SANDERLING (*CALIDRIS ALBA*) POPULATION STRUCTURE AND POLLUTANT EXPOSURE AT MAJOR WINTER AND MIGRATORY STOPOVER SITES INCLUDING CHAPLIN LAKE, SASKATCHEWAN

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By

Carla R. Labarrère

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ABSTRACT

Sanderling (*Calidris alba*) are a long distance migratory shorebird species found across a large range of coastal winter sites throughout North, Central and South America. As with many of the long distance migrant shorebirds, Sanderling have experienced significant population declines during the past 30 years, possibly due to pollution and other anthropogenic threats at wintering and migratory stopover sites. Sanderlings annually fly from their winter grounds to Arctic nesting grounds in Canada, migrating in an elliptical pattern, with significant numbers using the Central flyway in spring. This study aims to identify the population structure and wintering origins of Sanderlings that migrate northward along the Central flyway and stop in large numbers at Chaplin Lake, Saskatchewan, Canada. Despite the lack of research, this site is recognized for its hemispheric importance for shorebirds, particularly Sanderling (WHRSN Category 1). It also aims to identify the extent shorebirds are exposed to dioxins and dioxin-like compounds (DLCs), at selected stopovers across their range in North and South America to ultimately link the migratory patterns and potential risks of exposure to these contaminants.

Over 400 Sanderling were captured, measured and banded in Chaplin Lake, Saskatchewan on spring migration from 2012-2015. A total of 29 Sanderlings banded in Chaplin Lake were resighted, mostly during autumn migration following the elliptical migratory pathway along the east coast of North America, indicating band resightings alone were insufficient to determine wintering origin. A primary (P5) covert feather was sampled from 283 birds for stable isotope ($\delta^{15}N$, $\delta^{13}C$ and $\delta^{2}H$) analysis to infer the population structure and possible differences in winter origin. Additionally, feathers from 73 Sanderlings from Padre Island, Texas were similarly analyzed because birds from Texas Gulf coast were hypothesized to use the same migratory pathway as the Chaplin Lake population. Through a combination of isotopes, 3

distinct clusters of Sanderling were identified within the Chaplin Lake population, suggesting birds at this stopover winter over a broad geographic area. Clusters 1, 2, and 3 represented 28, 50, and 22% of Chaplin Lake population, respectively. The probability of the Texas Sanderling samples belonging to one of the three previously determined clusters was also estimated. The percentage of Padre Island, Texas birds assigned to clusters 1, 2, and 3 was 19, 25, and 56% respectively, implying strong overlap between populations. Using a combination of feather isotopic values, body morphometrics, known distributions and previously reported isotope data suggested possible origins of cluster 1, 2, and 3 as southern South America (e.g. Chile or Argentina), northwestern South America (e.g. Peru), and the Gulf of Mexico (e.g. Texas), respectively.

In order to assess the extent shorebirds are exposed to DLCs and conduct a preliminary hazard assessment, sediment samples were obtained from a set of wintering and stopover sites in North and South America to ultimately characterize potential toxicity risks. Sediment samples from migratory stopover or wintering sites in Canada, The United States, Colombia, Ecuador, Uruguay and Brazil were collected in partnership with local shorebird researchers. Following extraction, a novel application of the *in vitro* Luciferase bioassay method was used to assess the potency of the sediment extracts to activate the aryl hydrocarbon receptor (AhR) in the H4IIE-*Luc* cell line. Toxic induction of sediments ranged from 11.11 in Aracaju, Brazil to 20.43 pM 2,3,7,8-tetrachlorodibenzo-p-dioxin equivalent (TCDD-EQ) in Padre Island, Texas. Although 5 out of 8 sites showed TCDD-EQ values significantly above controls, all samples analyzed had concentrations of TCDD in sediment below published USEPA regulatory limits. Calculated TCDD exposure from the most contaminated site, Padre Island, was estimated to range from 0.0009 ng TCDD-EQ /day for a larger Willet (*Catoptrophorus semipalmatus*) to 0.0203 ng

TCDD-EQ /day in a small Semipalmated sandpiper (*Calidris pusilla*), which is below published toxicity hazard thresholds for birds. However, these results should be interpreted with caution, since sediment ingestion was the only route of exposure considered, whereas contaminants in the invertebrate diet may be more important.

The information collected about Sanderling migration ecology and connectivity has revealed new insight into the population structure and potential wintering origins at a key stopover site in the Central flyway. It is also an important step to determine the potential contaminant threats that shorebirds face during the annual cycle, specifically from DLCs caused by industrial pollution across their migratory range. This provides a basis to guide future work to determine the health and specific contaminant levels of this migratory shorebird population in the Central flyway which spends part of its annual cycle in diverse coastal areas of North, Central and South America.

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PREFACE

Chapter 1 of this thesis is a general introduction and Chapters 2 and 3 are written in manuscript style for future publication in scientific journals. Thus, there is some repetition of introductions, materials, and methods between chapters. Chapter 4 is a conclusion to both studies, with recommendations for future research.

CHAPTER 1

General introduction and research rational

1.1 Shorebird ecology

1.1.1 Migration ecology and staging areas

Approximately 215 species of shorebirds are distributed among 14 families in the order Charadriiformes, suborder Charadrii [1]. This diverse group occupies different habitats such as coastal, saline and freshwater wetlands, and arctic tundra [2]. They are adapted to feed on terrestrial or aquatic invertebrates. The most important prey are small crustaceans and bivalves. They also feed to a lesser extent on small polychaete worms and insects when available [3]. Many shorebirds are also known for their migratory habits. Species that breed in the northern latitudes often perform impressive long-distance flights [1]. Shorebird migration also includes some of the longest non-stop flights amongst birds [2]. For example, a marked Red knot (*Calidris canutus*) flew of 8,000 km in 6 days from Southern Brazil to the coast of North Carolina, U.S.A [4]. Evidences also suggested that bar-tailed Godwit (*Limosa lapponica*) would fly from Alaska to New Zealand for 11,000 km without stopping [5].

Shorebirds have a global distribution and are mostly associated with open habitats [1]. In the Western hemisphere they can be found wintering in primarily coastal areas of the United States and Central and South America [6, 7]. Two sites on the east coast, Tierra del Fuego, Argentina and Lagoa dos Peixes, Brazil have been recognized as significant areas of shorebird agglomeration [7]. A large number of shorebirds are also known to winter further north on the Pacific coast of North America, Baja California, the Gulf of Mexico and on shorelines of the southeastern Atlantic states, with fewer numbers along the beaches of Central America [8].

Many species demonstrate strong site fidelity, migrating annually from the Arctic breeding ground to the same wintering location [9, 10]. For example, Sanderlings are known to return to the same wintering sites and spend approximately 95% of their time within the same 5 km of beach. Movements between sites are rarely observed and performed typically by juveniles [10].

Some shorebirds have distinct breeding and wintering areas, using separate flyways [2, 11]. The Red knot migratory flyway is distinct for each of the recognized subspecies. The Red knot subspecies *Calidris canutus rufa*, migrates from Tierra del Fuego, Argentina to north of Canada uses Delaware Bay as the major stopover along northern flyway to the Eastern Arctic [4, 6]. However, newer tracking studies have revealed different migratory routes in birds of the same species, showing intra-specific variation [4, 12].

Shorebirds must stop to replenish fat stores, to moult, or to rest during the migration journey. The limited number of traditional staging sites makes this group of birds particularly vulnerable to environmental damage [11, 13]. Stopover sites are often well known locations on the coast. In the Central flyway, it also occurs at inland saline and alkaline lakes and wetlands in the Prairies of The United States and Canada [7]. Birds which migrate northward though the central flyway are also known to stop in central Canada, mainly on the saline/alkaline lakes found on the Prairies in Saskatchewan before completing their journey to the Arctic [7, 14]. A survey conducted in 2013 across the Prairies recorded 65,629 shorebirds of 29 species. Among these, 20 species and 95% of the birds were migrants [15] .

1.1.2 Shorebird population status

The most recent State of Canada's Bird Report [16] indicates that migratory shorebird populations have declined by almost half since 1970, experiencing some of the most substantial

declines of any guild. For example, Red knot (*Calidris canutus rufa*) estimates were up to a hundred thousand individuals 30 years ago, but the current population is about twenty-five thousand [4, 6]. Sanderling populations show similar trends. In 1972, the population was documented at 1.5 million, but the current population estimate is only three hundred thousand birds [7]. Similarly, Semipalmated sandpiper was classified as moderate concern because of their declining trend and common threats to their population [7, 17]. Endangered Piping plover (*Charadrius melodus*) populations breeding in the Canadian Prairies showed 32.4% decline since 1991 [18].

Over the last few years, the steep shorebird population declines have raised interest and concern of many researchers and institutions responsible for the conservation of shorebirds. Therefore, shorebird conservation plans were developed with the purpose of identifying current threats and management needs. The most likely threats affecting shorebird survival are loss of habitat, decreased availability of food, disease, predation and pollution [6, 7]. These issues are confounded by the large number of wintering and staging areas and the lack of information about shorebird migration ecology and connectivity making it difficult to establish where and when problems are occurring.

As shorebirds spend most of their annual cycle on the wintering grounds in Latin America or on migration, it is vital that they have adequate quantity and quality of winter and staging sites [6]. In Central and South America, winter habitats are rapidly disappearing due to the increased human development particularly in the coastal areas [4]. Additionally, industrial pollution such as leaching and inefficient waste disposal are an increasing concern. For example, oil and gas development has grown along with the number of accidental spills that may negatively affect migrating shorebird populations [6]. While there are many contaminants

capable of affecting shorebirds, dioxin-like compounds (DLCs) have largely been understudied despite their global distribution, toxicity, cumulative effects [19, 20], and the large annual quantities introduced into the environment [21, 22].

1.1.3 Study species and study sites

Sanderlings (*Calidris alba*) were chosen as targeted species for this project for several reasons: 1) their strong association with the marine environment through much of their annual cycle placing them at higher risk; 2) anecdotal and published evidence of oiling and/or mortality in these species exists; 3) their populations are exhibiting rates of decline similar to many shorebird species; and 4) they locally abundant and therefore relatively easy to study.

Sanderlings have a widespread distribution along coastal areas of North and South America during the temperate region's winter. They occupy mainly the Pacific coasts of Peru and northern Chile, the Gulf of Mexico, and southeast Atlantic coast of Brazil [8]. Sanderling migration is not extensively studied, but in general, Sanderlings migrate in an elliptical pattern. In spring the northern migration occurs distinctly along Atlantic coast of United States (mostly Texas and Delaware Bay), the Canadian (mostly Saskatchewan) and U.S. Prairies, and the U.S. Pacific coast. Then most appear to fly south through the Eastern flyway, occurring in large aggregation in Delaware Bay during fall [7]. Spring migration occurs from March until June, but the timing of the peak passage of Sanderlings varies by latitude. Fall migration occurs between mid-July and late October, with adults departing early in the season, and juveniles typically following a month later [7]. Below are highlighted some of the most important sites identified for Sanderlings during migration and winter.

• Canadian Prairies: The region is characterized by several shallow alkaline/saline lakes in Saskatchewan, Canada. The most important lakes are Chaplin Lake, Old Wives Lake, Reed Lake [14] and Quill Lakes [13]. They are considered an important staging area for many shorebird species including Sanderlings which approximately 97% of its prairie population is in Chaplin Lake [15]. The prairies may also support about 50% of the Western Hemisphere population during spring migration [7]. Chaplin Lake and Old Wives Lake combined reported a peak of approximately 55,000 birds during spring of 1994 [14]. In 2013, survey identified over 46,000 Sanderlings [15].

• Texas Gulf: Three main sites are located in Texas, United States. (1) Padre Island is located in South Texas, and consists of 70 miles of coastline. The peak number of Sanderlings at Padre Island National Shoreline (protected reserve portion of the Island) is over 5,000 birds during spring and fall migration [7]. It separates the Gulf of Mexico from the (2) Laguna Madre, a hypersaline lagoon important for over 100,000 shorebirds feeding and resting [23]. This is a large extension of land that goes from Port Mansfield, Texas to Tamaulipas, Mexico. Differently from the first two sites, the (3) Bolivar Flats Shorebirds Sanctuary is a human-made habitat. In the late 1800s in Galveston Bay, with the development of the North Jetty, the shore flow stopped resulting in accumulation of rich sediment and development of a complex invertebrate community into the mudflats. Consequently, this site became of huge importance for different species of animals, including birds. Over 100,000 shorebirds from 25 different species can be found stopping and wintering at Bolivar Flats [24].

• The Delta of the Iscuandé River: Located in Colombia, Naniño Department the area is constituted by sandy beaches, mangroves and muddy plains. The site holds approximately

30,000 shorebirds from 28 different species [25], including 1,000 to 2,500 Sanderlings according to 1980 survey [8].

• Salinas: Ecuasal company artificial lakes are located outside Salina city on the Province of Santa Elena, south west Ecuador. The salt plant created an artificial ecosystem that now supports a diverse macroinvertebrates community. As a result, this area attracts a variety of shorebirds including Sanderlings [26].

• Reserva Nacional de Paracas: located in Department of Ica, Peru approximately 200 km southeast of Lima, the area is winter ground for over 20,000 shorebirds. Sanderlings are among the predominant species with over 7,000 birds, approximately 8 % of the Pacific coast Sanderlings population [8, 27, 28].

• Bahía Lomas: located in the north coast of Tierra del Fuego, Chile the area is formatted by large sandy areas, and tidal or muddy plains. The local temperature during non migratory season (December to March) is around 6-12 °C [8, 29].

• Costa Atlántica de Tierra del Fuego: located in northeast strip of coastal area of Tierra del Fuego, Argentina approximately 100 km south of Bahía Lomas. The ecosystem is formatted mainly by sandy areas and muddy shoals, and is place for a large diversity of birds. Reports indicate that 135 species of birds can be found in this area, specially plovers and sandpipers, including Sanderlings [8, 30].

• Laguna de Rocha: Located in Department of Rocha, Uruguay is part of a 16,500 hectare of a complex wetland. The main lagoon is shallow and separated from the Atlantic Ocean by a relatively narrow sandbar. The lagoon complex is an important place for 24 species of shorebirds, including many species at risk. Sanderlings can be spotted from August to April in small groups [31].

• Lagoa do Peixe: This coastal lagoon in south of Brazil is one of the most important wintering and staging sites of the Atlantic coast. Over 6,600 Sanderlings were reported, representing 71% of the Atlantic Coast total population. The bird density could reach up to 69 Sanderlings per km [8].

• Aracaju: Located at Sergipe state, Northeast coast of Brazil, it represents an important site to shorebirds, especially during south migration. This area is mainly represented by sandy beaches and mangrove habitats. Shorebirds from 19 species were reported in census from Jan 2003 and Apr 2005, including Sanderlings, which were spotted in this location year around [32].

1.2 Migration strategies and assessment tools

The lack of information about established patterns of movement make it difficult to associate the migration movements to potential risks that animals face during the annual cycle. The study of animal migration ecology and connectivity is, therefore, vital for the effectiveness of proposed conservation plans [7, 33]. Different techniques have been applied to study the features of migratory movements of birds [4]. These methods can be classified as exogenous and endogenous. Exogenous methods involve external devices attached to the birds [34], ranging from a simple numbered band or coloured marker to complex devices such as satellite tags or geolocators. Resighting and recapture of birds with numbered bands and color leg markers have been the main method to determine stopover, breeding and winter locations [4]. But the utility of bands are limited to those species with a greater chance of recapture or resighting [34]. Radar tracking [35], geolocators and satellite-based technology have also been developed and applied to record individual or group pathways to better understand bird migration [4]. Radar tracking is a tool to identify local movements and sites of large groups of migrating birds. However, many

of these devices are prohibitively expensive compared to other techniques. Other downsides are the limited tracking distance, and the size of the tracker, which should be carefully chosen to adapt to birds size [35, 36]. Geolocators are a very impressive tool to track migration routes. They can continuously record the latitude and longitude of bird movement using intensity and timing of daylight, and they can collect and store data for a long period of time. The biggest issue with geolocators is that the scientist must recapture the bird to have access to the device to be able to download the storage data. This is particularly difficult for species of low recapture rate [4]. Endogenous methods to study migratory movements, including stable isotopes [34] and DNA markers [37], are not limited by the need for retrapping or following animals, since they do not need to be previously caught or marked.

1.2.1 Stable Isotopes

The use of stable isotopes as a marker of migration strategies improved the study of animal migration ecology and connectivity [33]. It has the advantage of being relatively inexpensive, can be applied to different species of animals and the combination of more than one isotope can improve results [38, 39]. Although most elements of the periodic Table have a stable isotope, just a few of them, such as carbon, hydrogen, nitrogen, oxygen and sulfur, are commonly used for wildlife studies. They have an abundant “light” isotope and a “heavier” but uncommon one and the ratio of the two relative to a standard are useful for inferring origin [38]. Physical and chemical process can result in different light and heavy isotopes ratios within distinct biomes and can be used as a marker that the animal carries with them [40, 41].

Stable isotopes in animal tissues reflect the isotopic signal of a local diet where the tissue is produced. Since their patterns vary spatially, it is possible to study where animals came from

using their isotopic profiles in select tissues [34]. It is possible to measure isotopes non-lethally in tissues such as blood; however the results will be representative of short-term dietary sources of assimilated foods [42]. In feathers, isotopes are assimilated during the period of moult and, because this tissue is metabolically inert after complete growth, it records past dietary information from the environment in which it was grown [38]. However, to be used as intrinsic markers of dietary and spatial origin some fundamental conditions must be respected: (1) birds need to migrate between places with different isotopic valuevalues and (2) they must retain one or more isotopes in the tissue of interest [41].

Previous studies have suggested that it is possible to use stable isotope values to assess wintering grounds of a long-distance migratory shorebird. In Red knots (*Calidris canutus*), isotope ratios of carbon and nitrogen in primary covert feathers could identify three distinct wintering locations [11]. The feather $\delta^{2}H$, $\delta^{13}C$ and $\delta^{15}N$ in Mountain plovers (*Charadrius montanus*) also varied with geographic location [43]. Significant differences were documented in $\delta^{2}H$, $\delta^{13}C$ and $\delta^{15}N$ in feathers of American Golden plover (*Pluvialis dominica*) and Pacific Golden plover (*P. fulva*) grown during summer and winter sites [44]. Information provided by isotope technique when correlated with evidence of environmental contamination could be used as powerful tool to understand dioxin and dioxin-like compounds exposure patterns in shorebirds.

1.3 Pollutants of concern: dioxin and dioxin-like compounds

1.3.1 Structure, properties and sources

Dioxins and “dioxin-like” compounds (DLCs) including the polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs), polychlorinated biphenyls (PCBs), and polycyclic

aromatic hydrocarbons (PAHs) are a broad class of compounds that have similar chemical structure and similar physical-chemical properties. Dioxin is a term often used to refer to a group of chemicals composed by 135 congeners of polychlorinated dibenzo-furans (PCDF) and 75 congeners of polychlorinated dibenzo-dioxins (PCDD) [45, 46]. PCDDs and PCDFs are stable nonpolar hydrophobic aromatic compounds [47]. They are commonly present in the environment, occurring naturally or through anthropogenic activities as unwanted by-products of combustion during industrial processes, such as chlorine bleaching of paper pulp, manufacturing of some herbicides and pesticides, fuel burning for agricultural purposes and waste incinerators; and non-industrial process such as backyard burning of waste, automobile fuel burning, and home heating [46-48]. High temperatures, alkaline media, and existence of UV-light can increase the formation of dioxins during the industrial processes [48]. PCDDs and PCDFs are globally distributed environmental contaminants of high toxic potency. They tend to accumulate in the body due to their high affinity for adipose tissues; consequently they are likely to accumulate in the food chain. Therefore, animals at the top of the food chain have a propensity to accumulate dioxin in their body [46]. The most studied dioxin is 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), also considered the most toxic congener. Therefore, the results of most of the studies involving other dioxins and dioxin-like compounds are represented as toxic equivalence relative to TCDD [46, 49].

Polychlorinated biphenyls (PCBs) refer to a group of 209 isomers and congeners with different numbers of chlorine atoms substituted in biphenyl rings some of which are considered dioxin-like compounds. PCBs can be divided into two groups, coplanar and non-coplanar. Coplanar PCBs have the phenyl rings at the same plan resulting in a rigid structure and activate the AhR, similar to PCDDs. Noncoplanar PCBs, in the other hand are not AhR agonists and are

not considered dioxin-like compounds [50, 51]. PCBs are man-made compounds globally produced and used in the past with large amounts found in the environment. They were created and marketed as mixtures of congeners. Monsanto Chemical Company was the only producer in the United States and they commercialized PCBs under the name Aroclor followed by four numbers (e.g. Aroclor 1254) where the first two numbers represent the 12 carbon atoms in the phenyl skeleton, and the other two represent the percentage of chlorine content by weight. For example, Aroclor 1254 has 54% chlorine by weight [52, 53]. Even though PCB manufacturing is no longer allowed in North America since 1979 under the Stockholm Convention on Persistent Organic Pollutants, their release into the environment still occurs from the disposal of large scale electrical equipment and waste (WHO 2010).

Polycyclic aromatic hydrocarbons (PAHs), also referred to as polynuclear aromatic hydrocarbons or polyaromatic hydrocarbons, are organic compounds with two or more fused six-carbon rings (benzene) that have hydrogen bonded to each carbon [54, 55]. This group includes about 100 compounds [56]. PAHs are formed during incomplete combustion [56] and can originate from natural sources such as forest fires, volcanoes, and from human activities such as oil production and release [57], combustion of fossil fuels and waste incineration [55, 58]. The physical and chemical properties vary with molecular weight and structure. Usually, high molecular weight compounds (four or more rings) are less water-soluble, less volatile and more lipophilic than lower molecular weight PAHs (two or three rings) [56, 59]. Most PAH compounds are persistent, toxic and widely distributed in the environment [19] with concentrations increasing significantly over the last century [59]. Analysis of the ice core from Greenland reported that the current level of PAHs is approximately 50 times higher than in pre-

industrial period. Interestingly, the same trend is found in the historical record of world petroleum production [60].

Ongoing demand for oil by industrialized societies increases the occurrence of oil spills and seeps, since it requires more oil exploitation, refining, and transporting activities [6, 7, 61]. The potential for oil contamination exists throughout shorebird migratory ranges. Large oil spills have caused significant ecological damage in the Gulf of Mexico [62], Argentina [63] and Alaska (U.S.) [64]. A single oil accident at a key stopover site might result in an enormous damage to fishes and water birds, including shorebird since they aggregate at key locations during the year [62, 65-68]. Also alarming, the presumed recovery of marine oil spill is estimated in up to 15% [62]. Despite the large proportion of accidents with oil spills, during the past decade, the number of those large disasters has decreased due to more rigorous regulation [69]. Thus, most marine environmental contamination is likely due to small-scale events from the daily transport and refining activities, offshore production [57], industrial and municipal discharges, disposal of waste oil and diesel (e.g., contaminated ballast from oil tankers), rivers discharge and urban runoff [66]. However, the accumulated volume of contaminants introduced in the marine environment by small spills can be frighteningly large. Between 1997 and 2010, 381 spills with less than $< 7.95 m^3$ occurred in Newfoundland, Canada at offshore production platforms [68]. Additionally, recent development of tar sands exploration represents another inland source of contaminant exposure to birds during migration [70, 71] [72].

1.3.2 DLC effects in birds

In humans and vertebrate wildlife, the major concern associated with DLC exposure is carcinogenicity [73-75]. However health risks in wildlife are associated with a range of toxic non-carcinogenic effects that differ according to the species sensitivity and exposure period [65, 76, 77]. Sublethal effects of DLCs exposure may include carcinogenesis, mutagenesis [75], altered endocrine function [70, 78] immunosuppression [79, 80], liver damage [77, 81] and hemolytic anemia [82].

During the pre-migratory period, it is possible to observe some physiological and behavioral changes including moult and an increase in body mass due to hyperphagia and increased deposition of fat [83]. This period is induced by variations in photoperiod [84]. In response to the change in photoperiod, endocrine mediated mechanisms such as thyroid hormones [85, 86], glucocorticoids [87] and leptin [88] are stimulated, and play an important role in the regulation of pre-migratory body changes. Thyroid hormones are indicated as important regulators of body weight, initiation of moult, lipid metabolism, thermoregulation, growth and reproduction in birds [70, 89, 90], but may also be playing a role in control of migration. Studies have demonstrated that exposure to PCBs may result in variation of thyroid hormones level [87, 91, 92]. European starling (*Sturnus vulgaris*) exposure to high level of Aroclor 1254 resulted in more disorientated migratory behavior, which may result in decreased migration performance [92]. PCBs may also be related to abnormal parental behaviour [93], lower growth rates [94] and immunotoxicity in birds [95].

Similar to PCBs, some evidence suggests that PAH may alter circulating blood concentrations of T_3 and T_4. Plasma levels of T_4 were assessed in nestling Black guillemot (*Cepphus grylle*) and Herring gull (*Larus argentutus*), and adult Leach's petrel (*Oceunodromu*

leucorhou), after oral dose of crude oil. In these three species, concentrations of circulating thyroxine (T_4) were greater than those of controls [87]. These results are supported by a study developed with nestling Tree swallows (*Tachycineta bicolour*) from the Athabasca oil sands. Concentrations of T_3 in plasma were elevated in birds from contaminated areas compared to those from the reference sites [70]. The consequences of altered levels of T_3 and T_4 in migratory birds have not been elucidated, but there are indications that in ring doves with elevated thyroid hormones, courtship and breeding behavior changed [96].

As shorebirds feed mainly on invertebrates to gain energy for moult and flight [7, 97], they are particular vulnerable to DLCs contamination through food chain biomagnification as invertebrate predators in marine ecosystems [20, 66, 77]. Many marine invertebrates also lack AhR mediated detoxification systems that cause bioaccumulation of DLCs that are then consumed by shorebirds [98-101]. This is of concern particularly in areas of high industrial activity, density populated, and in areas susceptible to petroleum contamination, especially coastal habitats [20, 66, 77]. Furthermore, there is a lack of information on where shorebirds are exposed to DLCs throughout their migratory cycle in the Americas. This is particularly relevant to shorebird conservation since many migratory shorebird species have been declining at an alarming rate.

1.4 Thesis objectives

Among the many species that use Chaplin Lake as a breeding or stopover site, here I am focussed on Sanderlings because of their declining population trends and lack of knowledge about winter origins of this large Central flyway population. This is especially important for understanding the possible threats they are exposed to during winter and migration. Among

these, little is known about the exposure to sublethal dioxin-like contaminants across their range; which is of concern due to global distribution, high avian toxicity, cumulative effects, environmental persistence, and large quantities introduced into the environment annually.

This study had two main objectives. The first one (chapter 2) was to assess variation and population structure of the large migratory population of Sanderlings on their northward migration while staging at Chaplin Lake (Saskatchewan, Canada) to identify potential wintering origins. I hypothesized that 1) the Chaplin Lake Sanderling population consists of different wintering groups since this unique stopover site can host up to 50% of the America Sanderling population; 2) Sanderling feather isotopes $\delta^{15}N$, $\delta^{13}C$ and $\delta^{2}H$ can be used to discriminate these groups since feathers are grown during late migration and early winter and site variation in longitude, precipitation, temperature, soil and plant characteristics among other information are known to reflect isotope values in bird feathers; 3) Sanderling of Padre Island, south Texas, United States would represent one group of the Chaplin population given the strong connectivity through the Central Flyway; and 4) morphological measurements and arrival timing of Sanderlings will differ among groups that are related to flight distance and climate of the wintering grounds.

The second objective of this study (chapter 3) was to assess environmental contamination from dioxin-like compounds at a subset of key stopover and wintering locations of shorebirds. Therefore, I hypothesized that 1) sediment collections and bioassays will reveal spatial patterns of environmental contamination among wintering and stopover sites important to shorebirds; and 2) dioxin and dioxin-like chemical exposure and hazard from sediment will vary among shorebird species due to feeding habits and size differences among species.

This work is an important step to identify key wintering locations and potential risks that shorebirds face at multiple stopovers during migration and winter. Additionally, the provided information could guide future studies and action plans regarding shorebirds conservation.

CHAPTER 2

Population structure of Sanderlings (*Calidris alba*) at a major stopover site in Chaplin Lake, Saskatchewan

2.1 Introduction

Many North American shorebirds are long distance migrants and are exposed to a large number of threats from their breeding grounds in northern Canada and the United States to their winter grounds throughout North, Central, and South America, as well as stopovers along their annual migration movements [1, 2, 6, 7, 26]. Due to the widespread geographic range of stopover and winter areas, threats such as loss of habitat, decreased availability of food, disease, predation and pollution are difficult to assess [6, 7]. Shorebirds, among other long-distance migrants currently under conservation concern are facing more threats than non-migratory birds [102]. Additionally, the lack of information about migration ecology and connectivity make it difficult to associate the migratory movements to potential risks that shorebirds face during the annual cycle.

Most shorebird species are associated with coastal water and inland habitats such as marine areas, estuarine and salt lakes, which are frequently disturbed and degraded [1, 6, 7, 103, 104]. Additionally, winter habitats in Central and South America are rapidly disappearing due to the increased human land development activity [6]. During migration, shorebirds must stop to replenish fat stores, to moult, or to rest during the migration journey. The limited number of traditional staging sites makes this group of birds particularly vulnerable to environmental changes [11, 13]. Furthermore, as shorebirds spend most of their annual cycle on the wintering

grounds in Latin America or on migration, it is vital that they have adequate quantity and quality of winter and staging sites [6].

The Canadian Arctic is the breeding ground of estimated 21 shorebird species. An alarming rate shows that more than 60% of those species are experiencing different rates of population declines [16, 103]. Among these, Sanderling (*Calidris alba*) are a long distance migratory shorebird breeding in the High Arctic tundra and found along coastal areas of North and South America during the winter. Sanderlings have experienced significant population declines during the past 30 years, possibly due to pollution and other anthropogenic threats at wintering and migratory stopover sites. In 1972, the population was documented at 1.5 million, but the current population estimate is only three hundred thousand birds [7].

In general, Sanderlings are known to migrate in an elliptical pattern but show large variation in routes often crossing between distinct flyways [9]. Spring migration occurs from March until June primarily through the central flyway (mostly Texas and the Canadian Prairies), with smaller numbers moving northward along the U.S. Pacific and Atlantic (i.e. Delaware Bay) coasts [9]. The Central Prairies may support up to 20-50% of the Western Hemisphere population during spring migration [7]. A unique stopover site in the Central flyway is Chaplin Lake, Saskatchewan, where approximately 97% of spring Prairie Sanderling populations has been reported [15] and is believed to support a large proportion of the hemispheric population [7, 9]. Despite its recognized importance as a Western Hemispheric Shorebird Reserve Network (WHSRN) site, Chaplin Lake is an understudied site and the Sanderling population structure and winter origins remain unknown [9, 105].

Based on mark-resight banding studies conducted in the 1980s and 1990s, wintering Sanderlings occupy mainly the Pacific coasts of Peru and Chile, the Gulf of Mexico, and

southeast Atlantic coast of Brazil and Argentina [8, 9, 105] but modern techniques have evolved to study migration strategies and winter origins of birds. In particular, stable isotopes have been extensively used because of their relatively inexpensive cost and the potential to be applied in different species of birds without the need for recapture [38, 39]. Stable isotopes in animal tissues reflect the isotopic signal of a local diet where the tissue is produced. Since their patterns vary spatially, it is possible to study where animals came from using their isotopic profiles in select tissues [34]. In feathers, isotopes are assimilated during the period of moulting, and, because this tissue is metabolically inert after complete growth, it records past dietary information from the environment in which it was grown [38]. Previous studies have suggested that it is possible to use stable isotopes to assess wintering grounds of long-distance migratory shorebirds. For example, Red knot (*Calidris canutus*) winter locations were identified by carbon and nitrogen isotopes in flight covert feathers [11]. Use of 3 isotopes can provide greater spatial resolution where δ^2H, δ^{13}C and δ^{15}N in feathers of mountain Plovers (*Charadrius montanus*) varied with geographic location [43]. Significant isotopic differences of δ^2H, δ^{13}C and δ^{15}N were documented for feathers grown during summer and winter of american golden Plovers (*Pluvialis dominica*) and pacific golden Plovers (*P. fulva*) [44].

Sanderling fall migration can occur for an extended period of time compared to spring migration [7], with moult into pre-basic plumage occurring at stopovers sites or on the wintering grounds. Moulting normally starts with feathers from the head, breast, and body, with flight feathers being the last ones to be replaced [106, 107]. The primaries are replaced in order, starting with P1 and other researchers have successfully used primary coverts (i.e. P5) to distinguish wintering origin as this is one of the last feathers to be grown [106, 107] . This study aims to (1) identify the feather isotopic variation and possible groups of wintering origins of a

large migratory population of Sanderlings on their northward migration through Chaplin Lake, Saskatchewan; (2) test whether morphological measurements differ among identified groups; and (3) apply statistical methods to estimate the probability of known Gulf Coast wintering origin Sanderlings belonging to one of the Chaplin Lake population groups.

2.2 Methods

2.2.1 Study area and Sanderling trapping

Sanderling were trapped during spring northward migration at Chaplin Lake (CL), Saskatchewan, Canada (50.441731°N 106.669028°W), a saline inland Prairie lake designated as Hemispheric Importance for shorebirds due to the large proportion of the western Sanderling population occurring here at one time (WHSRN). From 2012-2015, mist nets were set along the man-made dykes from dusk to dawn to capture the shorebirds, specifically Sanderlings. Nets were continuously monitored every 20-30 minutes and birds were extracted and processed immediately. In case of a large capture, birds were extracted from the nets and placed in cardboard boxes to allow movement and social contact until processing. A total of 405 Sanderlings were captured (see Table 2.1 for more details). Trapping was conducted throughout the peak spring migratory period from mid May to early June to capture arriving birds ideally from diverse origins.

Sanderlings were also captured on Padre Island (PI), Texas, United States (26.905842°N 97.370356°W). During late winter (February) of 2013 and 2015, a canon net was used to trap a total of 57 Sanderlings along the beach at Padre Island National Seashore (see Table 2.1). In 2013, an additional 28 Sanderlings were opportunistically collected at the same location after being killed by a beach vehicle and were also sampled for feathers. The cannon net was placed

on the ground, close to the shoreline, where birds routinely feed. Only when birds were in a safe position relative to the apparatus, the cannon was fired. The number of possible catches was considered before firing and based on the number of handlers available to safely and quickly retrieve birds. All birds captured were quickly removed from the net and held in keeping cages that permit free movements and social contacts. After banding and measurement they were immediately released at the capture site.

Figure 2.1. Map of study area and Sanderling trapping sites. Red stars represent the sites Chaplin Lake, Saskatchewan in Canada, and Padre Island, Texas in the U.S. The smaller maps beside each site show the area in more details.

2.2.2 Banding and morphological measurements

Sanderlings captured in 2012-2014 were banded with unique numbered Canadian Wildlife Service metal band, a plain white flag (signalling country of banding is Canada based on the Pan American Shorebird Program, PASP [108]) and a cohort colour band combination. The combination of colours is unique to birds for this site. The final bird identification was a light blue coloured band under a white flag on the upper right leg; an orange colour band on the lower right leg; a metal band on the upper left leg; and an orange coloured band on the lower left leg. In 2015, Sanderling were banded instead with a coded white flag with an alphanumeric code on the upper right, a CWS numbered metal band upper left and a single orange band on the lower left to permit individual identification. Right maximum flattened wing chord (mm), bill length (mm), right tarsus length (mm), body mass (g), and furcular fat score (score of 0-5 [109]) were measured in all captured Sanderlings. Sex cannot be determined in the hand for this species on migration and almost all Sanderling were aged as adults (After-second-year or ASY).

2.2.3 Feather stable isotope analysis

The fifth primary (P5) covert feather was cut with scissors from each bird. The P5 feather has been previously shown to be one of the final moulted primary feathers in migratory Calidris species and should therefore be grown on or near the wintering grounds [3, 106]. The covert feather was stored in plastic bags, and transported to the laboratory and stored until analysis. Of the 405 feather samples collected from Chaplin Lake and 85 from Padre Island over the 4 years, we randomly analyzed 283 and 71 samples from each location. Table 2.1 shows the number of feathers collected and analysed by year and site of sampling.

Table 2.1. Overview of number of Sanderlings captured and total number of covert feathers from Chaplin Lake and Padre Island analysed from 2012-2015.

	Chaplin Lake					Padre Island		
	2012	**2013**	**2014**	**2015**	**Total**	**2013**	**2015**	**Total**
Sanderlings captured	30	118	176	81	405	29	28	57
Feathers analyzed	12	113	79	79	283	26	26	71*

* Additional 19 feathers were recovered from carcass of Sanderlings possibly killed by a beach vehicle.

To remove potential surface impurities, feathers were washed with 2:1 chloroform:methanol solution, rinsed with deionized water and air dried in a fume hood overnight [110]. For δ^{15}N and δ^{13}C analyses, feathers were cut, homogenized, and approximately 1.0 mg samples were weighed into tin capsules. For δ^{2}H analyzes, approximately 0.35 mg of feather homogenates were weighed into silver capsules. The sealed samples were placed in 96-well microplates and sent for analysis through isotope-ratio mass spectrometry (IRMS).

The analysis of δ^{13}C and δ^{15}N was completed at the Stable Isotope Facility, UC Davis, California using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). Samples were combusted at 1000 °C and resulting oxides were removed in a reduction reactor. Carbosieve GC column (65°C, 65 mL/min) were used to separate N_2 and CO_2 before entering the IRMS. During analysis, glutamic acid (G-9) was used for elemental totals and size corrections; nylon (G-18) was used for drift correction; and isotope values were normalized to Nylon (G-18) and USGS-41 Glutamic Acid (G-17). Bovine liver (NIST 1577; G-13) was used as a check reference. All laboratory reference materials were calibrated to National Institute of Standards and Technology (NIST) Standard Reference Material. The final δ_{value} were presented relative to international standards (Vienna Peedee belemite for C; and Nitrogen air for N).

The analysis of δ^2H was completed at the Stable Isotope Hydrology and Ecology Laboratory of Environment Canada in Saskatoon, Canada using continuous-flow isotope-ratio mass spectrometry (CFIRMS: Isoprime, Manchester, UK). Samples were loaded into blank autosampler under He flow and combusted at 1350 °C in a Hekatek furnace coupled with a Eurovector (Milan, Italy) elemental analyser. Nonexchangeable δ^2H value of feathers was determined using calibrated keratin hydrogen isotope reference materials (CBS: -197‰; KHS: -54.1‰; SPK: -121.6‰) [111]. During analysis, keratin laboratory standards were used and calibrated against National Institute of Standards and Technology (NIST) Standard Reference Material. The final δ_{value} were presented relative to international standards (Vienna Standard Mean Ocean Water–Standard Light Antarctic Precipitation (VSMOW–SLAP) standard scale).

All stable isotopes values are presented in parts per thousand (‰) according to the equation 2.1 [112]:

$$\delta^{j/i}x = \frac{({}^{j}x / {}^{i}x)_{sample}}{({}^{j}x / {}^{i}x)_{standard}} - 1 \qquad (2.1)$$

Where ${}^{j}x$ = heavier isotope, and ${}^{i}x$ = lighter isotope.

2.3.4 Data analysis

Principal component analyse of the three stable isotopes indicated that none of the studied components could be excluded from the analysis without losing a significant amount of information. Additionally, all stable isotopes were found to be independent of each other. Therefore, all data analysis included a combination of δ^2H, δ^{13}C, and δ^{15}N. Isotope values were tested for normality using the Shapiro-Wilk W test.

Thirty different indices were tested in order to define the optimal clustering scheme to represent isotopically distinct Sanderling groups using multiple combinations of the number of clusters, distance measures, and clustering methods. Most techniques split the population into 2, 3, or 16 unique clusters. In order to identify the optimal number of unique cluster, five techniques were applied: K-means, partitioning around medoids (PAM), hierarchical cluster analysis using Ward method (HCA-W), hierarchical cluster analysis using complete linkage method, (HCA-C) and Normal Mixture Modeling for Model-Based Clustering. The PAM method with 3 clusters was selected because the results presented the most distinct set of clusters among the five applied techniques.

Feather isotope values of δ^2H outside the normal range of less than -100 ‰ or greater than 110 ‰ were excluded from the analyses (n = 12 samples from Chaplin Lake and 8 from Padre Island) as they were considered measurement errors or possibly bird moulting on the breeding grounds [33, 34, 40].

A $\log_{10}$ transformation was applied to improve normality of morphometric measures based on Shapiro-Wilk test. Analysis of variance (ANOVA) and post-hoc Turkey´s tests were used to compare Sanderling body measurements (mass, wing chord, tarsus, bill) among clusters, and a chi-square test to look for differences in fat score among clusters. A t-test was used to assess differences in body measurements between Sanderlings from Chaplin Lake and Padre Island.

A model to estimate the probability of a new bird (i.e., of known wintering origin in Padre Island, Texas) belonging to one of the determined Chaplin Lake group clusters was developed. The proposed method is based on the Kernel punctual intensity estimation method. This analysis aims to characterize the spatial distribution pattern of isotope ratios as “events”. As

a result, we can determine whether those events have a higher probability of occurring in the determined isotopic space. Therefore, we can estimate the probability of known and unknown (new) Sanderlings to belong to a given Chaplin Lake cluster (see Appendix A for details on the derivation of the models.

2.3 Results

2.3.1 Sanderling banding and resightings

During the 4 years of project, 415 Sanderlings were banded at Chaplin Lake and Padre Island. From the 382 Sanderlings banded in Chaplin Lake, 25, 113, 163, and 81 birds were banded in 2012, 2013, 2014, and 2015 respectively. In Padre Island, 33 Sanderlings were banded in 2015.

A total of 29 resightings were recorded. During the fall migration, 22 resightings of Sanderling banded at Chaplin Lake occurred throughout eastern Canada and the United States (Table 2.2). Other 8 sightings in late winter (January to April) were concentrated in the Gulf of Mexico (Florida, Texas) and New Jersey, Mexico, Panama, and El Salvador (see Table 2.2). It was not possible to identify in which year the resighted Sanderlings were banded, since we used the same colour combination for the project as a cohort during 2012-2014. From 2015, we used engraved (coded) flags with unique identifiers. A total of 114 Sanderlings were banded in 2015 with coded flags and 7 were later resighted.

Table 2.2. Resightings of Sanderlings banded in Chaplin Lake, Saskatchewan during May and June 2012-2015.

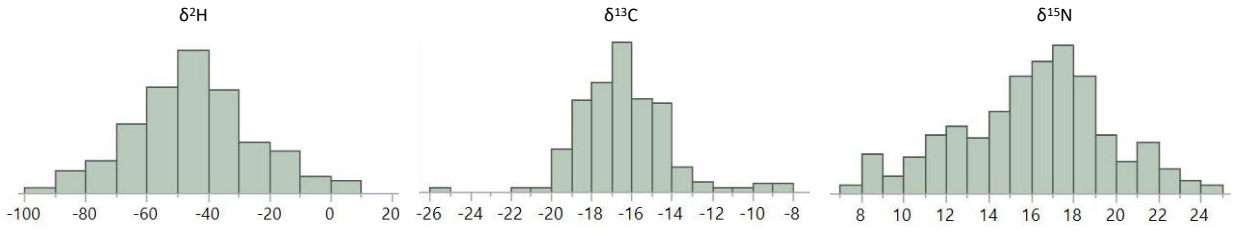
Resight year	Resight Date	State/Province	Country	Marker type
2013	August 13	Indiana	United States	FW
2014	July 30	Nova Scotia	Canada	FW
2014	August 7	New Jersey	United States	FW
2014	August 30	New Jersey	United States	FW
2014	September 22	New York	United States	FW
2014	October 2	Florida	United States	FW
2014	October 3	North Carolina	United States	FW
2015	January 27	Florida	United States	FW
2015	February 2	Panama	El Salvador	FW
2015	February 11	Florida	United States	FW
2015	March 15	Texas	United States	FW
2015	August 3	New Jersey	United States	FW
2015	August 5	Texas	United States	FEW
2015	August 7	Texas	United States	FW
2015	August 11	Quebec	Canada	FEW
2015	August 15	New York	United States	FW
2015	August 21	New Jersey	United States	FW
2015	August 27	Florida	United States	FEW
2015	September 9	New Jersey	United States	FW
2015	18 March to 29 April	Sonora	Mexico	FW
2015	September 18	Florida	United States	FEW

2015	September 18	Florida	United States	FEW
2015	December 10	Florida	United States	FW
2015	December 1	Texas	United States	FW
2015	August 9	Florida	United States	FEW
2015	October 18	Florida	United States	FW
2015	December 12	Florida	United States	FW
2016	April 4	Sonora	Mexico	FEW
2016	April 28	Texas	United States	FW

FW = plain white flag (cohort band); FEW = coded white flag (individual identification).

2.3.2 Population Structure

Feather samples of δ^2H, $\delta^{13}C$ and $\delta^{15}N$ of Sanderlings from Chaplin Lake were inspected for dispersion and broad patterns (Figure 2.1; Table 2.3). The feather δ^2H presented the widest range of values from -98.2 to 9.7 ‰ (mean = -45.1, n = 271). The remaining isotopes had a smaller but similar ranges from -25.3 to -8.0 ‰ for $\delta^{13}C$ (mean = -16.4, n = 271), and 7.4 to 24.5‰ for $\delta^{15}N$ (mean = 16.0, n = 271). Across the isotopic space using all 3 isotopes of δ^2H, $\delta^{13}C$ and $\delta^{15}N$, most of the individual Sanderlings were clustered in the center of the cloud (note 12 strong outliers of δ^2H were removed prior to analysis –see methods).



B

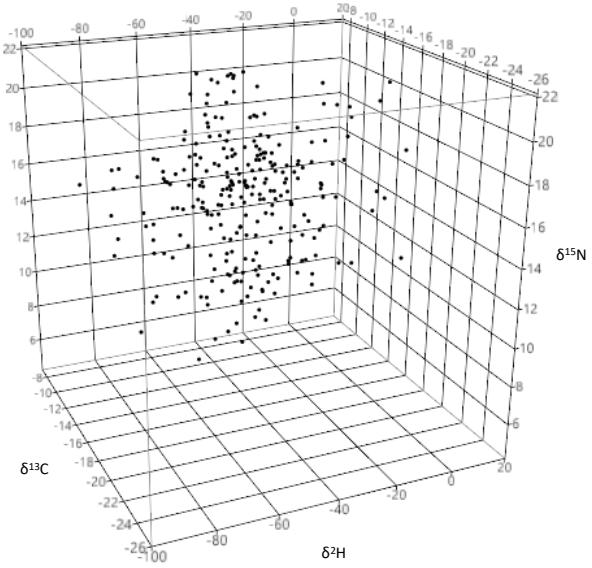


Figure 2.2. (A) Histograms and (B) 3D scatterplot of Sanderling captured in Chaplin Lake, Saskatchewan where each point is an individual's isotopic space based on feather δ^2H, δ^{13}C, and δ^{15}N values (‰).

Table 2.3. Mean ± standard error (S.E.), minimum and maximum values of δ^2H, δ^{13}C, δ^{15}N (‰) in feathers from Sanderlings captured at Chaplin Lake, Saskatchewan during 2012-2015.

	δ^2H	**δ^{13}C**	**δ^{15}N**	**n**
	mean ± S.E.	**mean ± S.E.**	**mean ± S.E.**	
	(min | max)	**(min | max)**	**(min | max)**	
Cluster 1	-68.1 ± 1.2	-17. 3 ± 0.2	16. 75 ± 0.4	77
	(-98.2 | -55.0)	(-25.3 | -10.8)	(7.4 | 23.5)	
Cluster 2	-43.8 ± 0.5	-16.2 ± 0.1	16.1 ± 0.3	135
	(-54.6 | -32.1)	(-19.9 | -9. 7)	(8.2 | 23.4)	
Cluster 3	-18.1 ± 1.3	-15.7 ± 0.4	15.0 ± 0.5	59
	(-31. 6 | 9.7)	(-19.9 | -8.0)	(7.7 | 24.5)	

Using the cluster technique, PAM, the best clusterization for this set of data included 3 cluster groups. The Chaplin Lake Sanderling population could be comprised of at least 3 broad

groups of birds. The percentage of birds in clusters 1, 2, and 3 was 28.4, 49.8, and 21.8 % respectively. The dispersion of those clusters is represented in Figure 2.4. All three groups showed the greatest separation along the δ^2H value range, likely consistent with latitude of origin (Figure 2.2, Table 2.3).

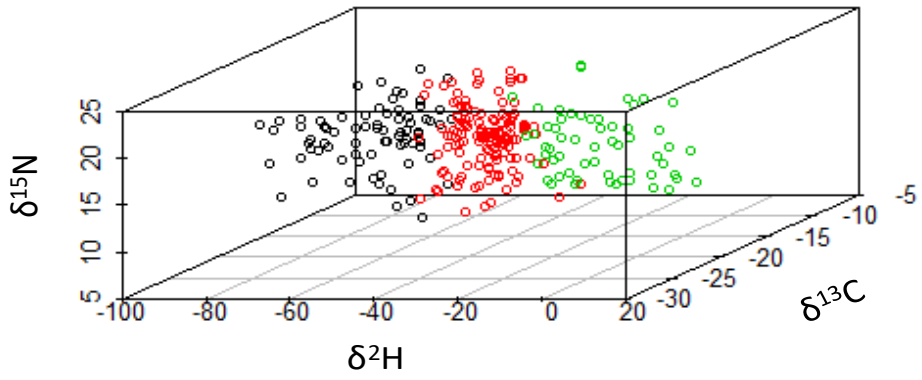


Figure 2.3. 3D Scatterplot of Sanderling feather stable isotope values from individuals captured in Chaplin Lake. Clusterization of δ^2H, $\delta^{13}C$, and $\delta^{15}N$ isotope values (‰) in feathers was determined using the PAM technique: Cluster 1 = black, 2 = red, and 3 = green.

2.3.3 Comparison of morphometric measurements among clusters

ANOVA and post-hoc Turkey´s procedure were used to assess whether Sanderling's body measurements differed among clusters which may improve the interpretation and significance of the cluster grouping. Mean wing measurements of cluster 1 (127.3 ± 0.4 mm) and cluster 2 (126.5 ± 0.3 mm) were similar, but differed for cluster 3 (124.5 ± 0.5 mm). Sanderlings from cluster 3 had significantly smaller wing sizes ($F_{2,263}$=9.48, $p<0.001$). Tarsus measurement also differed between Sanderling clusters ($F_{2,263}$=6.87, $p<0.01$). Cluster 1 Sanderlings had longer tarsus (28.0 ± 0.2 mm) than cluster 2 (27.1 ± 0.2 mm) and cluster 3 (27.0 ± 0.2 mm). No

difference in bill measurements ($F_{2,264}$=1.95, p=0.14) or body mass ($F_{2,269}$=1.70, p=0.18) were observed between the clusters.

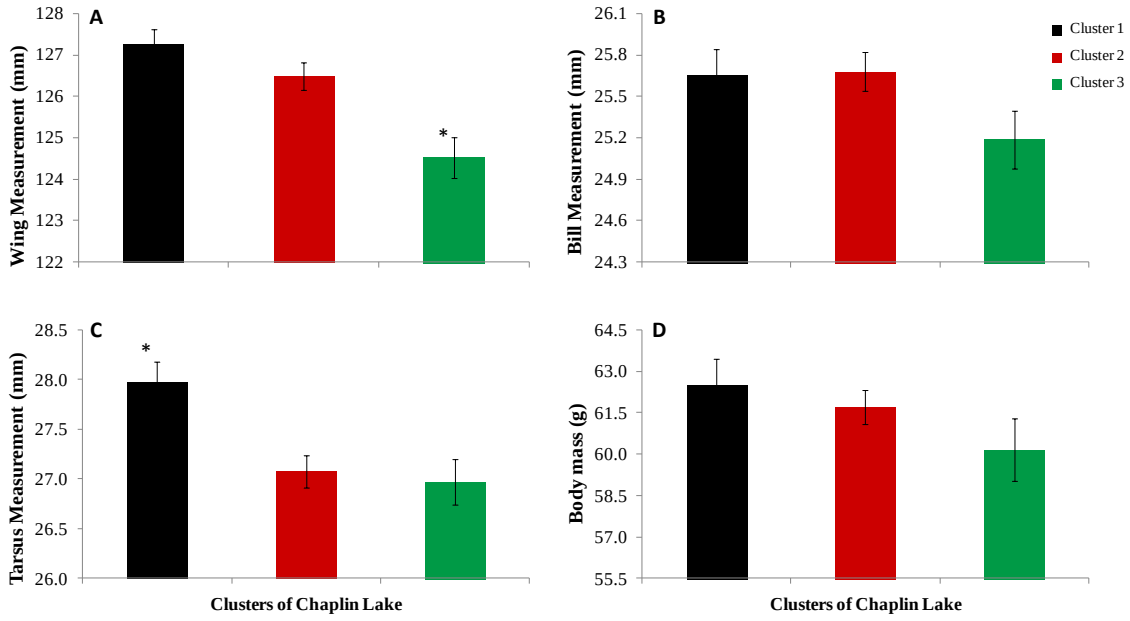


Figure 2.4. Mean ± SE of a) wing (mm), b) bill (mm),c) tarsus (mm), and d) body mass of clusters 1, 2, and 3 of Chaplin Lake Sanderling population measured in May and June 2012-2015. Stars represent significance of the measurement among clusters (Turkey, p<0.05).

Table 2.4. Mean ± SE of wing (mm), bill (mm), tarsus (mm), body mass, and fat score of clusters 1, 2, and 3 of Chaplin Lake Sanderling population measured in May and June 2012-2015.

	Wing (mm)	**Bill (mm)**	**Tarsus (mm)**	**Body mass (g)**	**Fat score**
Cluster 1	127.6 ± 0.4	25.7 ± 0.2	27.9 ± 0.2	62.5 ± 0.9	3.2 ± 0.1
Cluster 2	126.5 ± 0.3	25.7 ± 0.1	27.1 ± 0.2	61.7 ± 0.6	3.3 ± 0.1
Cluster 3	124.5 ± 0.5	25.2 ± 0.2	26.9 ± 0.2	60.2 ± 1.1	3.2 ± 0.1

The mean fat score of all birds was 3.3 ± 1.0 (Table 2.4). However, the distribution of the scores were different among clusters ($\chi^2_{(2, 259)}$ = 0.03, p < 0.01) (Figure 2.4). Cluster 1 and 2 had more heavy birds (fat scores of 5, respectively 18.8 % and 12.9 %) and relatively few thin birds

(fat scores 1, respectively 3.1 % and 3.2 %). Cluster 3 had a significant more lean Sanderlings with fat score of 1 (7.8 %) with few fat individuals with fat score of 5 (3.9 %).

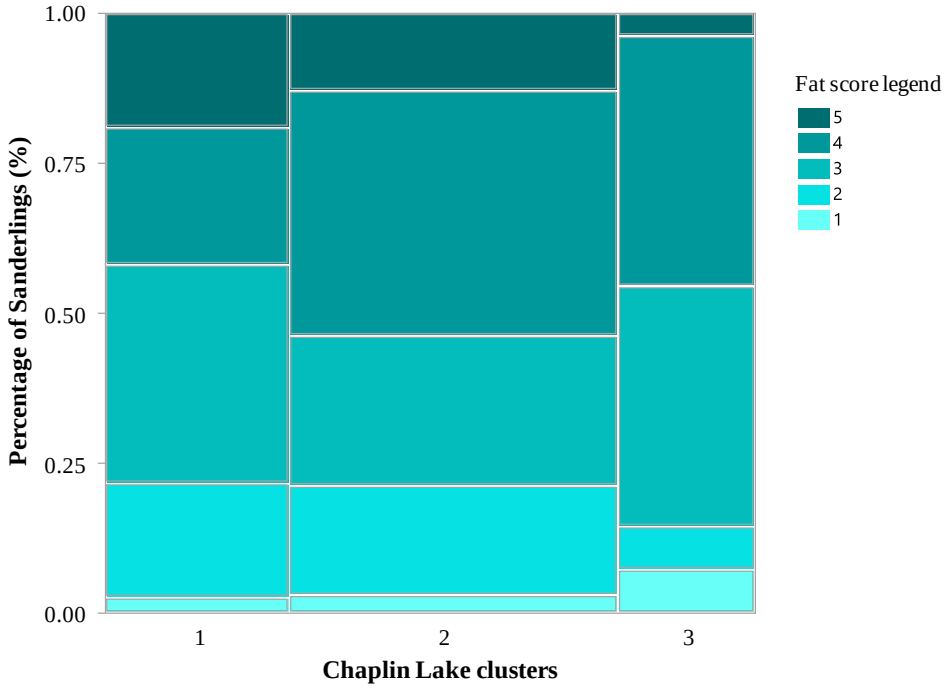


Figure 2.5. Percentage of Sanderlings in each fat score category for Chaplin Lake population clusters 1, 2, and 3.

Sanderlings that were captured later in the season exhibited higher fat scores than those caught earlier in the season. This was demonstrated in all three clusters. At the beginning of May (Day 133-140), birds from all the clusters presented average fat scores of 1.5 ± 0.7. At the end of the spring migration (Day 151-158), Sanderlings typically had a fat score of 4.1 ±0.7 out of maximum of 5 indicating increasing fuelling status during the migratory stopover.

The annual peak of the Sanderlings migration occurred at the end of May (day 148) (Figure 2.5). The peak capture date was the same for all 3 clusters whereas the mean capture date was 147.9 ± 0.4 for cluster 1, 148.1 ± 0.3 for cluster 2 and 147.9 ± 0.4 for cluster 3 ($F_{2,260}$=0.05,

p=0.95). ANOVA was used to access whether there was any difference in capture date by clusters, but no difference was observed ($F_{2,270}$=0.31, p=0.73).

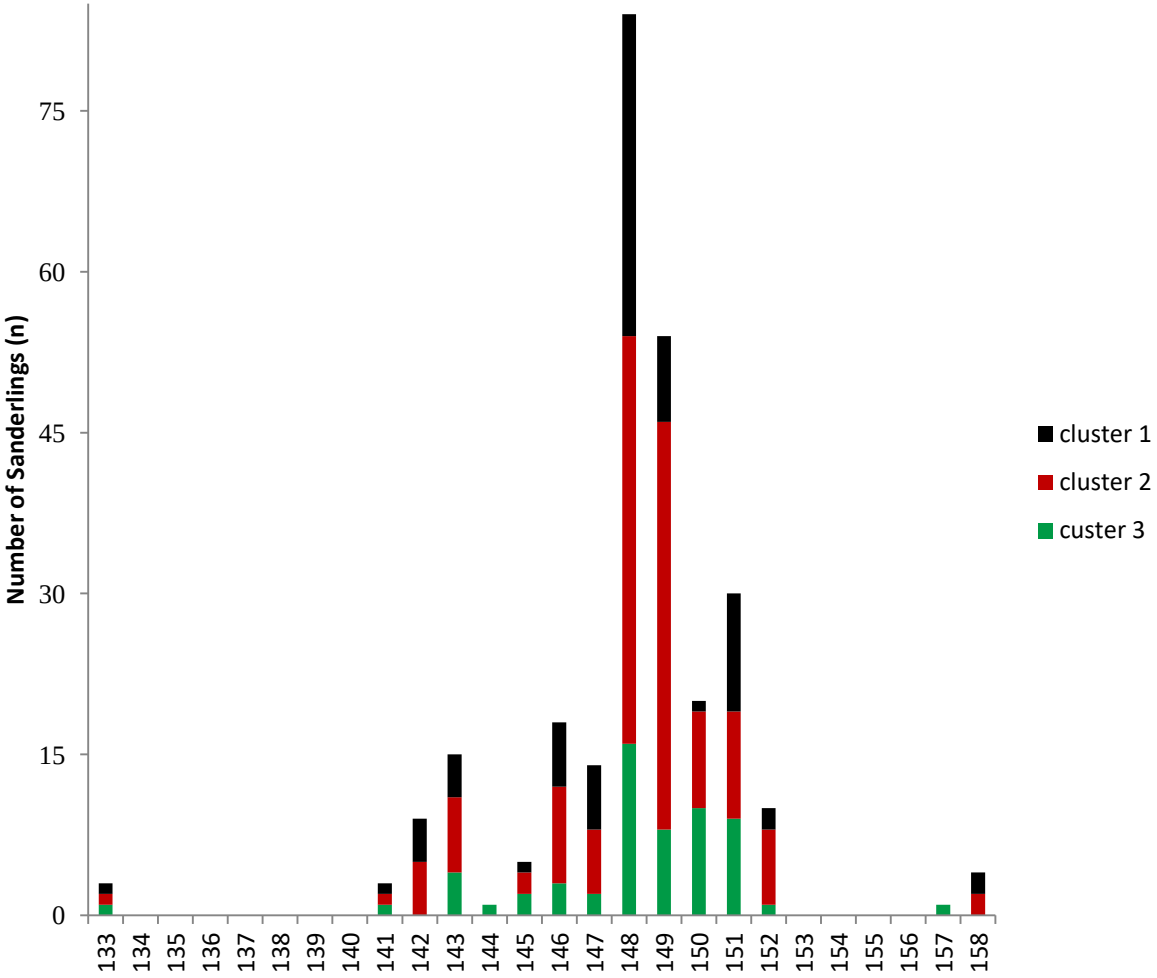


Figure 2.6. Percentage of Sanderlings captured by Julian date during spring migratory season (May to June) in Chaplin Lake (2012-2015).

2.3.4 Comparison of known wintering origin Sanderlings from Padre Island, Texas to Chaplin Lake population

Stable isotope profiles of Sanderlings from Padre Island were similar in comparison with birds from Chaplin Lake. Sanderling feather samples from Padre Island were distributed among all 3 clusters without any visual outliers that would indicate a missing cluster.

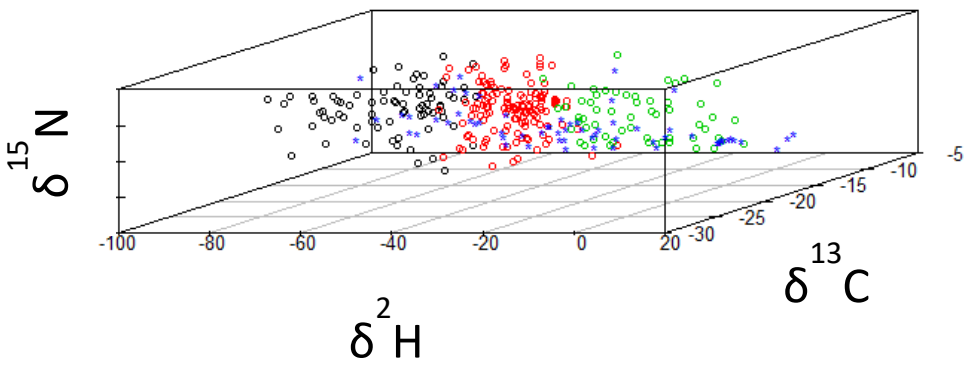


Figure 2.7. 3D Scatterplot of sanderling feather stable isotope values from individuals captured in Chaplin Lake during migration and Padre Island, Texas, United States during late winter. Clusterization of δ^2H, $\delta^{13}C$, and $\delta^{15}N$ isotope values (‰) in feathers was determined using the PAM technique: Chaplin Lake Cluster 1 = black, 2 = red, and 3 = green. Padre Island, Texas sanderlings = blue. Texas sanderlings overlapped with all 3 of the pre-determined Chaplin Lake population groups.

Using the previous cluster group separation, I could use the model to determine the probability of these "new" sanderlings from known winter origin in Padre Island, Texas as belonging to clusters 1, 2, and 3 (Table 2.5). Of the 63 Texas sanderlings studied, 12 (19%) were classified to cluster 1, 16 (25%) to cluster 2, and 35 (56%) to cluster 3 showing significant overlap across the groups.

Table 2.5. Probability of Sanderlings captured in Padre Island, Texas belonging to the previously determined cluster groupings of migrant Chaplin Lake population.

Chaplin Lake clusters	Number Assigned birds	% Assigned to each Cluster	Proportional probabilities for Texas birds to belong in Chaplin Lake clusters		
			Cluster 1	Cluster 2	Cluster 3
Cluster 1	12	19 %	0.93 ± 0.14	0.06 ± 0.1	0.00 ± 0.00
Cluster 2	16	25 %	0.07 ± 0.11	0.84 ± 0.13	0.07 ± 0.13
Cluster 3	35	56%	0.00 ± 0.00	0.05 ± 0.10	0.94 ± 0.10

Mean wing measurements of Sanderling in cluster 1 from Chaplin Lake and Padre Island were similar ($F_{1,87}$=0.66, p=0.42). However, birds from Chaplin Lake had shorter wings than Padre Island, which was statistically different in cluster 2 ($F_{1,143}$=7.05, p<0.01) and in cluster 3 ($F_{1,77}$=7.37, p<0.01) (Table 2.7). Mean tarsus lengths of Sanderlings from Chaplin Lake and Padre Island clusters were statistically similar for all three clusters (Cluster 1: $F_{1,94}$<0.001, p=0.05;cluster 2: ($F_{1,138}$<0.001, p=0.92); and Cluster 3: $F_{1,58}$<0.001, p=0.61). Bill length was not collected from Padre Island Sanderlings.

Table 2.6. Comparison of mean ± standard error (S.E.), minimum and maximum values of wing length (mm) and tarsus length (mm) of Sanderlings from cluster 1, 2, and 3 in Chaplin Lake, Saskatchewan (CL) and Padre Island, Texas (PI). Note: Bill length was not collected from Padre Island birds.

Cluster	Location	Wing (mm) n	Wing (mm) Mean ± S.E. (min | max)	Wing (mm) Signif.	Tarsus (mm) n	Tarsus (mm) Mean ± S.E. (min | max)	Tarsus (mm) Signif.
1	CL	76	127.2 ± 0.4 (119 | 134)	A	76	27.9 ± 0.2 (24.9 | 31.8)	A
	PI	12	128.1 ± 1.0 (122 | 134)	A	19	27.1 ± 0.3 (24.2 | 28.9)	A
2	CL	132	126.5 ± 0.3 (119 | 137)	A	132	27.1 ± 0.2 21.5 | 32.1)	A
	PI	12	129.5 ± 0.5 (126 | 132)	B	7	26.9 ± 0.4 (25.3 | 28.9)	A
3	CL	56	124.5 ± 0.5 (118 | 137)	A	56	26.9 ± 0.2 (24.3 | 30.8)	A
	PI	22	126.9 ± 0.5 (123 | 133)	B	3	26.5 ± 0.8 (25.1 | 27.8)	A

Means with different letters indicate significant difference between birds from different sites (Chaplin Lake vs. Padre Island) within each cluster (t test, p<0.01).

2.4 Discussion

2.4.1 Determining winter origin of Chaplin Lake Sanderling population

We attempted to determine the wintering origin of Chaplin Lake Sanderlings using a combination of band resightings and stable isotope values in order to better understand the migratory connectivity for this species and the importance of Chaplin Lake as a stopover site during northward migration. Over the years, the most widely used tool to determine migration and winter locations have been the recovery or resighting of bands and coloured leg markers [4]. However, its utility is limited by the need to band large numbers of birds and by the chance of recapture or resighting [4, 34]. Among the 415 Sanderlings banded in this project, 29 (give 6.9 %) were resighted and no Sanderling was recaptured. The resightings of Sanderlings during fall migration along the east coast of Canada and The United States is consistent with a previously described elliptical pattern of migration [3, 7, 9]. In spring, the Central northern migration occurs from March until June [7]. The peak passage of Sanderlings varies by latitude, with Chaplin Lake arrivals peaking at the end of May [14] as was also observed in this study. Most Sanderlings appear to fly south through the Eastern flyway [7, 9]. The number of birds migrating south through Chaplin Lake is considerably smaller compared to the spring migration [14]. Most birds banded in Chaplin Lake during spring migration were resighted along the East Coast of the United States. Sanderlings are likely migrating further south than our band resightings suggest. Banded Sanderlings in Chile and Peru have been resighted throughout the Central flyway [10]. But the lack of winter resights precludes our ability to identify more precise winter locations.

Chaplin Lake Sanderlings could be separated into 3 isotopically distinct groups. Hydrogen isotopes provided the greatest resolution with Cluster 1 (most negative) < Cluster 2 < Cluster 3 (least negative). However, the exact winter location of these Sanderlings cannot be

determined by stable isotope alone, since no birds were previously banded. However, feather isotopes values of other shorebird species such as the Red knot or white-rumped Sandpiper (*Calidris fuscicollis*) sampled on the wintering grounds have been previously published in the literature (Table 2.7). This information was compared to our Sanderling values to help interpret the isotopic wintering origins of the Chaplin Lake population. As observed in Table 2.7, very negative δ^2H can be found in feather of shorebirds wintering in southern South America.

Table 2.7. Sample of published data of feather isotopic values (‰), date of sampling and location of winter origin of multiple shorebirds species for comparison to the existing dataset.

Shorebird	Location	Date	δ^2H	$\delta^{13}C$	$\delta^{15}N$	author
Red Knot (*Calidris canutus*)	Argentina Rio Grande (TdF)	Nov 2012	−60.4 ± 1.28 *n*=6	−9.9 ± 0.30 *n*=14	18.1 ± 0.13 *n*=14	Atkinson et al., 2005 [11]
Red Knot (*Calidris canutus*)	Chile Bahia Lomas (TdF)	Feb 2003	-84.78 ± 5.04 *n*=4	−14.1 ± 0.13 *n*=11	16.4 ± 0.22 *n*=11	Atkinson et al., 2005 [11]
White-Rumped Sandpiper (*Calidris fuscicollis*)	Argentina Rio Grande (TdF)	Jan2001		-8.85 ± 0.07 *n*=2	19.8 ± 0.14 *n*=2	Farmer et al., 2003 [107]
White-Rumped Sandpiper (*Calidris fuscicollis*)	Argentina Laguna Mar Chiquita	Jan2001		-17.25 ± 3.97 *n*=7	10.95 ± 1.88 *n*=7	Farmer et al., 2003 [107]
White-Rumped Sandpiper (*Calidris fuscicollis*)	Argentina Laguna Dom Tomas	Jan2001		-20.5 *n*=1	11.00 *n*=1	Farmer et al., 2003 [107]
Pectoral sandpiper (*Calidris melanotos*)	Argentina Laguna Dom Tomas	Jan2001		-19.35 ± 3.60 *n*=2	9.30 *n*=2	Farmer et al., 2003 [107]
Greater Yellowlegs (*Tringa melanoleuca*)	Argentina Laguna Dom Tomas	Jan2001		-28.7 *n*=1	7.8 *n*=1	Farmer et al., 2003 [107]
Least Sandpiper (*Calidris minutilla*)	Quivira National Wildlife Refuge Kansas	Jul to Sep 2006 and 2007	-38 ± 17 *n*=51			Franks et al., 2009 [106]
Sanderling cluster 1 (*Calidris alba*)	Chaplin Lake Saskatchewan	May/Jun 2012-2015	-68.09 ± 1.18 *n*=77	-17.29 ± 0.21 *n*=77	16.65 ± 0.36 *n*=77	This study
Sanderling cluster 2 (*Calidris alba*)	Chaplin Lake Saskatchewan	May/Jun 2012-2015	-43.81 ± 0.51 *n*=135	-16.24 ± 0.14 *n*=135	16.09 ± 0.28 *n*=135	This study
Sanderling cluster 3 (*Calidris alba*)	Chaplin Lake Saskatchewan	May/Jun 2012-2015	-18.05 ± 1.31 *n*=59	-15.70 ± 0.37 *n*=59	15.03 ± 0.52 *n*=59	This study
Sanderling cluster 1 (*Calidris alba*)	Padre Island Texas	Feb 2013 and 2015	-64.76 ± 2.06 *n*=12	-16.59 ± 0.44 *n*=12	15.88 ± 0.82 *n*=12	This study
Sanderling cluster 2 (*Calidris alba*)	Padre Island Texas	Feb 2013 and 2015	-45.04 ± 1.25 *n*=16	-16.28 ± 0.30 *n*=16	14.63 ± 0.74 *n*=16	This study
Sanderling cluster 3 (*Calidris alba*)	Padre Island Texas	Feb 2013 and 2015	-21.17 ± 1.82 *n*=35	-12.64 ± 0.57 *n*=35	11.45 ± 0.54 *n*=35	This study

TdF = Tierra del Fuego

Isotopic values of $\delta^{13}C$, and $\delta^{15}N$ were very similar among all of the clusters so more data would be needed to compare these with known origin Sanderlings. These data must be interpreted with caution as there are many factors that affect isotopic values. Coastal areas are often difficult to determine isotopically. Published isomaps of South America are also more difficult to interpret latitudinal effects especially when compared to birds wintering in the Gulf of

Mexico [34, 39]. We also do not have good information of whether isomaps of hydrogen in precipitation or in the marine oceans are suitable for use with shorebird feathers. During the absorption process, isotopes can also undergo isotopic discrimination, which can create different isotopic values for each tissue [113].

The Sanderling population of Chaplin Lake could be identified in at least 3 broad but distinguishable groups using the combination of $\delta^{2}H$, $\delta^{13}C$, and $\delta^{15}N$ with most birds belonging to cluster 2 (49.8 %), followed by cluster 3 (21.8 %) and cluster 1 (28.4 %). Despite the fact that none of the stable isotopes could be excluded from the analysis without losing a significant amount of information, the $\delta^{2}H$ was visually the most important isotope to separate the different groups of Sanderlings. This might be due to the large range of values presented by $\delta^{2}H$ (-98.24 to 9.65 ‰). Or perhaps it is because $\delta^{2}H$ is a good stable isotope to distinguish latitudinal separation, acknowledging that Sanderling winter ranges extend from along the southern coasts of the United States all the way to Argentina [3, 7, 9].

2.4.2 Population structure revealed through Sanderling body measurement.

Sanderlings have a strong inter- and intra- winter ground fidelity [10], which could result in geographic variation of body measurements [114, 115]. Differences in body size have been observed in other shorebird species in relation to migration distance [4, 115, 116]. Sanderling clusters from Chaplin Lake did not differ in bill length or body mass. However, variation in wing and tarsus size indicates that the body size of cluster 1 > cluster 2 > cluster 3. Study on wintering Sanderling populations reported subtle differences in wing length with the largest wings in birds wintering furthest south in Mehuin, Chile (December to February) [105]. Geographic differentiation in wing length may be an adaptation to improve aerodynamics for longer

migration flight distances [114, 115]. Larger body size may also be caused by environmental factors, such as diet quality [117, 118], and temperature [114]. The larger size of Sanderlings wintering in higher latitudes (e.g. Chile) was justified as an adaptation to colder weather. Larger birds would have a proportionally smaller body surface for heat loss compared to smaller birds [114].

Average body mass, and fat score values were similar among the three Sanderlings cluster from Chaplin Lake, however the percentage of Sanderlings from each fat score was different among the clusters. In all three groups the predominance of birds presented a fat score of 3 and 4 but differences were observed in the proportion of lightest and heaviest birds. Cluster 1 birds which isotopically were the most negative δ^2H and the larger body size also had a greater proportion of fat individuals. Cluster 3 birds which were the most isotopically enriched in δ^2H with the smallest body size also had a greater proportion of thin birds. The variation observed could be due to conditions at their wintering origin or due to the date of capture and random weather responses such as fat reserves affected after peaks of cold weather and storms [119, 120]. Most of the birds were captured at the peak or end of the season, when they should be preparing to continue the migration to their breeding ground [7, 14]. Therefore, they should have acquired a larger reserve of fat to accomplish the long fly to the Arctic [84, 109, 121, 122]. We observed fat scores increasing during the migration season, demonstrating that capture date has a considerable influence on fat scores [123].

Regarding migration period, the number of Sanderlings captured was proportional to their abundance in Chaplin Lake [7, 14]. All three clusters had the highest capture rate around May 28 (Day 148), consistent with the season peak [7, 14]. The migration period for birds wintering in different latitudes along North, Central and South America is different. Birds wintering in

southern locations should start migrating earlier [6, 7] but we did not find any difference in capture date by cluster. Although, this assumes capture date is related to arrival date which may or may not be a correct assumption.

2.4.3 Probability prediction and implication for future work.

Based on the distribution probability of $\delta^{2}H$, $\delta^{13}C$, and $\delta^{15}N$ of Sanderlings feather captured in Chaplin Lake (CL), a model was created to estimate the probability of a new Sanderling belonging to one of the three previous determined clusters. Below are considerations for the use of this model: (1) the reported probability will be distributed among the clusters must total 1 (i.e. Cluster 1, 2, and 3 would have 0.1, 0.1, and 0.8, respectively); (2) if the new isotopic values are vastly different from the CL ones, the creation of a fourth cluster should be considered, meaning that the new bird (s) may not belong to any of the CL clusters; (3) the probability cut-off should be determined by the researcher; (4) the formula could be applied to a single Sanderling or to an entire population; (5) future researchers sampling a large number of Sanderlings also captured from CL, may consider adding the new birds to the initial model, as they will increase the power of the analysis; and (6) the model is very versatile and can easily be re-written and adapted to a new population of interest, including other species of birds and other animals.

When the model was applied to a new but known winter population of Sanderlings from Padre Island, the results revealed over half of the birds (56%) were from Chaplin Lake cluster 3 with smaller numbers belonging to each of the other 2 groups. Padre Island is considered both winter ground and stopover [3, 7]. The results from this study are consistent with this information that Sanderlings from, or stopping at, Padre Island were considered to migrate North

through the Central flyway [3, 7, 9]. Based on this information, and the isotopic values, the Padre Island birds might be made up of a mixture of mostly winter resident Sanderlings and a smaller number that are moulted further south and then migrated through Padre Island en route to Chaplin Lake.

Wing and tarsus measurements of Padre Island Sanderlings were evaluated for the similarity to Sanderlings from Chaplin Lake where we expected that if the new assignments were correct, there should be large overlap in morphometrics. Tarsus lengths, presented the same measurements among clusters from both locations, indicating possibly correct assignment of origin. Wing length of birds from cluster 1 were similar; however Sanderlings from cluster 2 and 3 of Padre Island had longer wings compared to Chaplin Lake suggesting some uncertainty.

2.5 Conclusion

The population of Sanderlings migrating north through Chaplin Lake appears to have originated from at least three different winter locations. Information collected in Chaplin Lake on feather isotopic values, body morphometrics, and reported data on migration strategy and isotope values were useful in providing evidence of potential wintering areas. Interestingly, the H isotope data, was able to distinguish different groups that are largely separated based on latitudes and suggests birds using Chaplin Lake as a stopover vary widely in their migration distance and winter origin.

The combination of δ^2H isomap [41], published data in shorebirds, and distribution and abundance of Sanderlings [3, 7, 8] suggests there are 3 different groups of birds migrating through Chaplin Lake with possible origins of cluster 1 in the southern portion of South America (e.g. Chile/Argentina), cluster 2 in northwestern part of South America (e.g. Peru), and cluster 3

in Gulf of Mexico (e.g. Padre Island). Evidence from the isotopes, large body size, and higher fuelling levels also support the notion that at least one of the Sanderling groups identified (Cluster 1) may have originated from a much further south – possibly southern Chile. By contrast, the opposite cluster 3 group had a smaller body size but lower body fat and likely wintered in the Gulf of Mexico region. This may suggest that this group is experiencing reduced fueling ability or later migration. In Texas, Sanderling foraging times were significantly greater and birds took longer to satisfy energetic requirements at this location during the non-breeding season [114]. Additionally, the isotope work confirms the assumption that Sanderlings found in late winter in the Texas Gulf are common to the Chaplin population but with significant overlap across all 3 clusters. Texas Sanderlings that were sampled were mostly winter residents but may also have included a mixture of migrants from further south. Alternatively, moult in Sanderling could be quite variable with some birds growing feathers further north than previously assumed. We had a small number of birds (n=16) with extremely negative δ^2H values that we excluded from analysis on the assumption that they moulted on the breeding grounds in the Arctic which is contrary to expectation.

The newly generated predictive isotope model proved to be useful for understanding origin and staging of migrating Sanderlings which could be an excellent tool for future studies in animal migration. Additionally, this study has demonstrated the importance of Chaplin Lake to shorebird migration, specifically to Sanderlings. This unique stopover contains a large accumulation of Sanderlings over a brief period that appear to originate from a large winter range in North and South America, demonstrating its conservation importance and value to birds using the Central flyway.

APPENDIX A

The proposed estimated probability of a new observed sanderlings $u_{NEW}{}^{*}$ belong to a given cluster *b* defined by:

$$P(B_{NEW} = b|u = u_{NEW}{}^{*}) = \frac{\hat{\lambda}_{sb}(u_{NEW}{}^{*})}{\hat{\lambda}_{s1}(u_{NEW}{}^{*}) + \cdots + \hat{\lambda}_{sb_{total}}(u_{NEW}{}^{*})}, \quad b = 1, \ldots, b_{total}. \tag{A.1}$$

Where $\widehat{\lambda s}_1(u_{NEW}{}^{*}) + \cdots + \hat{\lambda}_{sb_{total}}(u_{NEW}{}^{*})$ are the standardized kernels estimative of cluster 1,...,b_{total} for a new event observed $u_{NEW}{}^{*}$.

The described method was applied to the Sanderling population of Chaplin Lake and its 3 clusters: The intensity distribution of each one of the 3 clusters was presented in Figure A.1. The variable B (cluster) was defined as b=1, 2, 3.

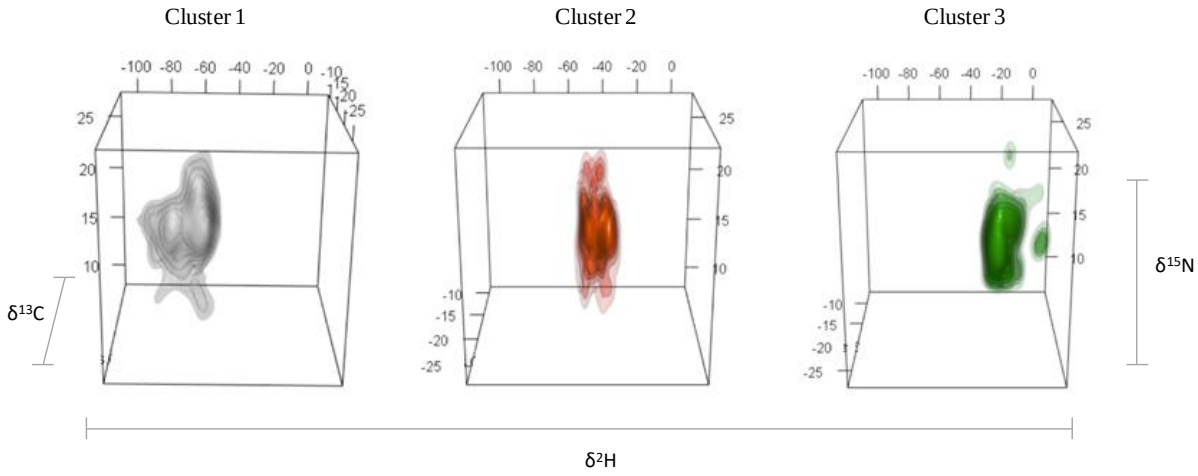


Figure A.1. Estimation of kernel intensity for each of the three pre-determined clusters of Sanderling from Chaplin Lake.

The resulting histogram of the kernel intensity distribution was presented in Figure A.2. Cluster 3 presented less concentrated values when compared to the other 2 clusters. Consequently, the odds of a new Sanderling belonging to cluster 3 would be underestimated. Therefore, the estimation of intensity needed to be standardized. The estimated intensity may

assume values from 0 to 1, thus all the values were divided by the maximum value of their respective cluster.

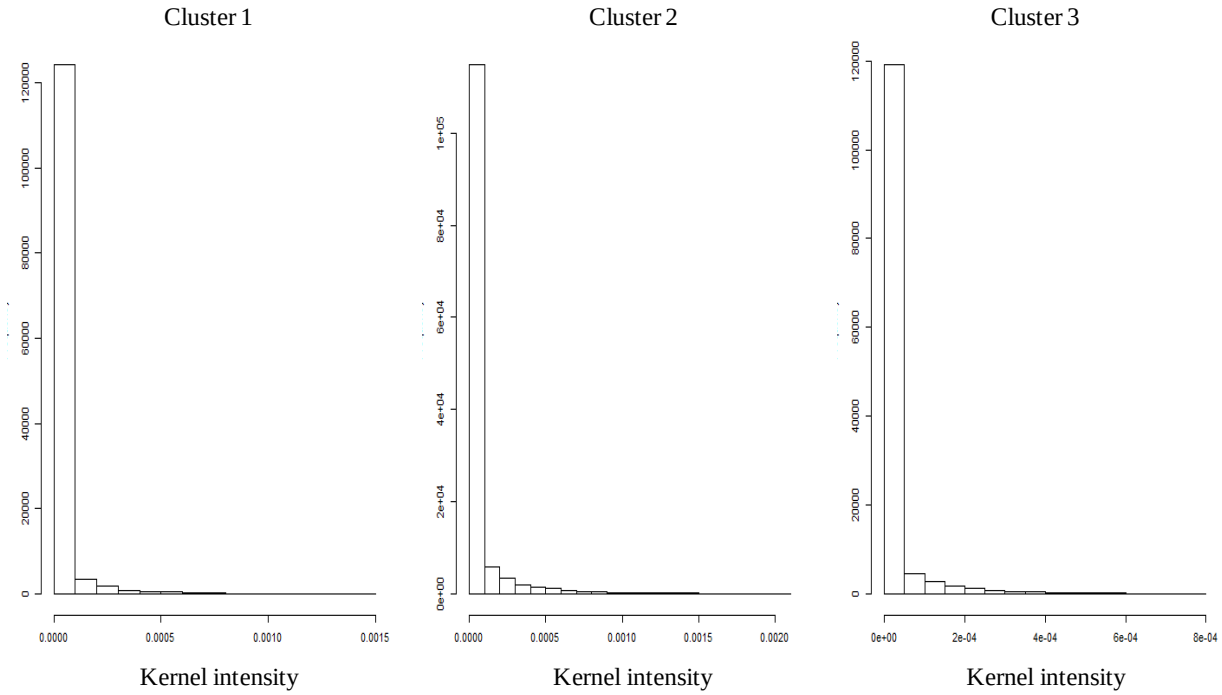


Figure A.2. Histogram of the estimation of kernel intensity of the three clusters of Sanderling from Chaplin Lake. The intensities were presented in different scales due to the large difference in values.

The probability of a new Sanderlings (B_{NEW}) belonging to the clusters 1, 2, and 3 of the Sanderling population of Chaplin Lake was demonstrated in equation A.2.

$$P(B_{NEW} = b|u = {u_{NEW}}^*) = \frac{\hat{\lambda}_b({u_{NEW}}^*)}{\hat{\lambda}_1({u_{NEW}}^*) + \hat{\lambda}_2({u_{NEW}}^*) + \hat{\lambda}_3({u_{NEW}}^*)}, \quad b = 1,2,3. \tag{A.2}$$

Where $\hat{\lambda}_1({u_{NEW}}^*) + \hat{\lambda}_2({u_{NEW}}^*) + \hat{\lambda}_3({u_{NEW}}^*)$ are the kernels estimative of the cluster 1, 2, and 3.

CHAPTER 3

Hazard assessment of industrial contamination at key stopover and wintering sites across the migratory range of shorebirds

3.1 Introduction

Shorebirds are known for their migratory habits, which includes some of the longest non-stop flights amongst birds [2].They are widely distributed in the world and are mostly associated with open habitats [1]. In the Western hemisphere they can be found wintering at a large variety of sites, mostly in coastal areas of the United States and Central and South America [6, 7]. In recent years, many shorebird populations have rapidly declined for reasons not well understood [3, 6, 7, 16]. Surveys of South America showed that the Red knot (*Calidris canutus rufa*) winter population in Tierra del Fuego, Argentina dramatically dropped from 67,500 in 1985 to 31,500 in 2004, and to 17,200 in 2006 [4] [124]. In Lagoa do Peixe, Brazil the peak count of Red knot during spring migration was just 9% of 10,000 animals counted in 1995 [124]. More recently survey indicated population of 4,000 Red knots [125]. In Delaware Bay, United States on of the most important stopovers for Red knots, the peak count in 1989 was 94,500 birds while in 2010 the peak was just 14,000 [124]. Following the same trend, Sanderling (*Calidris alba*) populations has also been reported to be declining. The population documented in 1972 was 1.5 million, but the current population is estimated at 300 thousand birds [7]. Similarly, other species such as Semipalmated sandpiper (*Calidris pusilla*) have been classified as moderate concern because of their similar declines and potential threats to their population [7, 17].

Several potential threats have been identified to affect shorebirds such as the loss of winter habitat, decreased availability of food, disease, higher predation rates, and pollution [6, 7].

Among all these possible threats, metal contamination in shorebirds has been demonstrated along the winter, stopover and breeding grounds [126-129]. Levels of mercury and lead were negatively correlated to shorebirds reproductive success [127] in Artic breeding populations. Blood mercury concentrations in Avocets (*Recurvirostra americana*) and Black-necked Stilts (*Himantopus mexicanus*) from San Francisco Bay, California showed that part of the population was within the range known to cause toxic effects [126]. Selenium levels in feathers of Red knots (*Calidris canutus*), Sanderlings and Semipalmated sandpipers from Delaware Bay, United States showed potential risk of selenium toxicity [128]. However, relatively little work has been done to evaluate organic pollutant exposure to shorebirds.

While there are many organic contaminants capable of affecting shorebirds, industrial pollution including dioxins and "dioxin-like" chemicals (DLCs) such as dioxins and furans, polychlorinated biphenyls (PCBs), and polycyclic aromatic hydrocarbons (PAHs) found in petroleum based products have largely been overlooked despite their global distribution, persistence, toxicity, cumulative effects [19, 20], environmental persistency [130], and large quantities introduced into the environment annually [21, 22]. Shorebirds are likely to be exposed to these compounds through ingestion of sediment and in their diet which is largely comprised of sediment dwelling invertebrates [129, 131].

The effects DLCs have been widely reported to affect birds including altered endocrine function [70, 78] such as variation of thyroid hormone levels [87, 91], disorientated migratory behavior [92], abnormal parental behaviour [93], lower growth rates [94], carcinogenesis and mutagenesis [75], immunosuppression [79, 80], liver damage [20, 67, 81]; and hemolytic anemia [82]. Studies have also alluded to impacts on fatty acid metabolism and fattening rates associated

with low level ingestion of petroleum based compounds [65] and increased metabolic rates [132].

Despite there are many ways to assess environment contamination exposure in birds, such as measurement of chemicals directly in the bird, there are ethical reasons that encourage the use of other less invasive techniques to do the assessment. Another common way to assess contamination exposure is through the foodweb. This technique is non-invasive, there is no need to catch the birds, and also would account for bioaccumulation. However, in the case of shorebirds it is difficult to collect a significant and representative sample of their diets. Shorebirds feed in a large variety of invertebrates, therefore account for a correct proportion of each invertebrate in the diet and also account for seasonal and local (winter vs. stopover) variation could decrease the results certainty. Additionally, the transportation and preservation could be an issue considering the multinational effort to assess shorebirds contamination exposure. The assessment of contamination in sediment on the other hand could be especially important for shorebirds. Sediment are easy to collect, transport, and preserve. The same sampling technique can be easily applied throughout all shorebirds winter and stopover sites with collaborators help. It also important for this group of birds considering the proportionally large amount of sediments they ingest during preying.

To assess possible environmental contamination in sediments of the vast range of shorebird wintering and stopover areas, a novel use of H4IIE-*luciferase* (H4IIE-*luc*) bioassay was applied. The H4IIE-*luc* bioassay has been widely used in ecological risk assessment studies to assess contamination of marine and freshwater habitats [133-137]. The method has been well validated in a variety of samples [138-140], including sediment [136, 137, 141, 142]. The H4IIE-*luc* bioassay is a bioanalytical tool based on genetically modified rat hepatoma cells containing

an inserted luciferase reporter gene into the genome [136, 143]. The modified cells produce fluorescent light by the luciferase enzyme when the aryl hydrocarbon receptor (AhR) is activated. AhR is a ligand-dependent transcription factor that can be activated by AhR agonists such as dioxins coplanar PCBs and some PAHs. The strongest AhR ligand is 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), therefore results of this bioassay are presented as TCDD equivalents (TCDD-EQ). The amount of light produced by the bioassay is directly proportional to the amount of AhR-active chemicals in the sample, namely dioxin-like compounds [144]. H4IIE-*luc* is a powerful tool as it measures the total potency of a sample to mediate AhR response, thus is can be used to study toxic exposure to complex matrices, such as contamination found in sediments [136, 141, 142, 145, 146].

The objective of this study is to (1) assess environmental contamination at a selection of key stopover and wintering locations for shorebirds across their migratory range in North and South America and (2) to identify potential DLC exposure risks that shorebirds face at certain stopovers during migration and the winter season.

3.2 Methods

3.2.1 Study sites

Sediment samples were collected from locations where shorebirds are known to stage or overwinter in order to monitor their exposure to regional sources of contamination. General information about each site can be found in Table 3.1. A total of 8 sites were investigated. Six out of 8 sites were sampled once in 2014. For the other 2 sites, Chaplin Lake and Padre Island, sampling occurred in consecutive years, 2012/2013 and 2013/2014 respectively.

In Canada, samples were taken at Chaplin Lake (CL), Saskatchewan in 2012 and 2013, and at Nelson River (NR), Manitoba in 2014. Samples were also taken from a major stopover and wintering site at Padre Island (PI), Texas (United States) in 2013 and 2014. Other collection sites in 2014 included Lagoa do Peixe (LP), Rio Grande do Sul and Aracaju (AR), Sergipe, south and northeast of Brazil respectively; at Iscuandé River (IR), Naniño (Colombia) in 2014; at Salinas (SA), Province of Santa Elena (Ecuador); and at Laguna de Rocha (LR), Rocha (Uruguay). See Table 3.1 for more details.

Figure 3.1. Map of the Americas representing the sediment sample sites (red stars).

3.2.2 Sampling

In each location, different areas were sampled except by PI in 2014 which is composed by only one sampling. Sediment samples were collected from up to 15 areas where shorebirds are known to feed and congregate (see Table 3.1 for detailed information). A polycarbonate tube was used to collect sediment from three random surface cores, from 2-5 cm in depth, along a transect line perpendicular to the water shore, at sites where there was no viable disturbance of the sediment. Three transects approximately 100 meters apart were sampled. All three cores of each transect, 9 cores in total, were preserved in air tight bags, then preserved in ice and kept out of light until transported to the local laboratory where it was frozen until shipment to University of Saskatchewan for preparation and analysis.

Table 3.1. Overview of location, GPS coordinates, year of sampling, and number of areas sampled for sediments at each site locations.

Site	Province/State, Country	Central GPS coordinates*	Year	Sample ID	Total areas sampled within site
Chaplin Lake	Saskatchewan, Canada	50.441731°N 106.669028°W	2012	CL12	10
		50.441731°N 106.669028°W	2013	CL13	10
Nelson River	Manitoba, Canada	57.12663°N 91.65765°W	2014	NR14	10
Padre Island	Texas, The United States	26.905842°N 97.370356°W	2013	PI13	11
		27.605689°N 97.207709°W	2014	PI14	1
Iscuandé River	Department of Nariño, Colombia	02.62680°S	2014	IR14	5

		78.057430°W			
Salinas	Province of Santa Elena, Ecuador	02.03160°S	2014	SA14	15
		80.44117°W			
Laguna de Rocha	Department of Rocha, Uruguay	34.40358°S	2014	LR14	11
		54.17025°W			
Aracaju	Sergipe, Brazil	10.993788°S	2014	AR14	9
		37.052491°W			
Lagoa do Peixe	Rio Grande do Sul, Brazil	31.275431°S	2014	LP14	12
		50.937624°W			

*For detailed sampling information see Appendix B

3.2.3 Sample preparation

Sediment samples were homogenized and lyophilized. The same quantity of dried sediment from all the areas sampled in one single location was added together, creating one pooled sample to represent each location. Each sample was extracted in triplicate. For each pooled sample, 10g of freeze-dried sediment was extracted overnight using 350 ml Dichloromethane (DCM) / n-hexane 1:1 volume in a soxhlet extractor. The obtained extracts were evaporated using rotavapor. The dried extract was rinsed out from the round bottom flask with approximately 7 ml n-hexane and transferred to amber glass tubes. The extracts were then evaporated using a gentle stream of nitrogen. After drying, 1 ml of DCM was added to each tube. Activated copper was added to the samples to remove possible elemental sulfur and then filtered using 0.2 µm nylon filters. The extract was divided into two portions. The solvent in one of the portions was evaporated and replaced with dimethyl sulfoxide (DMSO) for the bioassay and the other portion was reserved for future chemical analysis. The final concentration of the extract was 20 g of sediment dry weight (DW) per ml of DMSO.

3.2.4 Quality control

Field blanks were assessed to investigate potential contamination during sampling and collection equipment. Plastic bags containing 10g of sodium phosphate were used as a field blank and were opened at each sampling site, without adding samples to it. Travel blanks were used to assess contamination during transportation. As a travel blank, bags designated to keep samples were randomly chosen, never opened and exposed to the same transportation conditions. Up to five blanks were assessed in each location. Lab blanks (sodium phosphate) were used to assess equipment contamination. Chemical blanks were used to identify contamination from the solvents used in the extraction. As a chemical blank, 350 ml Dichloromethane (DCM) / n-hexane 1:1 volume was added to the soxhlet without any sodium phosphate. For each individual blank, 1g of sample was extracted by soxhlet method as described previously. Results from the H4IIE-*luc* test suggested that all blank samples were comparable to the control DMSO, except one field blank from Iscuandé River (Appendix C).

Recovery blanks were used to assess extraction efficiency. Control sand (SAND Sea Washed, Fisher Scientific, The United States) was spiked with known concentrations of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) kindly donated by Sean Kennedy's lab. Samples were extracted by soxhlet method as described previously. Results from the H4IIE-*luc* test present the concentration in the final extraction as mean relative luminescence (TCDD-EQ) as a function of the TCDD standard curve (0.61, 1.85, 5.55, 16.66, 50, 150 pM TCDD). The extraction efficiency of soxhlet was 95.35% for TCDD, as the concentration expected was 200 pM and the concentration detected was 190.7 ± 36.56 pM.

3.2.5 *In vitro* bioassay

The three separate sediment extractions of each sediment pool were analysed individually. The H4IIE-*luc* bioassay was performed by a method modified from [133]. Trypsinized cells from a culture plate were diluted to a concentration of approximately 15 x 10^5 cells ml^{-1} and seeded into 54 interior wells of a 96 well plate by adding 100 µl per well. Plates were incubated for 24 hours, the medium was changed and cells were dosed with DMSO, sediment sample extract, or TCDD (control) at a final concentration of 0.1% DMSO solvent (0.1% dose), which we validated previously to be non-cytotoxic. For dose-response characterization, sediment extracts were prepared at 2 concentrations, 100 and 50%. For TCDD dose response characterization, samples were prepared at six concentrations by 3-fold serial dilution (100, 33.0, 11.0, 3.3, 1.1, and 0.3%); the highest dose was 150 pM. All samples were tested in triplicate in the same plate. Luciferase assays were conducted after 24 h of exposure using a POLARstar OPTIMA microplate reading luminometer (BMG LABTECH, Ortenberg, Germany). Luminescence values were not blank corrected. See Appendix D for detailed protocol.

Cell viability and overall cytotoxicity were determined by the use of the Cell Proliferation Reagent WST-1 assay according to the product description (Roche Diagnostic GmbH, Mannheim, Germany). Reported mean response for the WST-1 cytotoxicity test presented no pattern of cytotoxicity between treatment and control DMSO (Appendix E).

3.2.6 Determination of risk

Considering that sediment is a good part of shorebirds ingestion, contamination in sediment should be carefully considered when assessing the risk of feeding from a determined

area. To estimate shorebirds chronic daily dose of environmental contaminants due to accidental sediment ingestion, the follow equation was used:

$$\text{Ingestion dose}_i = \frac{C_s \text{ x FIR x } PI_s}{BW} \quad (3.1)$$

Where,

Ingestion $dose_s$ = the daily intake of a given compound *j* due to sediment ingestion (ng *j* / day)

C_s = concentration of a given compound *j* in the sediment (ng *j* g^{-1} sediment dw)

FIR = food ingestion rate (dry Wt) for non-passerine birds [147]. FI (g/day) = 0.648 $Wt^{0.651}$ (g).

PI_s = fraction of sediment in shorebird diet (value between 0-1, ie. from your Table 3.4 below 0.03-0.30).

BW = body weight (g) (indicate from select shorebird spp in Table 3.4)

3.2.7 Data analysis and statistics

Bioassay response units were presented as mean relative luminescence (TCDD-EQ) as a function of the TCDD standard curve (0.61, 1.85, 5.55, 16.66, 50, 150 pM TCDD). Luminescence values were transformed to a percentage of the maximum response (%-TCDDmax) observed for a standard containing 150 pM of TCDD (= 100%-TCDDmax). The concentration of TCDD in the sediment was determined by converting the mean relative luminescence units from the bioassay to TCDD equivalency (TCDD-EQ) as a function of the TCDD standard curve (0.61, 1.85, 5.55, 16.66, 50, 150 pM TCDD). The values of TCDD-EQ

(pM) found in the H4IIE-*luc* bioassay were then transformed to absolute concentration (ng TCDD g^{-1} dw) of each location. In order to validate the assay, a TCDD dose-response curve was tested in addition to samples of extracts at full strength (100% extract) or half strength (50% extract). WST-1 test response units were compared between the control, blanks and samples to verify cell toxicity.

The Canadian tissue residue guideline for polychlorinated dibenzo-*p*-dioxins (PCDDs) for the protection of wildlife consumers of aquatic biota, was used to calculate whether the concentrations of TCDD-EQ in the sediments of studied locations were safe for birds [148]. A tolerable daily intake of 4.47 ng TEQ/kg bw/d has been determined for birds (TDI_{bird}) considering chronic effects of exposure to dioxins and differences in species sensitivity [148]. The values were expressed as toxic equivalency units (TEQs) of PCDD. However, the value was extrapolated to TCDD since toxic equivalency factor for 2,3,7,8-TCDD and PCDD for birds is known to be equal to 1 [149]. Therefore, this study considered 0.00447 ng TCDD-EQ/g bw/d as the TDI_{bird}.

Data are presented as mean ± standard error of the mean (S.E.M). Normality of the dataset was determined using Shapiro-Wilk test. A logarithmic transformation was applied when necessary to improve normality. An analysis of variance (ANOVA) followed by post hoc Dunnet's tests was used to test for differences between samples and DMSO control. In all the tests above, one replicate of the Aracaju (AR) sample was removed from the analysis after being identified as an outlier.

3.3 Results

The highest bioassay response was obtained from cells dosed with a standard containing 150 pM of TCDD. Therefore, those were considered the maximum response (= 100%-$TCDD_{max}$) (table 3.2). DMSO presented a response of 0.50% of $TCDD_{max}$. Sediment sample responses varied from 0.66 to 12.17% of $TCDD_{max}$. Of the 20 samples analysed, 9 were below 1%-$TCDD_{max}$, 6 were between 1-2%-$TCDD_{max}$, 3 were between 2-5%-$TCDD_{max}$, 1 sample was between 5-10%-$TCDD_{max}$, and just one sample was above the 10%-$TCDD_{max}$ (Padre Island, Texas, 2013).

Table 3.2. Mean and standard error of mean (S.E.M.) of H4IIE-*luc* bioassay results of sediment extractions from studied locations.

Sample ID	**Dose (%)**	**n**	**%-$TCDD_{max}$** [a]	**TCDD-EQ (pM)**		**Log_{10} TCDD-EQ**		**p-value** [b]
				Mean	**S.E.M.**	**Mean**	**S.E.M.**	
TCDD 150pM		8	100.00	167.83	11.22	2.22	0.03	<0.0001***
DMSO		8	0.50	0.84	0.09	-0.09	0.05	1.0000
CL12	100	3	1.08	1.81	0.60	0.21	0.13	0.3387
CL12	50	3	0.87	1.46	0.26	0.15	0.07	0.6750
CL13	100	3	0.84	1.41	0.27	0.14	0.08	0.7654
CL13	50	3	0.68	1.14	0.18	0.05	0.06	0.9964
NR14	100	3	1.37	2.30	0.54	0.33	0.09	0.0390*
NR14	50	3	1.17	1.98	0.62	0.25	0.12	0.1773
PI13	100	3	12.17	20.43	11.85	1.13	0.30	<0.0001***
PI13	50	3	3.21	5.38	1.66	0.68	0.16	<0.0001***
PI14	100	3	0.78	1.31	0.13	0.11	0.04	0.8742
PI14	50	3	0.73	1.23	0.12	0.08	0.04	0.9573
IR14	100	3	2.83	4.75	1.38	0.64	0.12	<0.0001***
IR14	50	3	1.22	2.05	0.42	0.29	0.10	0.1011
SA14	100	3	7.58	12.72	3.11	1.08	0.10	<0.0001***

SA14	50	3	1.69	2.84	0.95	0.41	0.14	0.0094**
LR14	100	3	2.49	4.18	1.70	0.55	0.18	0.0003***
LR14	50	3	0.93	1.57	0.42	0.16	0.12	0.6111
AR14	100	2	0.72	1.22	0.46	0.05	0.17	0.9990
AR14	50	2	0.66	1.11	0.08	0.04	0.03	0.9995
LP14	100	3	1.05	1.76	0.43	0.22	0.10	0.3020
LP14	50	3	0.79	1.32	0.33	0.10	0.10	0.9265

[a] Maximum response observed for a standard containing 150 pM of TCDD (= 100%-TCDDmax).
[b] Significance of TCDD-EQ (pM) compared to DMSO control for log transformed numbers.
*P ≤ 0.05, ** P ≤ 0.01, **P ≤ 0.001

Values of TCDD-EQ in sediment varied by location, the mean concentration ranged from 1.11 in Aracaju-2014 to 20.43 pM TCDD-EQ in Padre Island-2013 (Table 3.2; Figure 3.1). The control DMSO averaged of 1.25 ± 0.84 pM TCDD-EQ. Compared to DMSO controls, 5 out of 8 sites showed TCDD-EQ values significantly above controls ($F_{21,52} = 40.51$, $p < 0.001$). Extracts from the highest concentrate (100% extract) differed from controls at Padre Island, Texas (2013) (20.43 ± 11.85 pM TCDD-EQ), Salinas, Ecuador (2014) (12.72 ± 3.11 pM TCDD-EQ), Iscuandé River, Colombia (2014) (4.75 ± 1.38 pM TCDD-EQ), Laguna de Rocha, Uruguay (2014) (4.18 ± 1.70 pM TCDD-EQ), and Nelson River, Canada (2014) (2.30 ± 0.54 pM TCDD-EQ), thereby validating the response of the assay.

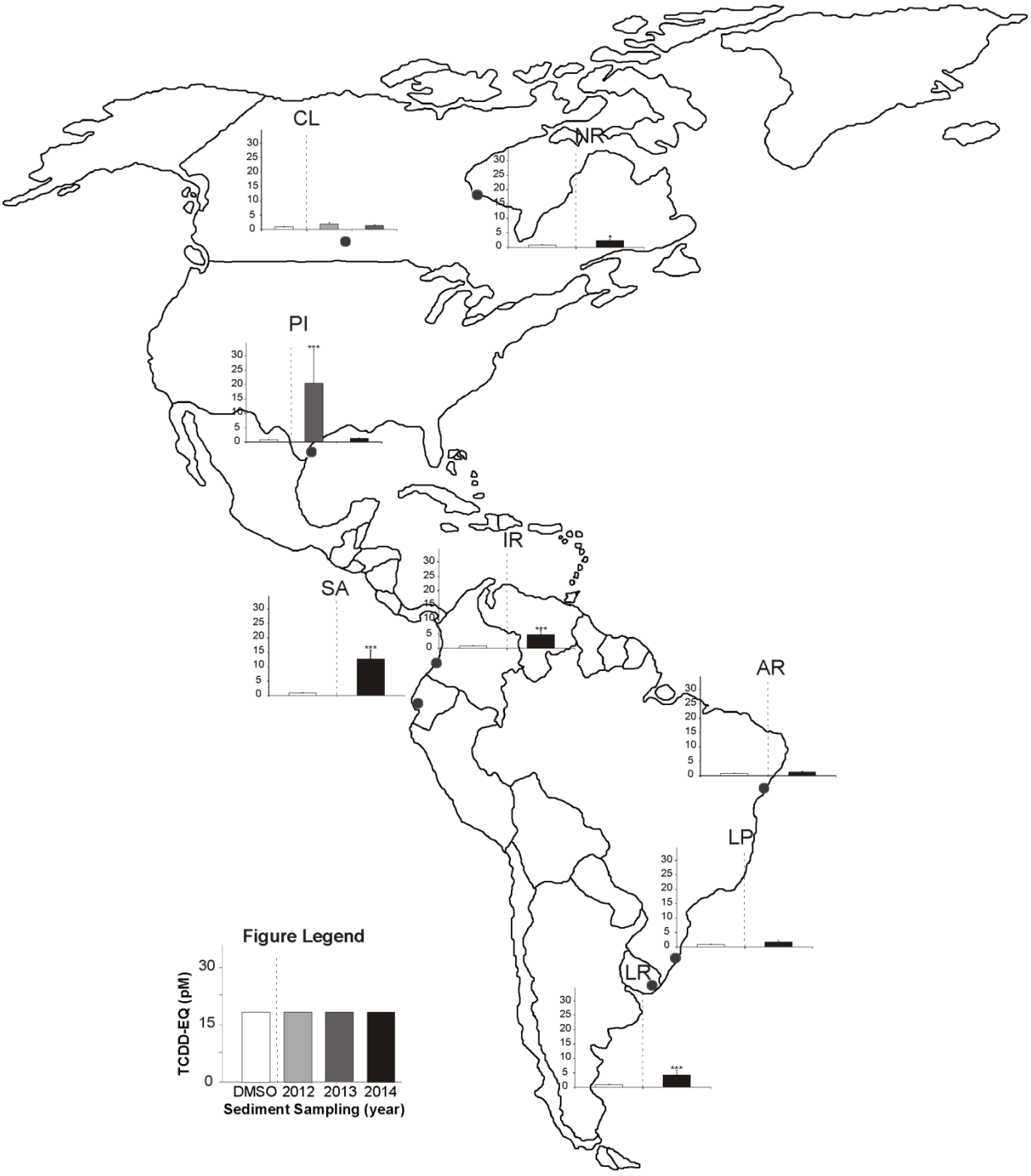


Figure 3.2. Mean dioxin equivalent concentrations (TCDD-EQ (pM)) in sediment extracts of important shorebird stopover and wintering grounds in North and South America compared to control samples. CL = Chaplin Lake, Canada; NR = Nelson River, Canada; PI = Padre Island, The United States; IR = Iscuandé River, Colombia; SA = Salinas, Ecuador; AR = Aracaju, north

of Brazil; LP = Lagoa do Peixe, south of Brazil; and LR = Laguna Rocha, Uruguay. Bars represent the highest dose extract (100%) relative to the controls (DMSO). Asterisks represent significance of TCDD-EQ (pM) compared to DMSO control for log transformed means of replicates. *P ≤ 0.05, ** P ≤ 0.01, **P ≤ 0.001.

The contamination by TCDD-EQ of each location in comparison to the US EPA reference value of 0.41 ng/g TCDD in marine/estuarine sediment is shown in Figure 3.2. Those values are a transformation based on the results found on H4IIE-*luc* bioassay. Therefore, the observed trends were the same as the results previous described. All samples were below the threshold reference value. However, the highest contamination was found in Padre Island-2013, which presented 0.3288 ng TCDD g^{-1} dw.

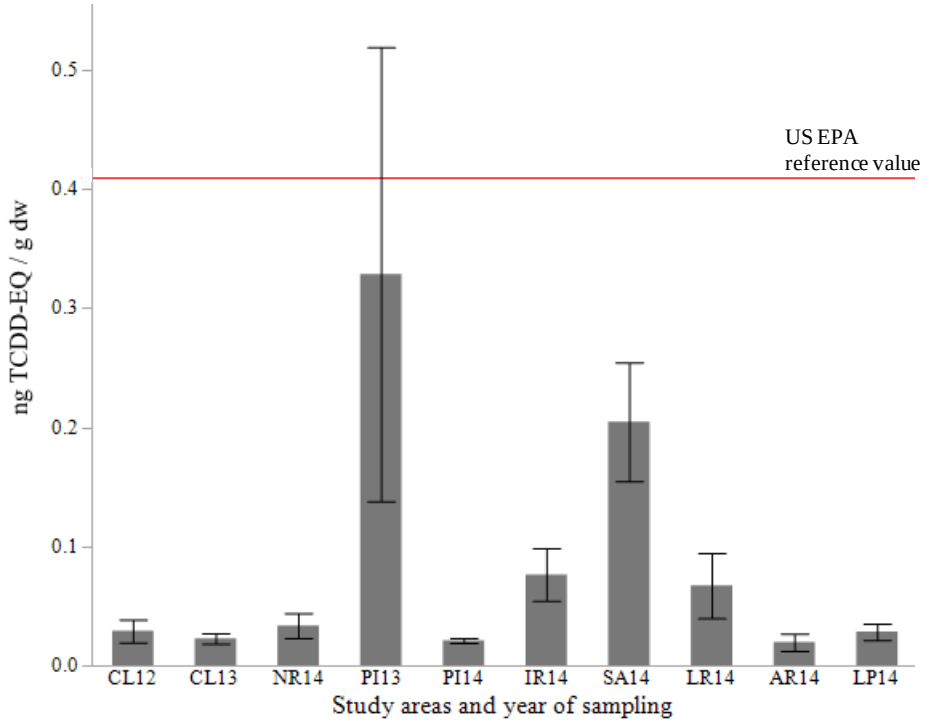


Figure 3.3. Concentration of TCDD-EQ (ng TCDD g^{-1} dw) in sediment collected in North and South America. Red line represents US EPA reference value of TCDD in marine/estuarine sediment (0.41 ng TCDD / g). CL12 = Chaplin Lake, Canada (2012); CL13 = Chaplin Lake, Canada (2013); NR14 = Nelson River, Canada (2014); PI13 = Padre Island, The United States (2013); PI14 = Padre Island, Texas (The United States) (2014); IR14 = Iscuandé River,

Colombia (2014); SA14 = Salinas, Ecuador (2014); LR14 = Laguna Rocha, Uruguay (2014); AR14 = Aracaju, north of Brazil (2014); LP14 = Lagoa do Peixe, south of Brazil (2014).

The ingestion of sediments by shorebirds occurs incidentally while they feed on invertebrate prey mainly in sandy areas. The rates of sediment ingestion and body mass are highly variable among species which can have a strong effect on the ingested dose (table 3.3) [131, 150, 151]. Literature on the percentage of sediment in shorebirds diet was available for only 6 species - reported as 3 to 30%. Body masses also varied from 21 to 330 grams. Therefore, I estimated TCDD-EQ ingestion from Padre Island, Texas (2013) would vary from 0.0009 ng TCDD-EQ/day in a Willet (*Catoptrophorus semipalmatus*) to 0.0203 ng TCDD-EQ /day in a Semipalmated sandpiper. None of the species exceeded the recommended TCDD tolerable daily intake [148].

Table 3.3. Shorebirds daily sediment ingestion and calculated TCDD-EQ intake from sediment collected from Padre Island, Texas (2003).

Shorebird species	Scientific name	Body mass (g)[a]	Percentage of sediment in diet (%)	Author	TCDD-EQ Ingestion dose (ng d^{-1})[b]	TDI_{bird}[c] (ng TCDD-EQ d^{-1})
Black-bellied plover	*Pluvialis squatarola*	160-277	29	Hui and Beyer 1998	0.0094	0.9766
Willet	*Catoptrophorus semipalmatus*	200-330	3	Hui and Beyer 1998	0.0009	1.1845
Stilt sandpiper	*Micropalama himantopus*	50-70	17	Beyer et al 1994	0.0087	0.2682
Semipalmated sandpiper	*Calidris pusilla*	21-32	30	Beyer et al 1994	0.0204	0.1184
Least sandpiper	*Calidris minutilla*	19-30	7.3	Beyer et al 1994	0.0051	0.1095
Western sandpiper	*Calidris mauri*	22-35	18	Beyer et al 1994	0.0119	0.1273

[a]The average body mass was used to calculate ingestion dose.
[b]Estimation was based on the ingestion dose formula.
[c]Tolerable daily intake for birds (TDI_{bird}), 0.00447 ng TCDD/g bw/d [148].

Figure 3.3 showed the estimated ingestion of DLC contamination by shorebirds for each one of the studied locations that had TCDD-EQ values significantly above controls. Contaminant intake was primarily determined by proportion of sediment in the diet. The best and worst case scenarios were demonstrated based on the sediment ingestion rates of 3 and 30%, respectively for 4 different body masses within the shorebird's potential mass range. Higher body mass had an effect of lowering the contaminant intake through biodilution. Though, I did not account for larger birds adjusting their consumption rates. Among all the estimates, the highest hazard was identified for Padre Island (2013) in birds of 25 g and ingesting 30% of sediment in diet (0.0208 ng TCDD-EQ / day). Even with 30% of sediment ingestion, the highest contamination intake of birds at larger masses of 75, 175 and 325 g was 0.0142, 0.0105, and 0.0085 ng TCDD-EQ / day respectively in Padre Island-2013. At 3% of sediment in diet, intake was below 0.0021 ng TCDD-EQ / day. None of the estimated intakes exceeded the guideline [148]. All DLC intake from sediment ingestion was less than 18.6% of the recommended TDI.

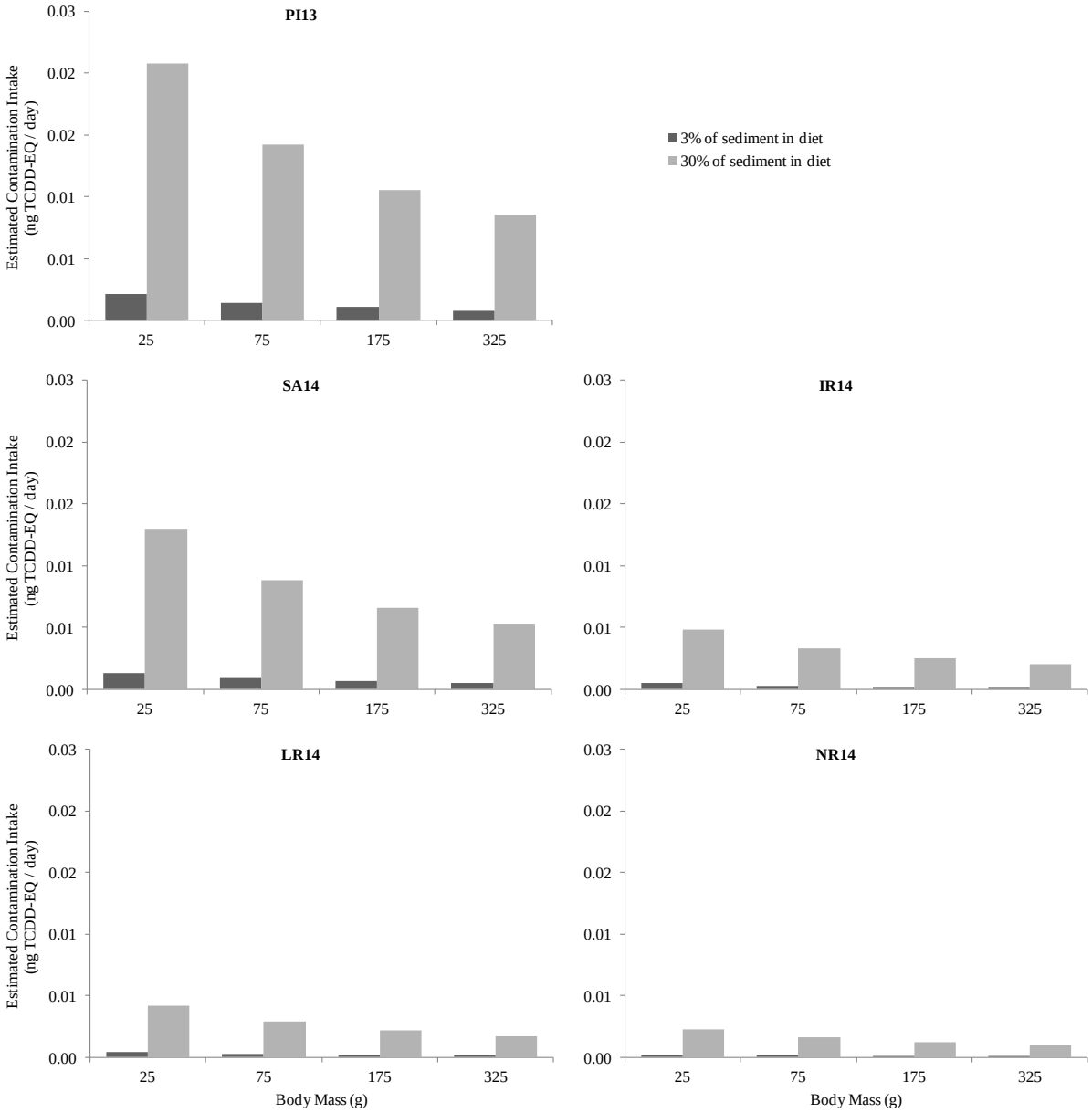


Figure 3.4. Daily contaminant intake (ng TCDD-EQ / day) by shorebirds estimated from the ingestion of sediment collected from important stopover and wintering grounds in North and South America. PI13 = Padre Island, United States (2013); SA = Salinas, Ecuador (2014); IR = Iscuandé River, Colombia (2014); LR = Laguna de Rocha, Uruguay (2014); and NR = Nelson River, Canada (2014). The TDI of birds mass 25, 75, 175, and 325 g was 0.1118, 0.3353, 0.7823, and 1.4528 ng TCDD / day respectively [148].

3.4 Discussion

3.4.1 Environmental contamination by DLCs

The potential for DLC contamination exists across all shorebird migratory routes [63, 64]. Most marine environmental contamination is likely due to small-scale events from the daily transportation of petroleum and refining activities, offshore production [57], industrial and municipal discharges, disposal of waste oil and diesel (e.g., contaminated ballast from oil tankers), rivers discharge, and urban runoff [66]. Additionally, development of tar sands exploration represents another inland source of contaminant exposure to birds during migration [70-72]. Thus, the sublethal exposure risk should be studied at large spatial scales.

The bioassay H4IIE-*luc* was performed on sediment collected from important locations for shorebirds to determine toxic potency of those areas. This bioassay is able to detect concentrations as small as 0.8 pM of TCDD [152]. As environmental samples may contain complex mixtures of contaminants, the results of this test were presented relative to the strongest AhR ligand, TCDD [136, 144]. Different from the samples collected in 2013, Padre Island-2014 did not show any toxic induction when compared to the control, possibly because this sample was collected in only one area, while Padre Island-2013 was a pool from 11 different areas (see Table 3.1). Compared to the US EPA reference value of TCDD in marine/estuarine sediment of 0.41 ng TCDD / g, all sediment sample means fell below the guideline. However, the results of Padre Island-2013 should be viewed cautiously as there was large variation, and at least 1 subsample exceeded the guideline value of 0.41 ng TCDD / g. Despite the fact that TCDD-EQ concentration in sediments collected from Iscuandé River-14, Laguna Rocha-14, and Nelson River-14 were below reference value, they were elevated above the control, which suggests DLCs are present in the environment from local or long range human and industrial activities.

Since 1951, Padre Island, Texas has been exploited for various petroleum activities, such as drilling for petroleum resources, and high traffic of oil transportation [153]. Consequently, soil contamination may have occurred due to those activities or accidental spills. Additionally, environmental contamination could be resulting from other activities like runoff from agriculture and industry [154]. Contaminated soil was reported in Padre Island National Seashore related to drilling and production of petroleum, yet the reported concentration represents negligible threats to human and environment in the short term, but the long term effects are unknown [153]. Residual exposure of animals to petroleum activities was demonstrated by analysis of PAHs in Peregrine falcons (*Falco peregrinus tundrius*) found along the Gulf Coast. The exposure could have occurred through direct contact with crude oil, or by ingesting avian prey that were in contact with oil after the 2010 Deepwater Horizon (DWH) oil spill. An increase of PAHs levels in the blood was found in falcons captured in 2010 compared to the levels found in 2011 [155]. In Salinas, province of Santa Elena, Ecuador, the manmade salt lakes were created in the 1960s for salt extraction by seawater evaporation [156]. Currently, the high salinity creates an ideal environment for the Brine shrimp (*Artemia salina*) community to grow, which attracts a large number of migratory birds [157, 158]. Many events are currently contributing to degradation of the salt lakes such as human disturbance, intensification of aquaculture, and contamination. The contamination may originate from surrounding human and industrial development and/or petroleum activities [157, 158]. Oil spills were previously observed in the municipality of Salinas [158]. Migratory birds, including shorebirds, were sighted with oil spots on their legs and wings [158]. However, only a small part of the oil contamination is estimated to be caused by spills; most of the environmental contamination is a result of extraction and transportation of petroleum [3, 6]. In the delta of the Iscuandé River, Colombia, the most important local threats

are the increase in agricultural lands and the growing urbanization further up the river. Along with the increase of sediment deposits in the low tide zone resulting from those activities, contamination from agricultural, and human waste are also a major concern [159, 160]. Laguna de Rocha, Uruguay is a coastal lagoon designated as Biosphere Reserve by UNESCO [161] and as a Nature Reserve by the Government of Uruguay [162]. As a preserved area, human and industrial activities are not present around the lagoon. However, many of those activities can be found associated within the main effluents of the lagoon, resulting in possible introduction of contaminants in the system [163]. The two northern stopover sites in Canada- Chaplin Lake and Nelson River, had low concentrations of TCDD-EQ in sediment. Nelson River, however, had samples above the controls, but sources of DLC contamination in this region remain unknown.

3.4.2 Potential DLCs exposure hazard that shorebirds face throughout South and North America

Shorebirds are known to ingest a large quantity of sediments while foraging [131, 150], and this trait can have a direct impact on the magnitude of contaminant exposure. As demonstrated, up to 20 % of the TCDD daily intake could be ingested from sediments alone. Despite the fact that TCDD daily intake in all assessed scenarios was below regulatory guidelines and estimated TDIs for shorebirds at the study locations, these results should be interpreted cautiously. This is a conservative estimate as it is taking into consideration only TCDD equivalents from sediment ingestion without considering other pathways of exposure. In particular, it does not consider contamination exposure through the shorebird's diet and possible bioaccumulation within their prey.

Within the Charadriformes shorebirds group, there is a large variety of species that differ by their migration strategies, choice of prey, and body size [3, 4, 6]. The assessment of

contamination, or exposure risk, of shorebirds as a large group has limitations, but an important first step toward furthering our understanding of site contamination, possible hotspots, and predicting risks to birds. However, these assumptions may underestimate risks to specific species whose life history traits makes them more vulnerable. For instance, birds of different body size will experience different contaminant intake and metabolic rates [147, 164, 165]. At a given location, all shorebirds would be exposed to the same level of environmental contamination; however, small species would have proportionally higher daily intake than larger species. Additionally, smaller species of shorebirds have lower TDI. Therefore, a higher contaminant intake per body mass, and a lower TDI put smaller shorebird species at a higher risk of exceeding the guidelines, and exhibiting toxic effects related to the DLC exposure. For this reason, body size [147, 165], percentage of sediment intake [131, 150, 166], type of prey [167, 168], foraging strategies (surface feeder versus deep sediment) [169, 170], and seasonal differences where birds exhibit hyperphagia during fuelling [171, 172] among other unique characteristics of given species should be considered for a complete understanding and determination of exposure to contaminants.

3.4.3 Use of the H4IIE-*luc* bioassay in conservation of migratory birds

Shorebirds use a diversity of habitats and have an extensive geographic range during their annual cycle [3, 7]. The distinctiveness of their migratory strategies and low connectivity poses a difficulty to identify the risks, especially for species of concern. Therefore, the use of toxicology techniques such as H4IIE-*luc* bioassay is a useful means to assess DLC contamination. Several studies have successfully used this technique to identify the main areas and contaminants of concern [136, 137, 142, 173]. This tool can help with species conservation plans to evaluate

contamination risk without having to capture birds. This is important preliminary information to determine the action plan for management and remediation of a given area.

3.5 Conclusion

All sediment samples analyzed presented a concentration of TCDD below the regulatory guidelines though most of the southern locations had some level of contamination. The ingestion of sediment from all study areas, despite their urban and industrial development, predicted no risk to shorebirds with respect to sediment contamination by DLCs. However, these results should be carefully interpreted, since it did not include other sources and exposure routes of contamination such as prey ingestion, which are likely to be equally or more important for exposure to organic contaminants. This method has potential to expand across a large network of stopover sites and prioritize sites of concern with follow up studies to determine the type and concentration of DLCs present. Due to the transboundary nature of shorebird contamination threats, it is critical to expand the multinational collaboration to include multiple key stopovers and winter sites in countries important to shorebird populations. Multinational efforts to assess contaminant risk across the range will improve our understanding of contaminant threats throughout the annual cycle, which will benefit the conservation of shorebirds.

APPENDIX B

All detailed information regarding sediment sampling was presented in the Table below.

Table B.1. GPS coordinate and date of sampling from all the sediments cores.

Site	Province/State, Country	Sample number	GPS coordinates	Sampling data
Chaplin Lake	Saskatchewan, Canada	1	50.44838°N 106.70932°W	05 Jun 12
		2	50.43745°N 106.64323°W	05 Jun 12
		3	50.43809°N 106.66433°W	05 Jun 12
		4	50.43801°N 106.66444°W	05 Jun 12
		5	50.43398°N 106.67241°W	05 Jun 12
		6	50.403397°N 106.67250°W	05 Jun 12
		7	50.43793°N 106.68412°W	05 Jun 12
		8	50.43796°N 106.68430°W	05 Jun 12
		9	50.44757°N 106.71008°W	05 Jun 12
		10	50.44762°N 06.7101999°W	05 Jun 12
		1’	50.44838°N 106.70932°W	27 May 13
		2’	50.43745°N 106.64323°W	27 May 13
		3’	50.43809°N 106.66433°W	30 May 13
		4’	50.43801°N 106.66444°W	30 May 13
		5’	50.43398°N 106.67241°W	30 May 13
		6’	50.40339°N 106.67250°W	30 May 13
		7’	50.43793°N 106.68412°W	29 May 13
		8’	50.43796°N 106.68430°W	29 May 13
		9’	50.44757°N 106.71008°W	27 May 13
		10’	50.44762°N 06.71019°W	27 May 13
Nelson River	Manitoba, Canada	1	57.11378°N 91.73368°W	04 Jun 14

		2	57.10674°N 91.75124°W	04 Jun 14
		3	57.12057°N 91.71358°W	04 Jun 14
		4	57.12337°N 91.69875°W	04 Jun 14
		5	57.12821°N 91.65999°W	04 Jun 14
		6	57.12825°N 91.67389°W	04 Jun 14
		7	57.12663°N 91.65765°W	04 Jun 14
		8	57.14735°N 91.58719°W	05 Jun 14
		9	57.14208°N 91.60819°W	05 Jun 14
		10	57.13542°N 91.62531°W	05 Jun 14
Padre Island	Texas, The United States	1	27.31438°N 97.33722°W	Feb 13
		2	27.20551°N 97.38966°W	Feb 13
		3	26.14358°N 97.17834°W	Feb 13
		4	26.24678°N 97.18144°W	Feb 13
		5	26.09409°N 97.16140°W	Feb 13
		6	26.12081°N 97.31647°W	Feb 13
		7	27.81998°N 97.06017°W	Feb 13
		8	27.84141°N 97.04215°W	Feb 13
		9	27.63313°N 97.21075°W	Feb 13
		10	27.64710°N 97.28074°W	Feb 13
		11	27.60517°N 97.20745°W	Feb 13
Padre Island	Texas, The United States	1	27.60517°N 97.20745°W	Feb 14
Iscuandé River*	Department of Nariño, Colombia	1	02.62256°S 78.04960°W	05 Feb 14
		2	02.66252°S 78.05070°W	05 Feb 14
		3	02.62680°S 78.05743°W	05 Feb 14
		4	02.61719°S 78.05755°W	05 Feb 14
		5	02.58770°S 78.04183°W	05 Feb 14
Salinas	Province of Santa Elena, Ecuador	1	02.16890°S 80.55027°W	27 Mar 14
		2	02.17326°S 80.54699°W	27 Mar 14

		3	02.14579°S 80.57215°W	27 Mar 14
		4	02.18266°S 80.54273°W	27 Mar 14
		5	02.01112°S 80.44246°W	15 Apr 14
		6	02.01684°S 80.44173°W	15 Apr 14
		7	02.03160°S 80.44117°W	15 Apr 14
		8	02.03875°S 80.44197°W	15 Apr 14
		9	02.04798°S 80.44437°W	15 Apr 14
		10	02.06730°S 80.45137°W	15 Apr 14
		11	02.12130°S 80.52361°W	18 Aug 14
		12	02.12532°S 80.53192°W	18 Aug 14
		13	02.12824°S 80.53624°W	18 Aug 14
		14	02.13055°S 80.56591°W	18 Aug 14
		15	02.13212°S 80.55956°W	18 Aug 14
Laguna de Rocha	Department of Rocha, Uruguay	1	34.40258°S 54.15448°W	01 Feb 14
		2	34.40461°S 54.16131°W	07 Feb 14
		3	34.40542°S 54.14335°W	07 Feb 14
		4	34.40567°S 54.16534°W	07 Feb 14
		5	34.40358°S 54.17025°W	07 Feb 14
		6	34.40186°S 54.16182°W	07 Feb 14
		7	34.41071°S 54.16162°W	07 Feb 14
		8	34.41190°S 54.17332°W	07 Feb 14
Aracaju	Sergipe, Brazil	1	10.96730°S 37.03490°W	15 Feb 14
		2	10.98300°S 37.04302°W	15 Feb 14
		3	10.99378°S 37.05249°W	15 Feb 14
		4	11.01494°S 37.06944°W	15 Feb 14
		5	11.13244°S 37.15543°W	15 Feb 14
		6	11.12840°S 37.14528°W	15 Feb 14
		7	11.12117°S 37.14001°W	15 Feb 14

		8	11.09089°S 37.12145°W	15 Feb 14
		9	11.06853°S 37.10631°W	15 Feb 14
Lagoa do Peixe	Rio Grande do Sul, Brazil	1	32.52736°S 52.39093°W	31 Jan 14
		2	32.38234°S 52.32001°W	31 Jan 14
		3	32.29481°S 52.26341°W	31 Jan 14
		4	32.18860°S 52.15384°W	31 Jan 14
		5	31.68008°S 51.40999°W	02 Fev 14
		6	31.36068°S 51.04123°W	02 Fev 14
		7	31.44440°S 51.16318°W	02 Fev 14
		8	31.38841°S 51.11294°W	02 Fev 14
		9	32.14885°S 52.01171°W	03 Fev 14
		10	32.13482°S 52.07953°W	03 Fev 14
		11	32.14310°S 52.07690°W	03 Fev 14
		12	32.13831°S 52.07274°W	03 Fev 14

*The coordinate was presented from just one sampling area. The remaining GPS coordinates were estimated based on site description and distance from the informed coordinate.

APPENDIX C

Sediment sample contamination from field sampling, sample transportation, laboratory technique and extraction chemicals were determined by the use of H4IIE-*luc* bioassay.

Table C.1. Overview of H4IIE-*luc* test results of TCDD (150 pM), control DMSO and blanks.

Sample ID	**n**	**TCDD-EQ (pM)**	**%-TCDD-EQ**
TCDD 150pM	8	167.83	100.00
DMSO	8	0.84	0.50
Chemical blank 100%	1	1.48	0.88
Chemical blank 50%	1	1.04	0.62
CL13 field blank 100%	1	0.98	0.58
CL13 field blank 50%	1	0.57	0.34
IR14 field blank 100%	1	8.81	5.25
IR14 field blank 50%	1	2.04	1.22
LAB blank 100%	1	0.91	0.54
LAB blank 50%	1	0.98	0.59
LP14 field blank 100%	1	1.16	0.69
LP14 field blank 50%	1	0.90	0.54
LP14 travel blank 100%	1	1.84	1.09
LP14 travel blank 50%	1	1.13	0.68
PI13 field blank 100%	1	0.77	0.46
PI13 field blank 50%	1	0.70	0.42
PI13 travel blank 100%	1	1.49	0.89
PI13 travel blank 50%	1	1.19	0.71

PI14 field blank 100%	1	0.96	0.57
PI14 field blank 50%	1	1.19	0.71
SA14 field blank 100%	1	1.81	1.08
SA14 field blank 50%	1	1.46	0.87
SA14 travel blank 100%	1	1.47	0.87
SA14 travel blank 50%	1	1.33	0.79

APPENDIX D

Preparation of Medium

1. Equipment, material and reagents:
    - Pump for filter
    - 2x 500 ml autoclaved bottle
    - 500 ml bottle top filter
    - 1000 ml graduated cylinder
    - Stir bar
    - Stir plate
    - Dulbecco's Modified Eagle's Medium (DMEM), Sigma D2906
    - 3.7 g sodium bicarbonate
    - Fetal Bovine Serum (FBS), 50 ml per each 500 ml of supplemented medium
    - Film hood
2. Method
    a. Add approximately 900 ml of nanopure water into a 1000 ml graduated cylinder, and place it on the stir plate.
    b. Place the stir bar in the graduated cylinder, and turn the stir plate on.
    c. As the water stirs, add the medium powder and the sodium bicarbonate.
    d. Wait until all the powder is dissolved and adjust the pH of the solution to 0.1 to 0.3 below 7.4 which is the desired final pH.
    e. Bring the volume up to 1000 ml with nanopure water.
    f. Bring it to the film hood. Take the appropriated procedures to avoid contamination.

g. Filter the medium using the bottle top filter and the pump.

h. Add FBS to one of the bottle and use it for the experiment. Add FBS to the other bottle just before using it.

i. Label the bottle with: sterilized DMEM medium for H4IIE cell culture, pH 7.4, date and name.

j. The medium can be storage up to 3 months at 2 – 8 °C.

Starting cells from frozen stock

1. Equipment, material and reagents:
    - Supplemented sterile medium
    - Culture plates
    - Pasteur pipette
    - Sterile PBS
    - Microscope
    - Incubator
2. Method
    a. Warm the medium to approximately 36 °C in water bath.
    b. Remove the cell vial from liquid nitrogen storage.
    c. Using gloves thaw the vial using the warm of your hands. Do not hold the vial at one position for too long as it can damage your skin.
    d. In film hood, add 10 ml of medium to a culture plate and transfer the cell suspension to this plate.
    e. Place the plate in incubator at 37 °C and 5% CO_2.

f. After 24, remove the place from the incubator and check for possible contamination and cell attachment on microscope. Do not open the lid!

g. Rinse the attached cell with sterile PBS. Repeat this process 3X.

h. Add 10-12 ml of medium.

i. Check the place on microscope to see if there are still cells attached.

j. Place the plate in incubator.

*This process is vital to remove any DMSO commonly found in the frozen solution that can be an issue for cell culture; and also to remove any dead cells and debris that can facilitate contamination.

k. At this stage the cells will need time to recover from freezing and to replicate. Therefore, it will take about 3 to 10 days for the next step. The length of this step will depend on the initial cell concentration.

l. Check the plate daily for signs of contamination. Observe replication rates and attachment of the cells.

m. The cells will be ready for the next step when cells cover 90-95 % of the plate. It is called a generation.

Replicating culture

1. Equipment, material and reagents:

    - Supplemented sterile medium
    - Culture plates
    - Sterile PBS
    - Trysin-EDTA

2. Method

    a. Rinse the dish with PBS. Repeat 2x.

    b. Add 0.5 to 1.5 ml of 1x sterile trysin-EDTA. Trypsin can be toxic for cells, so add as less as possible.

    c. Place the dish in incubator for approximately 5 minutes. Monitor the cells and stop the reaction when cells have detached from the plate.

    d. Stop the reaction by adding supplemented medium. Add 11.5 to 10.5 ml (bring the volume up to 12 ml).

    e. Add 2 ml of this suspended cell solution into a new plate. Add 10 ml of supplemented medium.

    f. Incubate for 24 h.

    g. After 24 hours, rinse the plate 3x with PBS and add 12 ml medium.

    h. Place the plate in incubator and monitor the cell coverage. The cells will be ready for another replication when cells cover 90-95 % of the plate.

    i. The cells will be ready to be dosed after 3-4 generations.

Dosing cells

1. Equipment, material and reagents:

    - Supplemented sterile medium
    - Culture plates
    - Sterile PBS
    - Trysin-EDTA
    - Hematocytometer

- 96 well plate (View-Plate^{TM})
- Repeat pipette
- Dosing solution

2. Method
   a. Rinse the dish with PBS. Repeat 2x.
   b. Add 0.5 to 1.5 ml of 1x sterile trysin-EDTA for approximately 5 minutes.
   c. Stop the reaction by adding supplemented medium. Bring the volume up to 12 ml.
   d. Determine the number of cells/ml with a hematocytometer.
   e. Dilute cell solution to a concentration of 80,000 cells/ml.
   f. Add 100 µl of cell suspension to a 96 well plate with repeat pipette. See template at Figure 3.C1.
   g. Add 100 µl PBS to the other wells.
   h. Wait 24h for cell attachment.
   i. Remove the old medium and add 100 µl supplemented medium dosed with 0.1% of dosing solution (TCDD, DMSO or sample).
   j. Exposures continue for 24 h.

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B		0.61 pM TCDD	0.61 pM TCDD	0.61 pM TCDD			DMSO		Sol. A 100%	Sol. A 100%	Sol. A 100%	
C		1.85 pM TCDD	1.85 pM TCDD	1.85 pM TCDD			DMSO		Sol. A 50%	Sol. A 50%	Sol. A 50%	
D		5.55 pM TCDD	5.55 pM TCDD	5.55 pM TCDD			DMSO		Sol. B 100%	Sol. B 100%	Sol. B 100%	
E		16.66 pM TCDD	16.66 pM TCDD	16.66 pM TCDD			Blank		Sol. B 50%	Sol. B 50%	Sol. B 50%	
F		50 pM TCDD	50 pM TCDD	50 pM TCDD			Blank		Sol. C 100%	Sol. C 100%	Sol. C 100%	
G		150 pM TCDD	150 pM TCDD	150 pM TCDD			Blank		Sol. C 50%	Sol. C 50%	Sol. C 50%	
H												

Figure D.1. A 96-well plate layout for H4IIE-*luc* bioassay. Each sample was analysed in

triplicates. In each plate, 3 different solutions were analysed. Solvent control is represented by DMSO. The empty columns are recommended to avoid cross-contamination, and they are filled with PBS.

Reading plate

1. Equipment, material and reagents:
    - PBS supplemented with Ca^{+2} and Mg^{+2}
    - Steadylite plus™ from PerkinElmer
    - POLARstar OPTIMA microplate reading luminometer (BMG LABTECH, Ortenberg, Germany)
2. Method
    a. Remove the plate from incubator and check whether the cells are attached.
    b. Dump all the liquid in specific container for proper disposal.
    c. Rinse the plate with 75 µl of PBS with Ca^{+2} and Mg^{+2} per well.
    d. Dump all the liquid.
    e. Attach the white stick to the back of the plate.
    f. Add 75 µl of PBS with Ca^{+2} and Mg^{+2} per well in all wells. Include a column of not seeded wells to correct for the background.
    g. Make the steadylite plus™ reagent solution according to the product description.
    h. Add 75 µl of the reagent solution to each well previously filled with PBS.
    i. Let the reaction happen for 15 minutes in the dark.
    j. Read the plate in a microplate reading luminometer.

APPENDIX E

Cell viability and overall cytotoxicity were determined by the use of the Cell Proliferation Reagent WST-1 assay according to the product description (Roche Diagnostic GmbH, Mannheim, Germany).

A total of 5 plates were used to analyse all samples. Data was shown as mean ± standard deviation of the mean (S.D.). Normality of the dataset was determined using the Shapiro-Wilk test. Analyse of variance (ANOVA) followed by post hoc Dunnet's tests was used to verify differences between samples and DMSO control. Nonparametric multiple comparison with control test was used for not normal distributed dataset.

Table E.1. Analysis of variance (ANOVA) (F and P values) of WST-1 test reading. Results are presented as number of replicates (n), mean and standard error of the mean (S.E.M.) of test readings.

Plate ID	ANOVA	Sample ID	n	Mean	S.E.M.	p-Value
1	$F_{10,11}$=7.38, p<0.01	DMSO	2	0.6057	0.1229	1.0000
1		CL13 100%	2	0.4723	0.0903	0.7284
1		CL13 50%	2	0.8219	0.0215	0.2696
1		PI14 100%	2	0.3999	0.0829	0.3129
1		PI14 50%	2	0.2937	0.0660	0.0601
1		TCDD 0.61	2	0.9358	0.0087	0.0446*
1		TCDD 1.85	2	0.6953	0.1060	0.9466
1		TCDD 150	2	0.4537	0.0172	0.6096
1		TCDD 16.6	2	0.5795	0.0803	1.0000
1		TCDD 5.55	2	0.6626	0.0203	0.9969
1		TCDD 50	2	0.4331	0.0411	0.4833
2	$F_{10,22}$=5.10, p<0.001	DMSO	3	0.7640	0.0711	1.0000
2		IR14 100%	3	1.1321	0.0655	0.0133*
2		IR14 50%	3	0.7900	0.0928	1.0000

2		NR14 100%	3	0.8891	0.0689	0.8081
2		NR14 50%	3	1.2263	0.0881	0.0016**
2		TCDD 0.61	3	0.6448	0.0761	0.8418
2		TCDD 1.85	3	0.8325	0.0508	0.9932
2		TCDD 150	3	0.8253	0.0782	0.9970
2		TCDD 16.6	3	0.9638	0.0799	0.3403
2		TCDD 5.55	3	0.9207	0.0468	0.6016
2		TCDD 50	3	0.9509	0.0732	0.4103
3	$F_{10,22}$=3.53, p<0.01	DMSO	3	2.5831	0.2041	
3		Chemical blank 100%	3	2.9559	0.3806	1.0000
3		Chemical blank 50%	3	3.1130	0.1407	0.4176
3		CL12 50%	3	3.0580	0.1154	0.4176
3		CL12 100%	3	2.6825	0.1221	1.0000
3		TCDD 0.61	3	2.2833	0.8235	0.9999
3		TCDD 1.85	3	3.2813	0.1278	0.4176
3		TCDD 150	3	0.7383	0.1189	0.4176
3		TCDD 16.6	3	2.6007	0.3036	0.9999
3		TCDD 5.55	3	2.7118	0.4023	1.0000
3		TCDD 50	3	2.5804	0.5034	0.9999
4	$F_{20,29}$=0.77, p=0.72	DMSO	2	0.4224	0.0924	NS
4		AR14 100%	2	0.3053	0.0832	NS
4		AR14 50%	2	0.4938	0.0798	NS
4		CL13 field blank 100%	2	0.3996	0.0322	NS
4		CL13 field blank 50%	2	0.4932	0.1061	NS
4		IR14 field blank 100%	2	0.4818	0.0344	NS
4		IR14 field blank 50%	2	0.4185	0.0548	NS
4		LAB blank 100%	2	0.5016	0.0988	NS
4		LAB blank 50%	2	0.5276	0.0192	NS

4		LP14 100%	4	0.4673	0.1284	NS
4		LP14 50%	4	0.7084	0.2873	NS
4		LR14 100%	4	0.6955	0.1530	NS
4		LR14 50%	4	0.7054	0.1556	NS
4		PI13 field blank 100%	2	0.5660	0.1718	NS
4		PI13 field blank 50%	2	0.7655	0.1756	NS
4		TCDD 0.61	2	0.3386	0.0376	NS
4		TCDD 1.85	2	0.3591	0.0250	NS
4		TCDD 150	2	0.3233	0.0082	NS
4		TCDD 16.6	2	0.3786	0.0513	NS
4		TCDD 5.55	2	0.3626	0.0361	NS
4		TCDD 50	2	0.3209	0.0105	NS
5	$F_{36,41}$=3.76, p<0.001*	DMSO	2	0.9297	0.1677	
5		AR14 100%	2	0.5269	0.0864	0.9935
5		AR14 50%	2	1.2385	0.3845	2.0000
5		CL12 100%	2	0.4217	0.2122	0.9935
5		CL12 50%	2	0.2902	0.1249	0.9935
5		CL13 100%	2	0.4279	0.0676	0.9935
5		CL13 50%	2	0.4133	0.0793	0.9935
5		IR14 100%	2	0.2493	0.0332	0.9935
5		IR14 50%	2	0.2650	0.0334	0.9935
5		LP14 100%	2	0.5357	0.0757	0.9935
5		LP14 50%	2	0.8519	0.1050	2.0000
5		LP14 field blank 100%	2	0.3404	0.1420	0.9935
5		LP14 field blank 50%	2	0.7552	0.2404	2.0000
5		LR14 100%	2	0.3329	0.0062	0.9935
5		LR14 50%	2	0.9810	0.1946	2.0000
5		NR14 100%	2	0.1011	0.0336	0.9935

5	NR14 50%	2	0.7484	0.2002	2.0000
5	PI13 100%	4	0.4750	0.0626	0.8142
5	PI13 50%	4	0.6938	0.3220	1.0000
5	PI14 100%	2	0.2673	0.0357	0.9935
5	PI14 50%	2	0.2815	0.0113	0.9935
5	PI14 field blank 100%	2	0.5086	0.1588	0.9935
5	PI14 field blank 50%	2	0.5024	0.1978	0.9935
5	SA14 field blank 100%	2	0.4236	0.1891	0.9935
5	SA14 field blank 50%	2	1.0710	0.2970	2.0000
5	SA14.1 100%	2	0.4586	0.0303	0.9935
5	SA14.1 50%	2	0.3819	0.0279	0.9935
5	SA14.2 100%	2	0.4032	0.0147	0.9935
5	SA14.2 50%	2	0.4826	0.0233	0.9935
5	TCDD 0.61	2	0.9701	0.2545	1.0000
5	TCDD 1.85	2	1.4045	0.0334	0.9935
5	TCDD 150	2	0.8106	0.1636	2.0000
5	TCDD 16.6	2	1.0700	0.0589	2.0000
5	TCDD 5.55	2	1.5534	0.2306	0.9935
5	TCDD 50	2	1.2804	0.0721	0.9935
5	TCDD standard solution 100%	2	0.3611	0.0753	0.9935
5	TCDD standard solution 50%	2	0.3930	0.0880	0.9935

*Plate 1 and 2 presented normal distribution. Plate 2, 3, and 4 presented not normal distribution and log transformed data was used for the analyse. *$P \leq 0.05$; **$P \leq 0.01$; NS, not significant

CHAPTER 4

SUMMARY AND CONCLUSIONS

4.1 Important findings about population structure of Sanderling from Chaplin Lake

Over 46,000 Sanderlings have been observed migrating through Chaplin Lake in a single year [15]. Although band resightings had limited use in determining winter locations, stable isotope results indicated that the migratory population of birds winter over a broad and overlapping geographic area. Using a combination of δ^2H isomap [41], published data in shorebirds, and distribution and abundance of Sanderlings [3, 7, 8] revealed 3 groups of Sanderlings with possible winter origins of cluster 1, 2, and 3 in southern South America (e.g. Chile or Argentina), northwestern South America (e.g. Peru), and the Gulf of Mexico (e.g. Texas), respectively. Body morphometrics among the clusters also lend support to this conclusion. Both wing and tarsus showed statistical difference among clusters. Sanderlings from Cluster 1 were hypothesized to winter in southern South America also had the largest body size with longer wing lengths. Larger body sizes could be an adaptation to colder weather as the larger body exhibits a proportionally smaller heat loss [114]. The larger wing length is also possible aiding flight distance [174-176].

Findings from this project indicate that stable isotopes are a great tool to broadly classify winter origins of Sanderlings. The combination of δ^2H, δ^{13}C, and δ^{15}N was the best approach to identify unique clusters. However, the hydrogen isotope had the greatest resolution and provided the best visual cluster separation. Additionally, it provides a good latitudinal categorization of values, which is appropriate for species that have a high latitudinal variation of winter grounds as found in migrant Sanderlings.

Despite the limited number of resightings, the information provided by them was useful to confirm that Sanderling migrating north through Saskatchewan tend to follow the elliptical pathway south. Resighting in the wintering grounds or wildlife tracking tools would be needed to address outstanding questions regarding specific winter ground locations and migration strategies.

4.2 Application of isotope method for assignment of Sanderling wintering origin

The statistical method of assignment probability was developed for Sanderling to facilitate future studies to determine wintering clusters of migrating birds. From this point forward, isotopic analyses from just one migrant Sanderling could be conducted to identify its winter origins. Inferences of origin and clusterization require analyzing a large quantity of animals to which I used 356 samples to originally develop the clusters. Additionally, Sanderlings collected from other wintering locations such as Argentina, Chile or Peru, could also be analyzed by this method to confirm the probability of this known origin bird belonging to one of the identified clusters. Although the Chaplin Lake assignment model is uniquely developed for this population, the formulae can be adopted to the study of any Sanderling across the flyway to determine if its isotopic value is bounded by the identified clusters.

4.3 H4IIE-*luc* cells: a approach to identify and quantify environmental contamination for conservation of bird populations

Ingestion is one of the main sources of contamination to shorebirds. As shorebirds feed, they incidentally ingest a large quantity of sediments, proportionally higher than other species. As described in chapter 3, the accidental sediment ingestion can have a significant impact on

shorebirds exposure to contaminants. Therefore, an intake of contaminants due to sediment ingestion is an important part of the dietary contamination intake.

The H4IIE-*luc* bioassay has been widely used in ecological risk assessment studies to assess contamination of marine and freshwater habitats [133-137]. This project extended the use of this technique for assessing environmental contamination of shorebird habitats. This was the first time that H4IIE-*luc* bioassay has been applied for studies related to bird conservation. The results demonstrated that the bioassay works for delivering information regarding status of industrial contaminants in coastal sediments. This technique can be applied to study contamination issues over multiple locations across a large area. For shorebirds in particular, H4IIE-*luc* bioassay could be used to study the breeding, stopover and winter grounds of species of conservation concern through a networked approach. H4IIE-*luc* of sediments is ideal as a first screening of shorebird's habitats due to their vast area used by migrants. More detailed studies could then be focused on areas found to have elevated levels (based on H4IIE-*luc* induction). Results from this study showed that most of the studied locations in the southern regions presented some degree of contamination. However, none of the samples exceeded the US EPA reference threshold value for sediments.

4.4 Linking Sanderling migratory patterns and potential risks of exposure to dioxin-like compounds (DLCs).

Sanderlings occupy habitats that are spread through a vast area, which makes it difficult to study possible threats they may face. As previously described in chapter 2, three clusters were identified in the Sanderling population of Chaplin Lake. These suggested winter origin of each cluster was South America (e.g. Chile and/or Argentina), the northwestern part of South America

(e.g. Peru), and Gulf of Mexico (e.g. Texas). Unfortunately, the sites analysed in chapter 3 did not include all the identified winter grounds, however none of the study sites exceeded contamination levels over the US EPA reference value. Padre Island, Texas contamination levels and calculated exposures were the highest among the studied locations. Although the mean value of Padre Island contamination was below the published sediment reference level, there are inherent errors and assumptions that indicate these should be considered preliminary. Additionally, contamination levels in bird's diet are likely to be much higher [177-179].

Assessment of the total concentration of contaminants in the environment is a great screening tool, and it also allows for assessing the hazard of contamination at stopover and wintering sites. Estimates of contamination intake should include the proposed model for contamination through accidental sediment ingestion in addition to prey. In this project, only contamination from sediment was assessed – an approach that avoided confounding effects of different shorebird prey availability and diets or ethical and logistical constraints of sampling live birds. The results showed that sediment has significant impact on the daily intake of contaminants. Contamination through sediment ingestion should be considered conservative since this does not account for diet exposure and bioaccumulation through the food chain which are important part of the risk evaluation.

Results of this project also demonstrated that despite shorebirds have a similar habit, diet, and potential exposure to the same contamination, the assessment of hazard and risk should be calculated for each species. Details such as body size [147, 165], percentage of sediment intake [131, 150, 166], and type of prey [167, 168] among other unique characteristics of given species are important in affecting exposure and contamination risk.

4.5 Implications for future research

Future researchers should aim to confirm the suggested wintering origins by studying isotopic value of feathers from Sanderlings captured at the hypothesized winter locations in South America. Another possible technique is the use of radio transmitters, geolocators and other intrinsic (DNA) markers to track migratory origins. Additionally, the influence of sex on the population clustering and body size would be an interesting research topic to explore further.

Regarding assessment of environmental contamination, future research should aim for studying additional wintering areas identified here, following up with areas of concern in more detail, and quantifying the chemicals and their source. Additionally, the assessment of contamination of the food supply would be of great importance for risk assessment and shorebirds conservation. Moreover, as the shorebirds use multiple habitats at different times of the year, an interesting study would be expanding the contamination assessment to a given area and do studies over time, thereby providing more information on risk during different phases of the life cycle.

Currently, shorebird conservation plans for Canada and the United States identifies pollution as a potentially important threat for shorebirds but relatively little work has gone into research on the exposure and effects of organic contaminants. Among possible contaminants, oil pollution have been consistently mentioned due to the number of offshore petroleum exploration, the long-term sublethal effects, the consistent small and non-reported spills, the potential for disastrous effects to shorebirds habitats [6, 7]. Moreover, due to the transboundary nature of shorebird contamination threats, it is essential to do more multinational collaborations to include other countries important to the survival of shorebirds. Multinational efforts will improve the relevance of projects, as shorebirds spend most of their time on the wintering or staging areas

outside of Canada. Collaborative efforts as used here in addressing pollution issues will immensely benefit the hemispheric conservation of shorebirds.

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