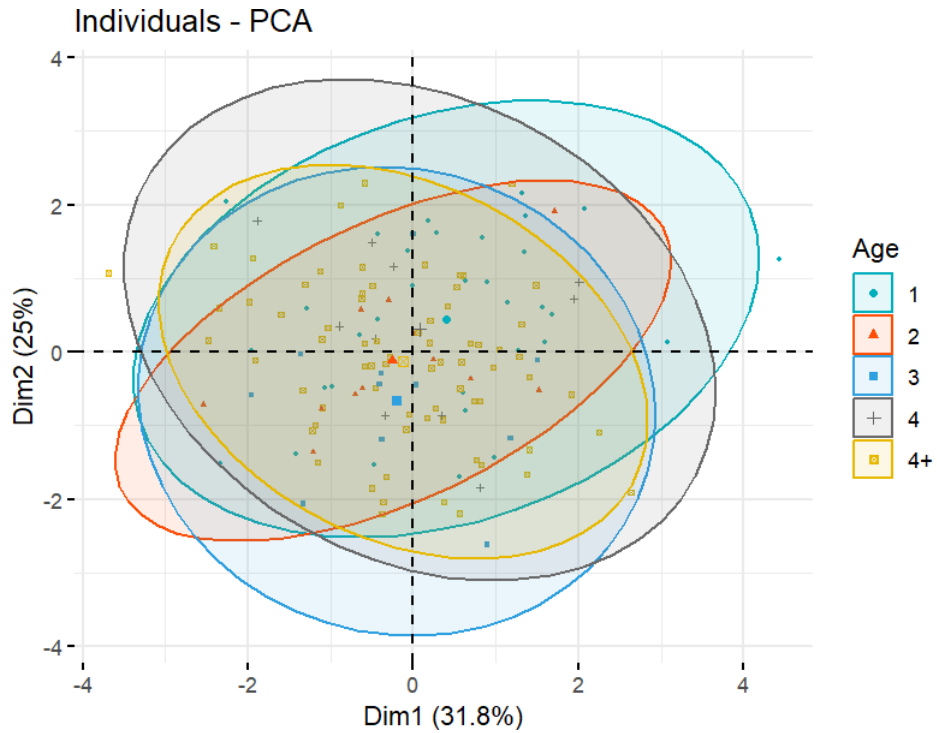


Figure 4: Biplot of principal components analysis (PCA) results for individual glaucous-winged gulls from each age group. Individual positions on the plot are based on PCA conducted on five physiological measurements of health including: glucose, hematocrit, hemoglobin, OXY, and d-ROMs levels.

Note : glucose and dROMs values were log transformed before PCA was conducted.

Dim1 = principal component 1; Dim2 = principal component 2.

Circles represent 95% concentration ellipses.

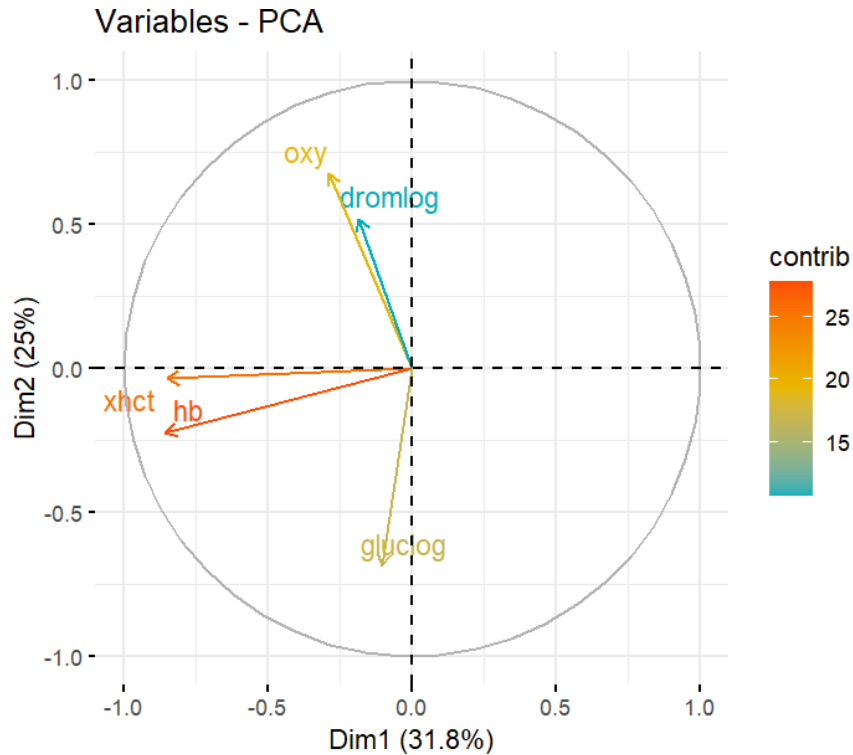


Figure 5: Variable correlation plots of components analysis (PCA) results for individual glaucous-winged gulls from each age group. Showing the relationship between each five physiological measurements of health including: glucose (glyclog), hematocrit (xhct), hemoglobin (hb), OXY, and d-ROMs (dromlog) levels.

3 DISCUSSION

In this study, we used a physiological approach to assess the health of a sentinel seabird species under increasing human-induced pressure, the Glaucous-winged Gull, which winters in the biologically important BC coastal ecosystem. We established baseline values for five health biomarkers, for five GWGU age classes providing a basis for long-term physiological monitoring in relation to future anthropogenic impacts in this region.

3.1 Covariation of handling time, size-corrected body mass, and physiological traits

We found a strong positive correlation between hemoglobin and hematocrit, so these biomarkers representing aerobic state indicate a similarity. Indeed, hemoglobin and hematocrit are both based on whole blood and therefore dependent on plasma volume, and are positively correlated with each other. In addition, we also found a positive correlation between antioxidant capacity (OXY) and reactive oxygen metabolites (d-ROM). This could suggest that a majority of the sampled population has a good antioxidant capacity. Indeed, it may be suggested that most individuals were able to counteract oxidative damage by increasing antioxidant levels and achieving oxidative equilibrium.

Handling time was also a covariate positively correlated with glucose, and it seems that the longer the handling time, the more glucose showed a slight increase (figure 2). This correlation could be explained by the fact that the time between capture and the end of blood sampling represents an acute stress factor. However, it has already been shown that birds can become hyperglycemic in the face of acute stress, due to a number of underlying physiological mechanisms (Remage-Healey & Romero, 2001; Vágási et al., 2020). We also found that there was a negative relationship between glucose and Total antioxidant titres, which could also be linked to the presence of acute stress, generating an hyperglycemia. We found that handling time and OXY approached a significant negative correlation (Table 4; $r=-0.140$, $P=0.074$), which would suggest that an increase in stress due to handling time would induce a decrease in anti-oxidants and therefore an increase in oxidative damage. This may well be due to the fact that it has already been shown that an increase in glucose due to an increase in corticosterone induces an increase in oxidative damage, i.e. a decrease in anti-oxidants (Vágási et al., 2020). However, the results of the glucose values must be tempered, because although the handheld device is an alternative to traditional laboratory devices useful in conservation-related monitoring (Bennett et al., 2017), it has functional limitations (Stoot et al., 2014). Moreover,

the reliability of POCT glucometer may be hampered by physiological conditions and species-specific differences, resulting in inaccurate results (Acierno et al., 2009; Lieske et al., 2002).

Finally, we also found a weak but approximately significant positive correlation between hematocrit and OXY. One might think that individuals with a good aerobic status would be likely to have a better antioxidant capacity.

3.2 Examining age related variation in health and body measurement

As we had hypothesized, we found that age had an effect on sized-corrected body mass variation, with sized-corrected body mass variation notably between first-winter individuals compared with full adults. There was no significance when looking at body mass, so it would seem that correcting body mass with structural size is more appropriate.

In term of oxidative status, it seems that first-winter GWGU have higher concentrations of reactive oxygen metabolites than adults, while total antioxidant titres do not seem to vary with age. This could support the hypothesis that juveniles with less experience of survival than adults (Marchetti & Price, 1989; Thorup et al., 2003) face more stress than sub-adults and adults.

With regard to aerobic status, our results seem to match the hypothesis we had made, as hematocrit (packed cell volume; PCV%) varied significantly with age, with first-winter GWGU having significantly lower PCV% than second-winter birds captured (Figure 3.b). This seems to be in line with data found in other seabirds, with younger birds having lower hematocrit levels (Elliott et al., 2015). In addition, there was a marginally significant effect of age on hemoglobin with first-winter GWGU having slightly lower hemoglobin values than full adult (4+) birds captured. This further reinforces the idea that first-year individuals have a lower aerobic status.

Our analysis of an overall “index of health,” using principal components analysis, similarly did not reveal clustering (of individuals) with differing health by age for the first component. However, PC2, a presumed measure of oxidative status, did vary significantly by age. With first year GWGU having significantly different PC2 than third year birds captured. We obtained very different results with the univariate approach, where age seems to have an effect on both aerobic and oxidative state variation, whereas the multivariate approach only allows us to observe that age has an effect on oxidative state variation. This may be due to the fact that the multivariate approach collapses variations down, but variations that could really be taken into account. Indeed, a lower aerobic status in first winters seems consistent with the

scientific literature. In both approaches, we observe that it is mostly first-year individuals who experience differences in health compared to subadults and adults.

This variation seems to show that first-winter juveniles are in poorer physiological health than sub-adults and adults. These results seem consistent with the scientific literature. Indeed, young seagulls under one year of age seem to have poorer general health, as they are still learning how to feed themselves, and are more often forced to deal with human food, which is easier to obtain (Greig et al., 1983). They also seem to have a more fragile immune system, and are more susceptible to disease and parasitism, which can be very costly, leading to a high mortality rate among the young (Sol et al., 2003).

Including first-winter GWGU sampling in the BC coast ecosystem health monitoring protocol appears to be of major interest. Indeed, in a sentinel context, these young birds seem to be more vulnerable than other age classes, making them more susceptible to disturbance. They are therefore likely to be the first to show physiological signs of the impact of harmful changes. During the first three years of this study, only the physiological health of adult GWGU was assessed. However, in view of our results, it would be interesting to build a baseline of physiological health reference range for juveniles too. This is in line with the aim of the "Salish Sea Gull Project" program, which seeks to conduct ecosystem health monitoring, in order to detect disturbances linked to environmental stressors at an early stage.

4 PERSPECTIVES

The results we have obtained open up new perspectives.

First of all, I'm going to redo the statistical analyses by grouping the age classes; with juvenile (first winter), subadults (second, third and fourth winter) and adults (4+); because we noticed that only GWGU under one year old have different physiological health parameters when compared to other age groups. This is to see whether pooling subadults together will lead to more correlation or a stronger degree, as well as perhaps other results with the multivariate approach.

New analyses will be carried out for the individuals of 2023, as we need to perform analyses on Triglycerides, in order to include these data in the nutritional status. This had not been done before because the kits were not available. We will also be obtaining genetic data giving the sex of each individual, so we will have to redo the statistical analyses with this new variable.

We also have other types of analysis carried out by other laboratories, which will enable us to study the link between age and variations in other health parameters. These include blood smear, inflammatory panel and a veterinary biochemical panel.

In addition, contaminant analyses are also being carried out on 2023 individuals, which can be coupled with physiological and veterinary analyses to determine what impact contaminants have on the health of these sentinel birds. But the contaminants can also be compared with new data on stable isotopes, also carried out on individuals from 2023, to determine which feeding behavior favors certain contaminants. A comparison could also be made between first winter GWGU and other age classes, to determine whether the feeding behavior of juveniles, often using human resources, has an impact on their exposure to contaminants.

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APPENDIX

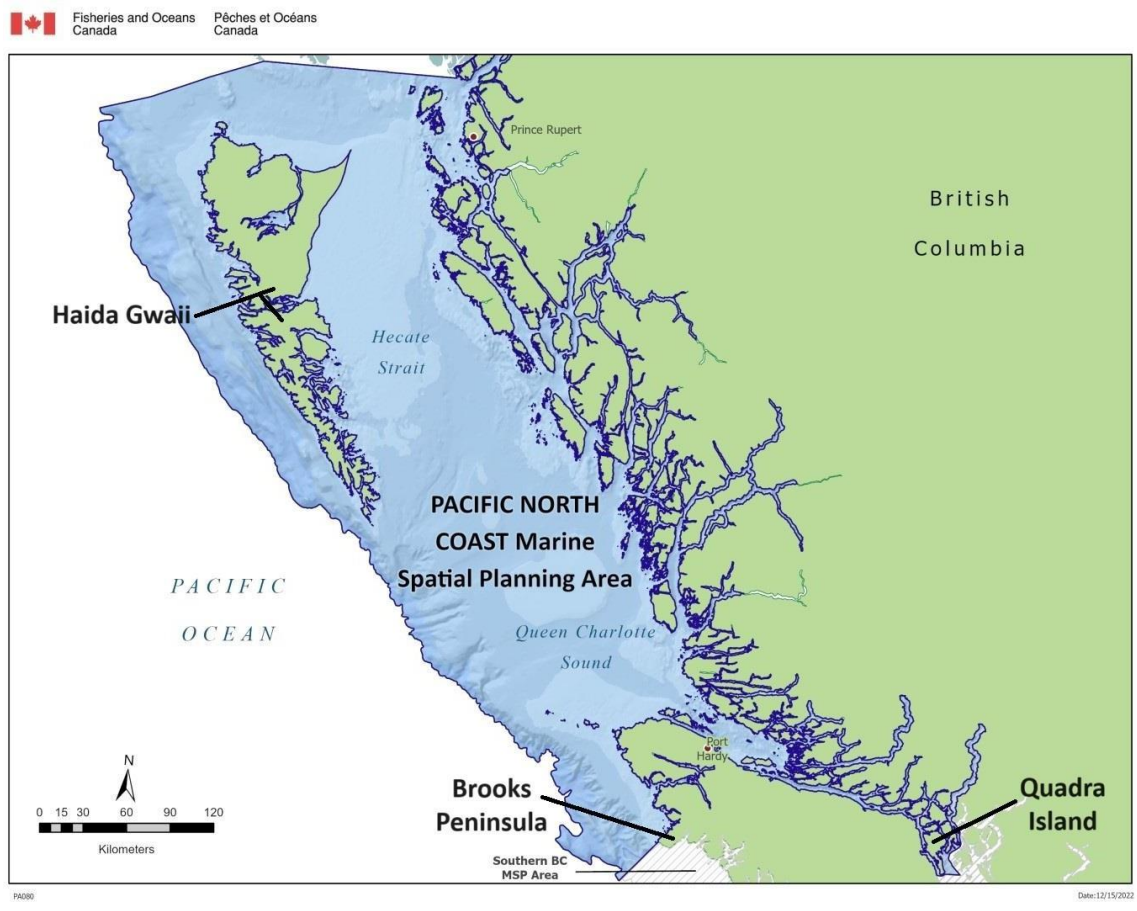
Appendix 1. The boundary of Salish Sea Canadian Marine Waters (in hatched red).

Note: Figure modified, originally from « Salish Sea Marine Emission Reductions Fund Applicant Guide 2021-2022 » led by ECCC.



Appendix 2. The boundary of Pacific North Coast Canadian Marine Waters (in blue).

Note: Figure modified, originally from “Pacific North Coast Integrated Management Area Plan (PNCIMAP)” led by ECCC.



Appendix 3. Map of the Trans Mountain expansion project. This map shows the route of the project, which begins just east of Edmonton, Alberta, and extends to Burnaby, British Columbia. (in orange).

Note: Figure from “What is the Trans Mountain Expansion Project?”, on the governmental website “Canada.ca” [2022-03-28].



A comparative analysis of physiological health parameters across age classes in a sentinel seabird wintering along the Canadian West Coast

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Abstract

The "Salish Sea Gull Project" was set up to monitor the health of a valuable coastal ecosystem facing increasing pressures from human activity. To this end, Glaucous-winged Gulls (GWGU) are used as sentinels, as they are generalist seabirds and sensitive to changes. Thus, monitoring the long-term physiological health parameters of the GWGU population would enable us to better understand and predict how they are affected by new stressors. However, little is known about the baseline health values of this species during the wintering period, and even less is known about it for the different age classes. We, therefore, wondered whether variations in individual physiological parameters are age-related, such as nutritional state, aerobic status, oxidation status, and physical condition. Our results showed that juveniles had lower aerobic and oxidative status. The monitoring protocol should include GWGU's juvenile samples, which are an even more sensitive sentinel.

Key words: *Age-related difference, avian physiology, sentinel specie, Laridae, reference range*

Une analyse comparative des paramètres physiologiques de santé entre les différentes classes d'âge chez un oiseau marin jouant le rôle de sentinelle.

Résumé

Le "Salish Sea Gull Project" a été créé pour surveiller la santé d'un précieux écosystème confronté à des pressions anthropiques croissantes. Pour cela, les goélands à ailes grises (GWGU) sont utilisés comme sentinelles, étant une espèce généraliste et sensible aux changements. Ainsi, le suivi à long terme des paramètres physiologiques des GWGU nous permettrait de mieux prédire l'impact que les nouveaux facteurs de stress devraient avoir. Cependant, nos connaissances sont limitées concernant les valeurs de référence de la santé physiologique des GWGU hivernant et de classes d'âges différentes. Nous nous sommes alors demandés si l'âge était responsable de variations des paramètres physiologiques individuels, comme l'état nutritionnel, d'aérobic, d'oxydation et la condition physique. Nous avons constaté que les juvéniles avaient un état d'aérobic et d'oxydation plus faible. Les juvéniles constitueraient des sentinelles sensibles et devraient être inclus dans le protocole de surveillance.

Mots-clés : *Différence liée à l'âge, physiologie aviaire, espèce sentinelle, Laridae, intervalle de référence*