Trophic transfer and spatial distribution of mercury in the Gulf of St. Lawrence using northern gannets (*Morus bassanus*) as biological samplers

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To the people who kept me going,

Andy, Megan, and myself.

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ABSTRACT

Contaminants are ubiquitous in marine environments, but they are not evenly distributed. Organisms at varying trophic levels and habitats are exposed to different contaminant loads due to biomagnification through food webs and spatial heterogeneity. This thesis seeks to understand how mercury, a contaminant of high concern for wildlife due to its neurotoxic properties, moves through the food web up to a top predator, the northern gannet (*Morus bassanus*) in the Gulf of St. Lawrence, and how different foraging habitats may influence the mercury loads in their prey. Chapter 1 reviews the current literature pertaining to mercury, the use of stable isotopes in ecotoxicology and the use of seabirds as ecological indicators. Chapter 2 compares the use of two stable isotope analysis methods to evaluate the rate of mercury biomagnification in the northern gannet food web. Compound-specific stable isotope analysis of amino acids (CSIA-AA) provides a method to estimate baseline stable nitrogen isotope values of food webs. The method allows less biased estimates of trophic positions than those provided by bulk stable isotope analysis, improving estimates of contaminant biomagnification. I calculated trophic positions with published CSIA-AA equations for four species of fish and for northern gannets and examined the effect of CSIA-AA-derived trophic positions on biomagnification metrics for mercury and compared to the more traditional bulk stable isotope approach. Trophic magnification factors (TMFs) produced by CSIA-AA were lower than that produced by bulk stable isotope analysis. The bulk technique yielded one of the highest TMFs ever reported in the scientific literature. My work demonstrates discrepancies in biomagnification assessed using different approaches that may go undetected when using a single approach. Chapter 3 seeks to understand how different foraging habitats may determine the contaminant load contained in gannets' diet, and what anthropogenic and biotic factors drive mercury contamination in the Gulf of St. Lawrence. To map the chemical landscape of mercury, I collected fish regurgitations from GPS-tracked northern gannets. I assigned mercury concentrations from fish muscle to the last known gannet foraging location, classified using Hidden Markov Models. In small fish species, trophic positions calculated with CSIA-AA values were the best predictor of THg. THg in large fish species was best explained by stable carbon isotopes, indicating inshore habitats had higher THg contamination than pelagic ones. Demonstrating where contaminants accumulate more efficiently is crucial to understanding what risks wildlife are exposed to based on their habitat and feeding ecology. The thesis develops novel tools for measuring contamination in our increasingly polluted oceans.

RÉSUMÉ

Les contaminants sont omniprésents dans l'environnement marin, sans toutefois être distribués de manière uniforme. Les animaux occupant des niveaux trophiques différents et des habitats divers sont exposés à des charges de contaminants variables en raison de la bioamplification et de l'hétérogénéité spatiale. Ce mémoire de maitrise étudie comment le mercure, un contaminant nocif pour la faune, est transféré à travers le réseau alimentaire jusqu'à une espèce de niveau trophique supérieure, le fou de Bassan (*Morus bassanus*) dans le golfe du Saint-Laurent. Le premier chapitre est une revue de la littérature actuelle du mercure, de l'utilisation des isotopes stables pour l'écotoxicologie, et l'utilisation des oiseaux marins comme indicateurs écologiques. Le deuxième chapitre compare l'utilité de deux méthodes d'analyse d'isotopes stables pour mesurer la bioamplification dans le réseau trophique du fou de Bassan. L'analyse d'isotopes stables d'azote dans les acides aminés (AISA-AA) permet de calculer la position trophique des animaux en évitant les lacunes associées à l'analyse des isotopes stables selon la méthode traditionnelle, ce qui peut améliorer les estimés de bioamplification. J'ai calculé le niveau trophique pour quatre espèces de poisson et pour le fou de Bassan en utilisant des équations trophiques publiées pour l'AISA-AA ainsi que pour la méthode traditionnelle. Avec cette information, j'ai pu évaluer l'effet du type d'isotope d'azote mesuré sur l'ampleur des facteurs de bioamplification de mercure. Les facteurs de bioamplification trophique (FBT) produits par le AISA-AA étaient plus bas que celui produit par la méthode traditionnelle, qui a produit un FBT représentant l'une des plus hautes valeurs dans la littérature. Mon travail démontre les divergences entre les FBT produits par la méthode traditionnelle et par l'ASIA-AA qui peuvent passer inaperçu lors de l'utilisation d'une seule approche. Le troisième chapitre a pour objectif de comprendre comment l'utilisation de l'environnement par les animaux peut influencer leur charge de contaminants, et comment les facteurs anthropiques ou biotiques peuvent déterminer la contamination de mercure dans différents habitats du golfe du Saint-Laurent. Pour cartographier la variation des concentrations de mercure dans le golfe du Saint-Laurent, j'ai échantillonné des régurgitations de poisson provenant de fous de Bassan suivi par GPS. Les concentrations de mercure dans les poissons ont été assignées au dernier endroit de quête alimentaire connu, que j'ai classifié en utilisant les modèles de Markov caché. Chez les petites espèces de poisson, le niveau trophique calculé à partir de valeurs AISA-AA était le meilleur indicateur des concentrations de mercure. Le mercure chez les grandes espèces était plutôt expliqué par les valeurs d'isotopes stables de carbone, indiquant que les habitats côtiers avaient de plus hauts

niveaux de contamination que les habitats pélagiques. La démonstration des endroits qui sont plus susceptibles à accumuler les contaminants est essentielle à la compréhension des risques posés aux animaux par leurs habitats et leur écologie. Ce mémoire développe de nouveaux outils pour mesurer la contamination dans les océans de plus en plus pollués.

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CONTRIBUTION OF AUTHORS

I conceived and designed the experiments for both data chapters with the help of Kyle Elliot and Raphaël Lavoie. David Pelletier (2017-2018) and Pauline Martigny (2021) conducted the fieldwork to collect samples I used, and all bird-banding permits are under Magella Guillemette. I analyzed the samples and data and wrote all three chapters myself with advice from Kyle Elliott and Raphaël Lavoie throughout. David Pelletier, Magella Guillemette, Marc Amyot, and Benjamin Barst provided editorial comments on the first manuscript (Chapter 2).

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$\delta^{13}C$ - stable carbon isotopes, the ratio of $^{13}C/^{12}C$

$\delta^{15}N$ – stable nitrogen isotopes, the ratio of $^{15}N/^{14}N$

$\delta^{34}S$ - stable sulfur isotopes, the ratio of $^{34}S/^{32}S$

AAs – amino acids

Ala – alanine

Asp – aspartic acid

BFR - brominated flame retardants

BMF – biomagnification factor

CH_3 – methyl group

CSIA-AA - compound-specific stable isotope analysis in amino acids

DDE – dichlorodiphenyldichloroethylene

DDT – dichlorodiphenyltrichloroethane

GC-C-IRMS - gas chromatography combustion isotope ratio mass spectrometry

Glu – glutamic acid

GPS – global positioning system

Hg – Mercury

Hg0 – elemental mercury

HMM – Hidden Markov Model

Ile – isoleucine

Leu – leucine

MeHg – methylmercury

nLeu – norLeucine

PCBs - polychlorinated biphenyls

PFASs - per- and polyfluoroalkyl substances

Phe – phenylalanine

POPs - persistent organic pollutants

Pro – proline

SIA – stable isotope analysis

TDF – trophic discrimination factor

THg – total mercury

TMF - trophic magnification factors

TMS – trophic magnification slope

TP – trophic position

Val – valine

GENERAL INTRODUCTION

Contaminants threaten the health of entire ecosystems, from plants to predators. Mercury, in its organic form, is of particular concern, due to its neurotoxic nature and ubiquity around the globe (Wolfe et al. 1998, Durnford et al. 2010). Anthropogenic disruptions to the natural mercury cycle increase emissions regionally (Morel et al. 1998, Driscoll et al. 2013), and cause mercury to be transported long distances and to contaminate ecosystems globally (Durnford et al. 2010). The effects of mercury contamination can have important consequences on wildlife, from reducing reproductive success to death (Wolfe et al. 1998, Scheuhammer et al. 2007, Zheng et al. 2019). It is therefore crucial to monitor and understand how mercury behaves in the environment, how mercury is transported globally, and how mercury biomagnifies through food webs to contaminate organisms.

Mercury contamination in aquatic ecosystems is especially concerning. Globally, oceans are an important sink of mercury, accumulating high concentrations through various processes such as riverine discharge and atmospheric deposition (Lamborg et al. 2014, Liu et al. 2021). Once mercury enters aquatic habitats, it can have a residency period of 20-30 years (Gworek et al. 2016). Aquatic food chains are also typically longer than terrestrial ones, and contaminants have more opportunities to biomagnify through the food web (Cabana and Rasmussen 1994). Marine wildlife is therefore at high risk of important mercury contamination.

The Gulf of St. Lawrence is a large and valuable ecosystem in Atlantic Canada, in terms of ecological, commercial, and cultural significance. The Gulf hosts a diversity of physical habitats, influenced by coastlines, geological formations, bathymetry, and depth (Nozères et al. 2015). Species richness and diversity are high and include a suite of demersal and pelagic fishes, invertebrates and phytoplankton, seabirds and marine mammals (Desgranges and Jobin 2003, Nozères et al. 2015). The area is also greatly important for shipping routes, connecting the North Atlantic to the Great Lakes via the St. Lawrence Seaway, which receives over 200 million tonnes of cargo annually (SLSMC 2020). The increased presence of humans and their activities have been shown to disturb many marine species and entire food webs. The greater presence of shipping vessels in the Gulf can help introduce more non-indigenous plants into the ecosystem, and the proximity of vessels to wildlife can hinder the foraging behaviour of marine mammals (Cohen et al. 2007, Lesage et al. 2017). Fisheries can cause important forage fish stocks to collapse, causing a cascade of events for seabirds such as shifts in diet, foraging range, and

breeding success (Chapdelaine and Rail 1997, Montevecchi 2007, Savenkoff et al. 2007, Guillemette et al. 2018). As such, this highly dynamic ecosystem is undergoing changes which, in many cases, are impacting wildlife in unknown ways.

The distribution of contaminants in the Gulf is therefore an important challenge for conservation and management. Understanding the dynamics of mercury contamination across habitats is necessary to properly evaluate the risks organisms are exposed to. The tools investigators use to study these risks are critical to forming correct and sound conclusions that pertain to the transfer of mercury through food webs. The advancement of new technologies and tools to track contamination in marine environments in more precise ways is also critical. New methods can help increase the accuracy of measurements (e.g., trophic level) and extent of the sampling area, while reducing time and money spent in the field.

Wildlife has been widely used as ecological indicators, as their study allows researchers to gain insight into the state of ecosystems, and resource availabilities or distributions (Zacharias and Roff 2001, Boucher et al. 2020). Top predators are often used because they integrate biological spatial and temporal variations into their tissues during their lifetime and can be used as proxies for other organisms feeding at high trophic levels. Seabirds are especially good ecological indicators because of their relevant diet usually composed of fish. In addition, they are highly accessible during the breeding season and can be sampled repeatedly at their colony, as they must regularly return to it to raise their fledgling (Piatt et al. 2007). Northern gannets (*Morus bassanus*) have been used as ecological indicators in the past to monitor various contaminants. They have successfully been used to link thinning eggshells and reduced reproductive success to dichlorodiphenyltrichloroethane (DDT) contamination (Elliott et al. 1988), and to examine temporal trends in mercury, organochlorines, polychlorinated biphenyls, and brominated flame retardants (Champoux et al. 2015, Champoux et al. 2017). As such, northern gannets are relevant ecological indicators to use to study the prevalence and dynamics of mercury contamination in the Gulf of St. Lawrence.

The research aim of this thesis is to understand how the food-web dynamics and spatial distribution of mercury influences the contamination of wildlife in the Gulf of St. Lawrence using the gannets as a model organism. The main research aim will be addressed through the following objectives:

1) Evaluate and contrast biomagnification metrics describing the transfer of mercury through food webs using traditional and new methods of stable isotope analyses in the fish-to-gannet food web.
2) Investigate the biotic and anthropogenic drivers of total mercury variation across the Gulf of St. Lawrence using regurgitated fish samples collected from GPS-tracked gannets.

A pair of northern gannets (*Morus bassanus*) at the Bonaventure Island colony. Photo by Roxanne Turgeon

Chapter 1: Literature Review

This section will review the important concepts connected to and explored within my thesis. First, the types of contaminants, their effects on ecosystems, and the ways through which they accumulate in wildlife tissues will be discussed, before focusing on mercury. The different sources of mercury, as well as its toxicity, will be reviewed. I then discuss the stable isotope tools that have previously been used to study mercury contamination and biomagnification in the past as well as newer methods, before reviewing the use of wildlife, seabirds especially, as ecological indicators.

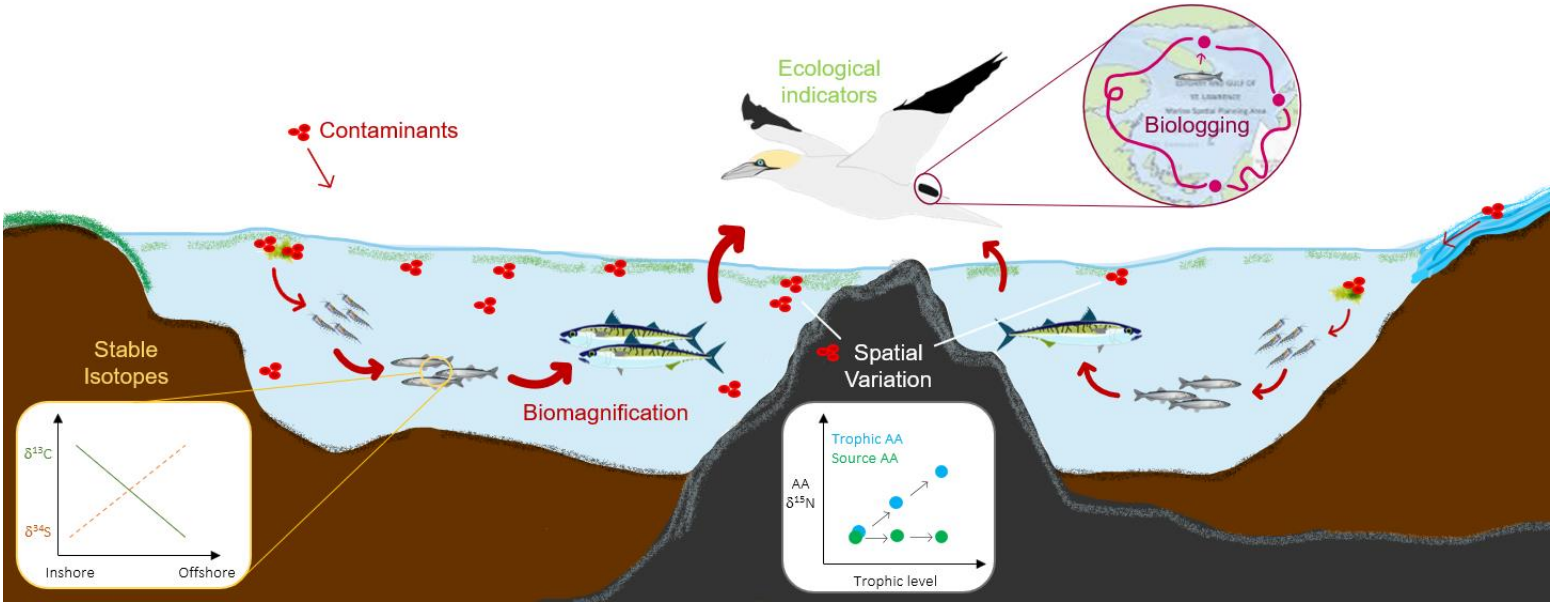

Figure 1.1. Summary figure showing the different topics discussed in this literature review. Contaminants enter aquatic ecosystems through processes such as atmospheric deposition (top left) or riverine inputs (far right). Contaminants then biomagnify through the food web to accumulate to high concentrations in top predators (note the thickness of the arrows representing the severity of contaminant transfer). Stable isotopes of $\delta^{13}C$ and $\delta^{34}S$ can inform an organism's foraging habitat (bottom left), and stable isotopes of $\delta^{15}N$ in amino acids (AA) can provide baseline-corrected trophic positions to help study biomagnification (bottom center). Different habitats can be contaminated to different extents (see left vs right basins), requiring the use of compound-specific stable isotope analysis in amino acids (bottom center). Mobile predators can be used as ecological indicators of their foraging environment (top center) and biologging devices can record valuable foraging and migratory behaviours (top right).

1. Contaminants in wildlife

A wide range of contaminants enter the environment and threaten the health of ecosystems. Contaminants come from both natural and anthropogenic sources and can negatively impact the health of plants and animals. Heavy metals such as lead, arsenic, mercury, and cadmium have been well-studied and their health effects range between altered metabolism, reproductive failure, compromised immune systems, and death (Scheuhammer et al. 2007, Dietz et al. 2019). Other well-known contaminants include pesticides, like dichlorodiphenyltrichloroethane (DDT) and neonicotinoids, pharmaceuticals, and flame retardants (Sauvé and Desrosiers 2014). The impacts on health linked to these contaminants are wide-ranging. Contaminants have spread worldwide via long-range transport, even to remote areas through the air, and water currents and are transported by biota (Tong et al. 2022), putting fauna and flora in these ecosystems at risk of health issues from indirect contamination of their habitat (Battaglin et al. 2018). Therefore, it is crucial to study the dispersal and distribution of contaminants locally, regionally, and globally.

Contaminants can have long-lasting effects and pose an important risk to wildlife, even years after being banned. Legacy contaminants are compounds that were phased out of industries or banned by international treaties, like the United Nations Stockholm Convention on persistent organic pollutants (POPs). This convention entered into force in 2004 and banned the use of common compounds, such as DDT, polychlorinated biphenyls (PCBs), chlordane, dieldrin, and many other chemicals used as pesticides, in industry, or those formed as unintentional by-products (Fiedler et al. 2019). The Stockholm Convention includes chemicals that have the potential to persist in the environment, be transported over long distances, accumulate in organisms during their lifetime, and cause adverse effects on health (Fiedler et al. 2019). However, even though most legacy contaminants have been out of use for close to two decades, and even longer for others (such as DDT), some POPs continue to plague ecosystems and contaminate wildlife. For example, in the Coatzacoalcos River, Mexico, where POPs were phased out in 1991, PCBs, DDTs, and other POPs continued to cause oxidative damage to DNA in wild reptiles, fishes, amphibians, and invertebrates decades later (González-Mille et al. 2019). The persistence of legacy contaminants can negatively impact ecosystem integrity over long periods of time.

Environmental contamination is of particular concern in top predators because contaminants will biomagnify up the food web and bioaccumulate in long-lived organisms. Bioaccumulation occurs when organisms accumulate contaminants from nutrient assimilation in their tissues during their lifetime (Atwell et al. 1998). Thus, longer-lived animals will be more contaminated due to longer exposure to chemicals through their diet (Morel et al. 1998). Biomagnification is the process whereby the concentration of a compound will increase with each stepwise increase in trophic level (Kelly et al. 2007). This phenomenon occurs because animals feeding at higher trophic levels consume large quantities of lower-trophic level organisms, and the amount of contaminant assimilated exceeds that of the amount excreted, leading to an increase in predator contaminant burdens relative to their prey. This means that long-lived, high-trophic consumers, most often carnivorous or piscivorous mammals, birds, and fish, are at higher risk of suffering health effects from contaminants. Further, the efficiency of biomagnification can differ between and within habitats depending on the contaminant of interest (Fioramonti et al. 2022). For example, Choo et al. (2019) found that brominated flame retardants (BFR) accumulated to higher concentrations in benthic foraging fish, than in pelagic fish, likely to due different rates of biomagnification and bioaccumulation between the two habitats. Therefore, understanding the life history and diet of an organism is essential to studying contaminants and their transfer through the environment.

Emerging contaminants, such as per- and poly-fluoroalkyl substances (PFASs), are chemicals which are not regulated, or for which little is known in terms of their toxicity and potential detrimental effects on the environment (Petrisor 2004, Sauvé and Desrosiers 2014). Unstudied or “emerging” chemicals can trigger waves of research investigating their hazards, thus labelling a contaminant as “of emerging interest” (Petrisor 2004). This occurs when they are detected in important resources, like drinking water. As the field of ecotoxicology continues to evolve, new contaminants become of concern for the environment and human health. However, some compounds are neither new and emerging, nor legacy contaminants, but are of continuous concern due to their prevalence and ubiquity across ecosystems, such as mercury.

1.1 Mercury and methylmercury

Mercury (Hg) is a metal that naturally occurs in the Earth’s crust, and that is remobilized into the atmosphere by the natural biogeochemical cycle, rather than being exclusively man-made

(Jackson 1997). Some natural sources of Hg emissions include forest fires, volcanic eruptions, and Hg-enriched soils and vegetation from previous Hg deposition (Morel et al. 1998, Mason 2009, Driscoll et al. 2013). However, most of the current global Hg emissions are caused by disturbances to these Hg-rich areas by humans, exacerbated by re-emissions into the atmosphere (Mason 2009, Pirrone et al. 2010). Humans have greatly disrupted the natural Hg cycle: since the beginning of the anthropogenic era, Hg emissions have increased drastically, mainly due to activities such as mining, metal processing, biomass burning, and industry (Morel et al. 1998, Driscoll et al. 2013). The gaseous elemental form of Hg (Hg0) is highly volatile, and once it enters the atmosphere, it can be transported over long distances to remote ecosystems (Durnford et al. 2010), where it is deposited mainly in the soil and water (Morel et al. 1998, Lamborg et al. 2014). Because of ongoing climate change, Hg is increasing in the atmosphere, and long-range transport facilitates the contamination of remote habitats (O'Driscoll et al. 2005). Lamborg et al. (2014) estimated that the quantity of Hg present in oceanic waters has increased by 150% due to anthropogenic disruptions to the Hg cycle. Further, Hg is known to have a long residency time in aquatic environments, between 20 to 30 years (Gworek et al. 2016). Consequently, living organisms are continuously and increasingly exposed to Hg through most facets of their environment and are at great risk of suffering from toxicity.

Hg is a contaminant of major concern because it is easily taken up by organisms and transferred through the food web. Organic forms of Hg, like methylmercury (MeHg), are especially bioavailable and toxic. MeHg is produced by sulfate-reducing bacteria in anoxic and aquatic environments through the process of methylation - the addition of a methyl group (CH_3) to the Hg molecule (Ma et al. 2019). MeHg readily enters food webs by bioaccumulating in plants and biomagnifying between prey and their predators (Raj and Maiti 2019). Diet is the most important mechanism of exposure to MeHg and Hg in animals and higher-level consumers accumulate high concentrations in their tissues through bioaccumulation and biomagnification (Atwell et al. 1998). MeHg is also an important threat to humans, who intake MeHg primarily through the consumption of seafood (Díez 2009, Lavoie et al. 2018). MeHg is a powerful neurotoxin in humans, causing developmental delays in fetuses and children, diseases such as cerebral palsy, and in severe cases, death (Díez 2009). A famous decades-long occurrence of mass MeHg poisoning through shellfish consumption caused hundreds of deaths in the Minamata Bay community in Japan, which became known as Minamata disease (Díez 2009). In 2013, the

Minamata Convention on Mercury came into force to reduce global mercury emissions and protect the health of coastal communities (UNEP 2013).

MeHg is particularly concerning in food webs due to its toxicity. In most organisms, MeHg can act as 1) a neurotoxin, causing ataxia or paralysis, 2) an immunotoxin, reducing the capacity of the immune system to fight off infections, or 3) it can damage genetic material in fully developed organisms (Wolfe et al. 1998). The main toxicological effect of MeHg in fish pertains to reproductive impairment, by inhibiting gonadal development, changing behaviour, and disrupting the production of sex hormones (Scheuhammer et al. 2007). For example, MeHg slowed growth and impaired the immune system in juvenile walleye (*Sander vitreus*), which increased the likelihood of mortality (Friedmann et al. 1996). Birds and mammals are also susceptible to MeHg contamination causing changes to usual behaviour, immune function, and leading to reproductive failure (Scheuhammer et al. 2007). In common loons (*Gavia immer*) for example, adults with higher MeHg loads left their eggs unincubated more often and for longer than loons exposed to lower concentrations of Hg (Evers et al. 2008). Little auks (*Alle alle*) with higher mercury burdens were physiologically limited in their foraging activities (Grunst et al. 2023). In vampire bats (*Desmodus rotundus*), high MeHg contamination impaired immune function and increased individuals' susceptibility to pathogens and bacteria (Becker et al. 2017). Little is known about the long-term effect of MeHg contamination in reptiles, herpetofauna, and invertebrates, but it is suspected to contribute to population declines as one of the multiple stressors threatening populations (Scheuhammer et al. 2012). It is vital to understand the behaviour of Hg compounds at every level of the food web because of their ability to easily travel through the environment and the food chain and to cause important physiological effects.

1.2 Spatial distribution of mercury

Contaminant concentrations vary between different ecosystems and habitats. Understanding where contaminants accumulate is hence important to evaluate threats to fauna and flora. Some nutrients and pollutants have been shown to exist in gradients in marine habitats. The Mediterranean Sea is well known for having higher Hg concentrations than any other marine site (Martins et al. 2006, Pinzone et al. 2019). The vertical stratification in the ocean produces favourable environments for Hg methylation in deeper waters, meaning deeper foraging species often have higher MeHg burdens in their tissues than those that forage in the epipelagic zone

(Anderson et al. 2009, Houssard et al. 2019). High Hg concentrations have been linked to industrialized areas; in the western Pacific Ocean, an important Hg hotspot was identified near East China and Yellow Seas, likely due to increased anthropogenic inputs (Médieu et al. 2022). The same study also identified the western United States and Mexico as areas rich in Hg. In the southwestern Pacific Ocean, Houssard et al. (2019) found increasing Hg concentrations between Papua New Guinea and New Caledonia. Although Hg emissions are higher in East Asia, Lippold et al. (2022) found that polar bears (*Ursus maritimus*) in the Russian Arctic did not display higher Hg than populations in Norway and Canada. This demonstrates that even when Hg emissions are higher in the environment, concentrations in wildlife may reflect patterns due to other processes. In a wider scope, it is crucial to monitor contaminants in general, as they remain prevalent in the environment and can cause harm to wildlife, human, and ecosystem health.

2. Stable Isotopes

Stable isotope analysis has been a key tool for understanding the spatial distribution of contaminants. Isotopes are different forms of elements that have unique atomic masses (West et al. 2006). Stable isotopes do not decay like their radioactive counterparts (West et al. 2006), and the differences in mass between two isotopes of the same element determine their reactions in metabolic processes and their signatures in wildlife (Peterson and Fry 1987). Stable isotope signatures are expressed as delta notation (δ) in permil (‰), and are calculated using the following equation (Peterson and Fry 1987):

$$\delta X\ (‰) = \frac{(R\ sample - R\ standard)}{R\ standard} \times 1000 \qquad (1)$$

Where X represents an element of ecological interest, usually carbon, nitrogen, sulfur, oxygen, or hydrogen, and R is the ratio of heavy to light isotope of the element (e.g. $^{13}C/^{12}C$) compared to a certified standard. Organisms take up carbon, nitrogen, and sulfur isotopes from their environment mainly through their diet (West et al. 2006).

Stable isotope signatures can change throughout the food web as predators feed on prey and metabolize the isotopes, incorporating them into their tissues (DeNiro and Epstein 1978). This process is called discrimination, and is essentially the difference between the predator's signatures and its prey's signatures (Peterson and Fry 1987):

$$\Delta\,(‰) = \delta Predator - \delta Prey \quad (2)$$

The discrimination rates can be tissue- and species-specific but some general trends have been established (Hobson and Clark 1992b). The discrimination of $\delta^{15}N$ is typically considered to be 3.4‰ per trophic level (Post 2002). Further, different tissues turnover, or regenerate, at different rates, and therefore, represent different seasons and timeframes (Hobson and Clark 1992a). For example, Hobson and Clark (1993) showed that $\delta^{13}C$ in American crows (*Corvus brachyrhynchos*) red blood cells had a half-life of approximately 30 days, whereas the plasma fraction had a half-life of just 2.9 days. The isotopic signatures in muscle typically represent recent (over a few weeks) dietary intake (Winter et al. 2019), while feathers can record isotope values only during feather formation (Bond 2010). Hence, there is an important need for research to determine the turnover rates in many tissues and taxa.

2.1 Stable isotopes capture ecological trends

As stable isotopes change between organisms and through the environment, they can be useful ecological tracers to study various biological processes. Stable isotopes of nitrogen and carbon change as they transit through food webs, which can help researchers to study animal diets and trophic linkages (Post 2002). Isotopic signatures of carbon and oxygen in migratory animals' tissues generated during overwintering periods (i.e., away from breeding grounds) can inform on life history (McMahon et al. 2013). Continental gradients of hydrogen and oxygen isotopes can help establish previously unknown migration areas (Hobson 1999). In contaminant studies, nitrogen, carbon, and sulfur are most used to trace the movement and origin of harmful chemicals in food webs as they are the most abundant isotopes and are linked to diet (Cabana and Rasmussen 1994, Post 2002, Connolly et al. 2004).

Bulk nitrogen isotopes (as represented by $\delta^{15}N$) are commonly associated with an animal's trophic position, due to the large discrimination factor of nitrogen that occurs between trophic levels (3 - 5‰; Peterson and Fry 1987). $\delta^{15}N$ signatures increase in a stepwise manner between trophic levels and can help differentiate organisms feeding at different trophic positions. The use of $\delta^{15}N$ can help determine previously unknown prey items (Hobson et al. 1994). Additionally, because $\delta^{15}N$ is associated with increasing trophic levels, these isotopes have commonly been used to assess the biomagnification of contaminants through food webs. One notable study,

conducted by Cabana and Rasmussen (1994), showed that $\delta^{15}N$ was a better predictor of Hg contamination than trophic position in lake ecosystems, due to its continuous nature, and a greater sensitivity to dietary breadth, such as omnivory. Since then, using $\delta^{15}N$ to assess trophic and contamination levels has been standard practice (Won et al. 2018).

Carbon ($\delta^{13}C$) stable isotopes mainly inform on foraging habitats and can differentiate between terrestrial, coastal, or marine foraging areas. Groups of primary producer organisms have different photosynthetic pathways (C3 vs C4 producers) and as such, carbon isotopic signatures vary between habitats predictably (DeNiro and Epstein 1978). In marine environments, $\delta^{13}C$ is typically lower (~ -24‰) when associated with phytoplankton-based (i.e., offshore, pelagic) food webs, while inshore and benthic habitats are higher (~ -17‰; Peterson and Fry 1987, France 1995, Hobson 1999). Freshwater habitats can also display gradients of $\delta^{13}C$ where the offshore, or pelagic zones, of lakes are typically much lower (~ -36‰), and the lacustrine benthos regions are comparatively higher (~ -26‰), while riverine benthos tend to display intermediate signatures (France 1995). Estuaries are also transitional habitats and can have variable $\delta^{13}C$ values (-16 to -25‰; Fry 2013). As carbon moves through the food chain, slight discrimination occurs between diet and animal tissues. This discrimination factor seems to depend on the diet composition and tissue type, and studies have reported $\delta^{13}C$ discrimination ranging between 0.4‰ to 2.6‰ in a variety of organisms (DeNiro and Epstein 1978, Roth and Hobson 2000, Post 2002). The application of $\delta^{13}C$ to ecological studies thus rests on prior knowledge about the isotopic composition of the ecosystem of interest.

Sulfur ($\delta^{34}S$) signatures can also be useful to determine foraging habitats for organisms of interest. $\delta^{34}S$ displays a gradient between inshore (lower; ~ 4‰) and offshore (higher; ~ 20‰) habitats in marine environments, as well as between surface (higher; ~ 20‰) and benthic waters (depleted; ~ -24‰; Connolly et al. 2004, Elliott and Elliott 2016). $\delta^{34}S$ is a great asset to differentiate between marine inputs such as estuaries, nearshore, and pelagic waters (Elliott and Elliott 2016). When the use of stable isotopes became widely used, sulfur was considered to have a small trophic discrimination factor between trophic levels (Peterson and Fry 1987, Connolly et al. 2004). However, more recent studies have found significant fractionation between diet and consumer tissues, as well as variability in discrimination rates between tissues, in seabirds, suggesting that the dietary fractionation of sulfur is a variable that researchers should be wary of

when using $\delta^{34}S$ (Craig et al. 2015, Rosciano et al. 2023). $\delta^{34}S$ has also been found to predict Hg concentrations in seabird tissues more consistently than $\delta^{15}N$ (Elliott and Elliott 2016, Góngora et al. 2018).

Even though $\delta^{13}C$ and $\delta^{34}S$ are used for similar purposes, they do have opposite relationships between nearshore/benthic and offshore/pelagic habitats. Combining the use of both these stable isotopes can elucidate feeding patterns for animals which would be confounding when only using one isotope. For example, $\delta^{34}S$ in polar bear (*Ursus maritimus*) guard hair helped correctly identify inshore feeding habitats for these animals in the late summer, while the $\delta^{13}C$ indicated pelagic feeding (Stern et al. 2021). The use of $\delta^{13}C$ alone to determine the feeding habitats would have resulted in a misinterpretation of the ecological data. Lavoie et al. (2015) used threshold values for both carbon (separating natural freshwater, aquaculture, and ^{13}C-rich waters) and sulfur (differentiating marine from freshwater habitats) to assign feeding habitats to individual double-crested cormorants (*Nannopterum auritus*) and Caspian terns (*Hydroprogne caspia*). $\delta^{13}C$ and $\delta^{34}S$ can also be used together to categorize organisms into known feeding habitats.

Stable isotopes vary both spatially and temporally in ecosystems, and understanding these fluctuations is important to correctly interpret ecological trends. While this variability is what allows researchers to gain insight into ecological processes in the first place, some spatial or temporal variability may go unnoticed which may skew conclusions that are drawn from data. The Suess effect, for example, consists of the temporal decline of $\delta^{13}C$ ratios in marine habitats over decades, due to the anthropogenic addition of carbon to the environment through fossil fuel burning (Revelle and Suess 1957). Studies that compare samples over extended periods (i.e., decades) must take the Suess effect into account when analyzing their data, to avoid drawing any biased conclusions (Keeling 1979). Stable isotopes of $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ also vary in space. As discussed earlier, $\delta^{13}C$ and $\delta^{34}S$ vary in marine environments, which allows researchers to identify foraging habitats. $\delta^{15}N$ can also vary spatially, due to fluctuations of primary producers (Brault et al. 2018). $\delta^{15}N$ fluctuations in marine environments can be caused by abiotic factors, such as depth and the underwater landscape, and biotic variables, such as microbial activity and primary producer biomass (Graham et al. 2010, McMahon et al. 2013). This so-called “baseline” of continually shifting $\delta^{15}N$ values in primary producers is important to consider when comparing habitats at different times, or that are geographically different.

2.2 Compound-specific stable isotope analysis in amino acids

The analysis of stable nitrogen isotopes in individual amino acids (compound-specific stable isotope analysis in AAs; CSIA-AA) is relatively new and offers an efficient measure of both baseline and consumer isotopic values. Amino acids (AAs) are molecules that make up proteins in animal tissues. Some AAs, such as glutamic acid, have higher discrimination factors between prey and predators (McClelland and Montoya 2002), resulting in higher $\delta^{15}N$ in predator tissues. AAs that follow this pattern are classified as "trophic AAs" and include glutamic acid, aspartic acid, alanine, isoleucine, leucine, proline, and valine (Chikaraishi et al. 2009). Other AAs are termed "source AAs" because they have very small discrimination factors between prey and predator tissues. Source AAs give insight into the isotopic composition of organisms at the base of the food web, like primary producers, because of this small or null discrimination (McClelland and Montoya 2002). This category includes phenylalanine, methionine, lysine, and tyrosine (Chikaraishi et al. 2009).

In their seminal work, McClelland and Montoya (2002) reported that the discrimination factor for phenylalanine was null, but this has been refuted by more recent studies (Chikaraishi et al. 2009, Ohkouchi et al. 2017). Some studies have reported small (~ 0.7‰) but significant discrimination factors of phenylalanine (Phe), which they caution can have an important impact when sampling high trophic consumers (Chikaraishi et al. 2009, Ohkouchi et al. 2017). As Phe is passed through multiple consumers, e.g., four trophic positions, the small fractionation rate becomes more important (~ 2.8‰), constituting close to one trophic position (McMahon and McCarthy 2016). However, the fractionation rate differs between different organisms and is mostly attributed to diet quality (McMahon et al. 2015). A high-quality diet (diet items' AA composition highly resembles the consumer's AA composition) will result in lower fractionation rates. While the consensus remains that Phe fractionates the least between all AAs and it can still be used as a proxy to measure baseline isotopic composition, Phe must be used cautiously as a source AA and must be corrected for small levels of fractionation in certain cases. Glutamic acid (Glu) and Phe are most used due to the quality of the resulting AA data: these two AAs are consistently reported to vary the least in the output following analysis (Chikaraishi et al. 2009, Chikaraishi et al. 2014).

The most common way of calculating baseline-corrected trophic positions with AAs is as follows (McMahon and McCarthy 2016):

$$TP_{CSIA} = 1 + \left[\frac{\delta^{15}N_{Glu} - \delta^{15}N_{Phe} - \beta}{\Delta^{15}N_{Glu} - \Delta^{15}N_{Phe}}\right] \quad (3)$$

Where $\delta^{15}N_{Glu}$ and $\delta^{15}N_{Phe}$ are measures of nitrogen isotopes in the consumer's tissues, β represents the difference between Glu and Phe AA $\delta^{15}N$ in primary producers of the food web of interest (Chikaraishi et al. 2009). The denominator represents the trophic discrimination factor (TDF) for Glu and Phe, calculated by subtracting the fractionation coefficient specific to Phe from the fractionation coefficient for Glu. Other methods to calculate baseline-corrected trophic positions are similar, but use the average of many trophic AAs in place of Glu (Ohkouchi et al. 2017). CSIA-AA also allows for corrections of baseline variability when calculating biomagnification slope coefficients (Won et al. 2018, Elliott et al. 2021).

However, because the fractionation of AAs differs between groups of organisms and even between tissues, some trophic calculations must be adjusted. For example, Quillfeldt and Masello (2020) used different β coefficients and discrimination rates to calculate trophic positions from blood and feathers originating from the same Gentoo penguins (*Pygoscelis papua*) and other seabirds. This is due to the metabolic processes involved in the formation of different tissues, which fractionate $\delta^{15}N$ at different rates (Chikaraishi et al. 2014). Therefore, when using different tissues to compare organisms, or when comparing certain carnivorous consumers to their prey, it may be important to use taxon-specific variables within the more general equation 3.

2.3 Stable isotopes in ecotoxicology

Because CSIA-AA helps correct for baseline variability, this approach is ideally suited for studying the distribution of contaminants across ecosystems and through time. The calculation of accurate trophic positions in organisms when conducting food web contamination studies is essential to reporting ecological phenomena accurately, and to avoid overextending or underestimating the significance of the research (Won et al. 2018). In Pacific seabirds, for example, a shift in diet over two decades (1973 – 2006) made it appear as though Hg loads were decreasing over time (Elliott et al. 2021). However, when dietary shifts were accounted for, these

temporal trends disappeared. In many Canadian lakes, differences in the rate of Hg biomagnification in gulls (*Larus* spp.) were elucidated by gradual declines in trophic positions by individuals in some lakes and not in others (Dolgova et al. 2018, Hebert and Popp 2018).

Moreover, a smaller subset of samples analyzed for CSIA-AA can be used to trophic-correct samples analyzed only for bulk $\delta^{15}N$ (Dolgova et al. 2018). Due to the high analytical costs associated with CSIA-AA, it is much more accessible to analyze a smaller subset of samples and to develop a general correction for bulk data, as done by Dolgova et al. (2018). However, these authors were applying the trophic correction to samples that came from relatively closed ecosystems (i.e., lakes), thus expecting the baseline corrections in a single lake to be rather robust. To my knowledge, no papers have attempted to apply this type of baseline correction to samples analyzed for bulk $\delta^{15}N$ in a more open and dynamic ecosystem, such as estuaries or gulfs.

In summary, stable isotopes can be vital ecological tracers that can help elucidate the structure of food webs, determine key areas in an organism's life history, and track the transfers of energy and contaminants through food webs. The use of bulk stable isotopes, and more recently, CSIA-AA, allows researchers to gain knowledge about ecosystems and the processes within. While stable isotopes can be sampled from fauna, flora, and soils; wildlife remains the principal entity studied by this approach.

3. Ecological indicators

Wildlife organisms are useful monitors of ecosystem contamination, as they integrate and accumulate signatures over extended periods. Biotic sampling is an affordable, and often a telling way to interpret the effects of abiotic factors, such as weather patterns and temperature regimes on ecosystems (Piatt et al. 2007b). Wildlife health reflects the availability of resources and the extent of contamination and disease in a food web. Factors such as survival rate, breeding success, and foraging effort can inform on resource distributions or availability, which can be influenced by abiotic factors (Cairns 1987, Piatt et al. 2007a, Boucher et al. 2020). The early clearing of sea ice in recent decades, for example, has led to the increased body condition of bowhead whales (*Balaena mysticetus*) and Arctic char (*Salvelinus alpinus*) because they could exploit phytoplankton blooms earlier (Harwood et al. 2015). Fluctuations in contaminant concentrations can point to dietary shifts toward new prey species that are expanding their range

and that are exposed to different sources and types of contaminants (Braune et al. 2014, Harwood et al. 2015). The decrease in beluga (*Delphinapterus leucas*) liver Hg burdens, while environmental concentrations remained constant, indicated a probable prey shift in available prey species in the Beaufort Sea (Loseto et al. 2015).

Top predators are especially useful, as they can be used as proxies for entire food webs and can integrate information across space and time. Large carnivores require sufficient prey to survive and successfully reproduce and are therefore sensitive to changes in lower levels of the food web. Predators are unlikely to switch to new prey unless their usual resources are limited, so changes to their isotopic signatures are typically indications of an ecological shift in the food web (Hanson et al. 2018). Polar bears, for example, were observed to increase their isotopic niche – that is, diversifying their prey and foraging in new areas – in years of poor ringed seal (*Pusa hispida*) reproduction (Boucher et al. 2020). A poor year for polar bear health can indicate an important ecosystem shift that is limiting important resources at lower trophic levels, such as the lack of keystone forage fish. Contaminants bioaccumulate and biomagnify to important levels in top predators, and are more easily tested for than the small, sometimes undetectable, concentrations in shorter-lived primary consumers and mid-level predators (Cabana and Rasmussen 1994, Atwell et al. 1998, Senn et al. 2010). Crocodilians, for example, are generalist feeders in fresh- and salt-water wetland ecosystems, which offers a unique opportunity to examine contaminant signatures in their habitats (Schneider et al. 2013). They have large and infrequent meals, thus their tissues record ‘pulses’ of nutrients. These pulses can then be contextualized and related to environmental conditions (Schneider et al. 2013). Considering these studies, researching a single predator species can help further knowledge about multiple prey groups and ecosystem mechanisms.

3.1 Seabirds

Marine environments can be challenging to monitor due to the mobility of aquatic species and the variable nature of habitats (Zacharias and Roff 2001). Seabirds offer an accessible opportunity to monitor this ever-changing environment through wildlife monitoring practices (Piatt et al. 2007b). Seabirds, during the breeding season, are central place foragers: they forage in marine waters and cover large areas but must return to their breeding grounds regularly (Schreiber and Burger 2001). Seabird behaviour, combined with breeding success, can indicate

shifting prey distributions or assemblages (Brisson-Curadeau et al. 2017, Guillemette et al. 2018), while factors such as survival can signal important abiotic shifts (Cairns 1987, Piatt et al. 2020). Many species of seabirds are colonial breeders, which facilitates sampling and allows for efficient and inexpensive data collection. Samples such as prey, feathers, blood, eggs, as well as behaviour can provide information about the contamination and distribution of resources in the environment (Furness et al. 1986, Hobson and Clark 1993, Montevecchi and Myers 1996).

Seabird behaviour and mortality have often indicated large-scale shifts in the marine ecosystems they exploit. For example, in the northwestern Atlantic, a cold temperature shift in the early 1990s forced northern gannets (*Morus bassanus*; hereafter gannet) to switch to cold-water prey, which resulted in important trophic shifts for this species (Montevecchi and Myers 1996, Montevecchi 2007). Further, even though sea temperatures returned to pre-shift values in the mid-1990s, gannets and fisheries continued to catch the newer cold-water species, and the return of warm-water species in gannet diets lagged by a decade (Montevecchi 2007). Similarly, breeding failures at seabird colonies globally have foretold the collapse of economically important fisheries stock, like the anchoveta (*Engraulis ringens*; Peru), Atlantic herring (*Clupea harengus*; Norway), capelin (*Mallotus villosus*; Barents Sea), and sandlance (*Ammodytes* spp.; North Sea) since the 1950s (Furness and Camphuysen 1997, Piatt et al. 2007b). Temporal Hg trends in black-legged kittiwakes (*Rissa tridactyla*) showed how available prey in the Barents Sea shifted in two subsequent events from the typical forage fish (Arctic cod; *Boreogadus saida*) to Atlantic prey like capelin, and later to Atlantic herring (Tartu et al. 2022). These examples show how biotic samplers can provide more insight into ecological phenomena which may appear to be resolved through the lens of abiotic sampling.

Seabirds integrate biological signatures from their prey and can offer insight into the transfer of contaminants as they biomagnify up food webs (Monteiro et al. 1998, Arcos et al. 2002). Contaminant levels can be difficult to track in lower-level consumers because of low concentrations, but as these contaminants magnify through many trophic levels and reach seabirds, the likelihood of detecting concentrations increases (Mallory and Braune 2012). Studying contaminants in seabirds and their prey can paint a clearer picture of how contaminants behave during trophic transfer (Monteiro et al. 1998) and how a consumer's metabolism can impact their contaminant burden (Le Croizier et al. 2022). Seabirds are also more susceptible to

certain types of contaminants than primary consumers, like fat-soluble compounds such as organochlorine compounds, which can more easily bind and accumulate in seabird tissues than in lipid-poor organisms such as invertebrates (Furness and Camphuysen 1997). While contaminants are present at every level of a food web, concentrations may only become high enough to study in predator populations, making seabirds a wise choice to study the trophic transfer of pollutants.

The introduction of tracking devices to ecotoxicological studies has helped produce refined isotopic and chemical landscapes from predator tissues. Some biologging devices are much more precise than others. For example, GPS record latitudinal and longitudinal coordinates while geolocators give rough position estimates based on the light cycle. Accelerometers inform on animal movement on three axes and time-depth recorders offer insight into the vertical habitat use of animals. Biological samples with a short turnover rate like plasma can provide isotopic and chemical information in the short term, which can then be associated with a precise geographic point, representing diving locations or a mean foraging point—the exact point where prey meets predator and contaminants thus move up a trophic level (Jaeger et al. 2010, Carpenter-Kling et al. 2020). The use of geolocation can also help account for anthropogenic additions to diet, such as seabirds feeding on fisheries' discards, which can skew the isotopic and contaminant signatures found in tissues (Ceia et al. 2015). In addition, tracking devices can help researchers identify individuals that may be foraging in significantly different habitats, such as at different oceanic fronts (Carpenter-Kling et al. 2020), or those that overwinter in vastly different areas (Fort et al. 2014, Albert et al. 2021). Understanding where seabirds forage is crucial to accurately mapping out isotopic and chemical landscapes on a finer scale.

Few studies have used tracked seabirds to sample contaminants in different habitats. On an Ocean scale, during the non-breeding season, Fort et al. (2014) used geolocators deployed on little auks to identify the mean wintering area in the Northwestern Atlantic and relate the geographical position to Hg in feathers. In the same region, Pollet et al. (2022) showed how Hg exposure in GPS-tracked Leach's storm-petrels, sampled from blood, could be influenced by foraging behaviours (depth of foraging ground and latitude). A study on GPS-tracked gulls (*Larus* spp.) off the French Coast found that increased Hg was associated with marine diets, rather than terrestrial foraging (Jouanneau et al. 2022). In the Pacific Ocean, Ito et al. (2013) used GPS devices on streaked shearwater (*Calonectris leucomelas*) to demonstrate how different

foraging areas could impact POPs concentrations in preen oil, which represented short-term exposure to contaminants, during the most recent tracked foraging trip. On a much smaller scale, a study in the St. Lawrence River showed that GPS-tracked male ring-billed gulls (*Larus delawarensis*) were exposed to higher polybrominated diphenyl ether concentrations when they foraged in waste management facilities than males that foraged in the river, or agricultural areas (Gentes et al. 2015). Another study in the same region showed that landfills are important habitats contributing to the exposure of ring-billed gulls to halogenated flame retardants and organophosphates (Kerric et al. 2021). Even fewer studies have used seabirds to sample prey, and these studies did not use biologging devices to identify the likely origins of the fish (Góngora et al. 2018, Le Croizier et al. 2022). Thus, integrating the opportunistic sampling of prey into GPS-tracked seabird studies could expand the possibilities of using seabirds as biological samplers.

While top predators like seabirds are useful monitors for ecosystem processes, caution is advised when interpreting data. Seabirds are only useful monitors when researchers understand the species' response to environmental variability (Fleming et al. 2016). Knowledge about the life history and foraging ecology is crucial to using seabirds as monitors. Some species may be specialists, reflecting the changes in a well-defined component of the ecosystem, while others may be generalists that reflect and integrate ecological signals from many species and habitats (Monteiro et al. 1999). Moreover, the migratory movements of seabirds can impact the ecological data that they integrate. Stable isotope and contaminant signals may reflect the prey exploited in their overwintering grounds and differ between individuals breeding at the same colony but overwintering in different locations (Fort et al. 2014). Because seabirds are usually long-lived and mobile, they also take up contaminant loads from many different habitats, and over time these contaminants can be incorporated into tissues in complex ways (Monteiro and Furness 1995). Consequently, researchers require long-term data sets to use seabirds as ecological indicators, to identify temporal trends (Furness and Camphuysen 1997).

3.2 Northern gannets

Northern gannets are a relatively well-studied seabird which breeds in Eastern North America and Western Europe. They breed colonially on the rocky cliffsides of the Atlantic Ocean (Mowbray 2002). Gannets are typically monogamous and raise a single brood per year, consisting of a single egg (Montevecchi et al. 1984). They exhibit biparental care, although

males and females have different brooding strategies. Males are present at the colony (named gannetry) more often, but females tend to incubate for longer periods (Fifield et al. 2014). Nestlings are altricial (i.e., hatch in a vulnerable state and require parental care and protection) and remain in the nest for 90 days on average, before fledging (Montevecchi et al. 1984). Following the breeding season, gannets migrate south to overwinter thousands of kilometres away from their breeding grounds (Fort et al. 2012). North American individuals typically overwinter along the eastern coast of the United States and in the Gulf of Mexico, while European gannets will migrate anywhere between Europe and Northern Africa (Fort et al. 2012, Fifield et al. 2014).

Gannets are abundant and are commonly observed along coasts where they forage. Populations on both sides of the Atlantic consistently grew from the mid-1980s to the early 2000s, until the global population was estimated to be 430 000 breeding pairs (Chardine et al. 2013). This estimate excludes non-breeding juveniles which are more difficult to monitor. 70% of gannets breed in the United Kingdom, and the largest gannetry is located on Bass Rock, Scotland (Wanless et al. 2006). Of the six North American colonies, three are located on the island of Labrador, and three are in the Gulf of St. Lawrence, including Bonaventure Island, Québec which is the largest North American gannetry (Chardine et al. 2013). Gannets are extremely faithful to the colony they hatched at and will rarely immigrate to a new one, except to form a new colony when resources become too limited (Wanless et al. 2006, Fifield et al. 2014).

Gannets can travel long distances to forage for their chicks. Foraging habits are different between sexes and between gannetries. Females are more selective in their choice of foraging areas than males are, and they have been shown to dive to deeper depths (Lewis et al. 2002). Breeding individuals from larger colonies travel farther and longer to forage (Lewis et al. 2001). As the breeding season progresses, the fish resources surrounding the colony are depleted or relocated to more suitable microhabitats and gannets must travel further to feed themselves and their nestlings (Elliott et al. 2009, Weber et al. 2021). Additionally, foraging parents may make single-day trips or multi-day trips to forage for the best resources for their nestling (Garthe et al. 2003). This variability in foraging technique means that gannets, as a colonial entity, cover extremely large areas surrounding their gannetry throughout the breeding season. Therefore, these seabirds

integrate information from a large ecosystem and allow researchers to monitor ecosystem indices.

Gannets are generalist foragers that exploit the epipelagic zone of marine waters, rarely diving deeper than 10 metres (Garthe et al. 2000). In North American colonies, gannet diet is dominated by capelin and Atlantic mackerel and can include a variety of prey such as Atlantic herring, sandlance, squid, and other small fish (Garthe et al. 2007, Guillemette et al. 2018). Gannets regurgitate spontaneously when disturbed or stressed, so cataloguing their diet has been an easy task for researchers (Montevecchi 2007, Guillemette et al. 2018). Gannets' breeding success is greatly dependent on the availability of forage fish, especially Atlantic mackerel (Guillemette et al. 2018). Therefore, gannets may be sensitive to ecosystem shifts that affect fish distribution, such as warming temperatures (Montevecchi 2007, Franci et al. 2015). This also makes them excellent indicators of regime shifts in fish stocks, like the sudden dominance of rockfish (*Sebastes* spp.) in the diet of Bonaventure Island gannets during the 2020 and 2021 breeding seasons (P. Martigny, unpublished data).

They have also been the subject of regular monitoring since 1969 and can inform researchers of the temporal trends of a range of contaminants. Gannets breeding at Bonaventure Island specifically have been used as ecotoxicological study organisms in the past. A seminal study showed that chronic exposure to organochlorines (DDT and dichlorodiphenyldichloroethylene – DDE), caused eggshell thinning in gannets and important reproductive failure in the colony for close to a decade (Elliott et al. 1988). These effects were found after the banning and consequent global reduction in organic contaminant emissions caused by the Stockholm Convention. Even decades after the initial discovery of thinning eggshells due to DDT and DDE by Elliott et al. (1988), Champoux et al. (2015) reported thinning by about 10% relative to the pre-DDT eggshell thicknesses. More recent studies examine the temporal trends of contaminants in northern gannet eggs, describing the decrease of mercury, organochlorines, PCBs, flame retardants, DDT/E, and other contaminants (Champoux et al. 2015, Champoux et al. 2017).

4. Conclusion

Stable isotopes and wildlife monitors can be used effectively to evaluate Hg contamination in the environment and food webs. Namely, amino acid-specific stable isotope analysis can allow researchers to correct for baseline variation when calculating trophic positions using source and

trophic amino acids. The consideration of baseline variation when assessing the biomagnification of contaminants is critical to understanding ecological patterns. Generalist feeders that cover large distances can be especially useful to study ecological contamination. Northern gannets, as foragers that travel over a large portion of the Gulf of St. Lawrence, reflect ecological signatures from the ecosystem through their breeding success and can inform on prey abundances through their regurgitations. By combining bulk and amino acid-specific stable isotope analysis and gannets as monitors, it is possible to sample fish from different foraging areas to assess the biomagnification potential and spatial distribution of mercury in the Gulf of St. Lawrence.

NOTE ON CHAPTER 2

Chapter 2 corresponds to a manuscript with the same title that has been submitted to the peer-reviewed journal *Environmental Pollution*. Co-authors (Benjamin Barst, David Pelletier, Magella Guillemette, Marc Amyot, Raphaël Lavoie, and Kyle Elliott) have all contributed comments to the presented version. Field collections were conducted by David Pelletier and Magella Guillemette. The bulk of the work was conducted by myself (data processing, analysis and interpretation, writing the manuscript, and making figures and tables).

A northern gannet flying in the Gulf of St. Lawrence. Photo by Ryan Boswarthick © 2022

Chapter 2: Compound-specific stable isotope nitrogen analysis of amino acids shows that bulk methods provide higher estimates of mercury biomagnification in the Gulf of St. Lawrence

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Abstract

Compound-specific stable isotope analysis of amino acids (CSIA-AA) provides a method to estimate baseline $\delta^{15}N$ values of food webs, allowing less biased estimates of trophic positions for organisms. Greater accuracy in trophic position can improve estimates of contaminant biomagnification. We calculated trophic positions with various CSIA-AA equations for four species of fish and northern gannets (*Morus bassanus*) from the Gulf of St. Lawrence. We examined the effect of CSIA-AA-derived trophic positions on mercury biomagnification metrics (trophic magnification factors (TMF) and biomagnification factors) and compared these with trophic position estimates and metrics obtained from traditional bulk stable isotope analysis. The TMFs for the CSIA-AA equations ranged from 10 to 19, and bulk stable isotope analysis produced a TMF of 43, one of the highest TMFs recorded yet in the literature. Biomagnification

factors between prey and northern gannets ranged from 20 to 38 using dietary observations and stable isotope mixing models. Our study demonstrates discrepancies in mercury biomagnification assessed using different approaches that may go undetected when using a single approach.

Keywords: mercury, seabird, CSIA-AA, trophic dynamics, BMF

1. Introduction

Mercury is a naturally occurring metal found in air, water, and soil, which cycles in the environment (Driscoll et al. 2013). Humans have greatly disrupted the natural mercury cycle, drastically increasing emissions through activities such as mining, metal processing, fossil fuel combustion, industry, and small-scale artisanal gold mining (Driscoll et al. 2013, Esdaile and Chalker 2018). Mercury is a contaminant of concern in ecosystems and food webs because of its potential to cause harm to living organisms. The organic form of mercury, methylmercury, is ecologically relevant because it biomagnifies through food webs, leading to high concentrations in top predators (Atwell et al. 1998, Anderson et al. 2009). Methylmercury can impede reproductive success, cause malformations, change behaviour in maladaptive ways, and even cause death (Friedmann et al. 1996, Scheuhammer et al. 2007, Ackerman et al. 2016). Thus, understanding the dynamics of mercury, both in its inorganic and organic forms (total mercury; THg) through food webs and into top predators is vital to preserving wildlife health.

Bulk stable isotope analysis (bulk SIA) is widely used to study the biotic transfer of contaminants through the environment. Stable isotopes can provide insight into diet, foraging habitats, and the transfer efficiency of contaminants through food webs (Hobson et al. 2002). $\delta^{15}N$ values are commonly linked to the trophic position, as $\delta^{15}N$ values increase predictably with each trophic step (Peterson and Fry 1987). As such, $\delta^{15}N$ can be used to calculate biomagnification metrics, linking trophic position to THg concentrations. $\delta^{13}C$ values are used to infer foraging habitat, where signatures are differentiated by the main type of vegetation due to differences in photosynthetic pathways (DeNiro and Epstein 1978). Higher $\delta^{13}C$ values are associated with benthic/inshore algae and lower $\delta^{13}C$ values are associated with pelagic phytoplankton (France 1995). $\delta^{34}S$ values help differentiate between benthic, inshore, and offshore habitats and further refine habitat estimates provided by $\delta^{13}C$ values (Connolly et al. 2004). $\delta^{34}S$ values are lower in benthic environments due to sulfate reduction and are higher in

the oceanic water column (Connolly et al. 2004, Elliott and Elliott 2016). When combined, these three SIA ratios can provide powerful insight into food web dynamics and the movement of THg from the base to the top of a food web (Cabana and Rasmussen 1994).

Bulk SIA is particularly useful to assess a consumer's trophic position when calculating biomagnification (Won et al. 2018). Biomagnification metrics rely on the trophic position and contamination level of each organism in the food web to assess the rate of increase of contaminants. However, bulk SIA may provide a skewed estimate of trophic positions, due to baseline differences (e.g. primary producer) $\delta^{15}N$ signatures (McClelland and Montoya 2002, McMahon and McCarthy 2016). These baseline differences can be due to biotic (e.g. nitrogen fixation, denitrification) or abiotic (e.g. runoff, atmospheric deposition) factors, and are most evident when studying organisms that forage in or originate from multiple locations (Montoya 2007). The analysis of stable isotope values of amino acids (compound-specific stable isotope analysis; CSIA-AA) is meant to remove this bias stemming from baseline differences (McClelland and Montoya 2002). The $\delta^{15}N$ values of certain amino acids (AAs) increase in a stepwise manner with trophic position, as is expected with bulk $\delta^{15}N$ values (i.e. "trophic AAs", most commonly glutamic acid; McClelland and Montoya 2002). The $\delta^{15}N$ values of other AAs do not increase significantly between trophic levels (i.e. "source AAs", most commonly phenylalanine; Chikaraishi et al. 2009). When using trophic and source AAs in combination, $\delta^{15}N$ signatures can be corrected for potential baseline differences to estimate an organism's trophic position. Comparisons of trophic positions for organisms from different habitats may therefore be more robust (McClelland and Montoya 2002, Chikaraishi et al. 2009), and improve contaminant biomagnification relationships. Previous work on THg and methylmercury biomagnification has shown CSIA-AA produces higher biomagnification metrics than bulk SIA approaches in some studies (Elliott et al. 2021, Zhang et al. 2021), but with other contaminants, the opposite relationship is observed (Kobayashi et al. 2019). The mechanisms behind these relationships have not yet been identified.

Seabirds have been used extensively as health indicators of marine ecosystems (Furness and Camphuysen 1997, Montevecchi 2007, Elliott and Elliott 2013). Particularly, their foraging ecology has helped to predict important ecosystem shifts, such as fish stock collapse and shifting ranges for forage fish (Montevecchi 2007, Guillemette et al. 2018). Their use as indicators of marine food web contamination is also highly relevant, as most seabirds are piscivorous

predators, and are likely to accumulate contaminants at relatively high concentrations (Le Croizier et al. 2022). Northern gannets (*Morus bassanus*; henceforth "gannets") are large, piscivorous seabirds that travel great distances during the breeding season to feed their nestling (Garthe et al. 2007, Montevecchi et al. 2012, Guillemette et al. 2018). In the Gulf of St. Lawrence (Atlantic Canada), they feed on commercially relevant fish such as Atlantic mackerel (*Scomber scombrus*), Atlantic herring (*Clupea harengus*), as well as on capelin (*Mallotus villosus*), and sandlance (*Ammodytes* spp.) (Guillemette et al. 2018). During the breeding season, gannets regurgitate spontaneously when stressed at their nest site (Guillemette et al. 2018). Thus, northern gannets provide researchers with the non-lethal opportunity to study THg biomagnification to a top predator from their regurgitated prey samples.

Our objectives were to 1) estimate trophic positions of fish and gannets from the Gulf of St. Lawrence using CSIA-AA, and 2) contrast CSIA-AA with bulk SIA when assessing the biomagnification of THg in this food web. We hypothesized that 1) CSIA-AA would provide lower trophic position estimates compared to bulk SIA, because baseline variation is accounted for and this is consistent with previous observations (An et al. 2020), and 2) THg biomagnification would be greater when using CSIA-AA compared to bulk SIA, as has been reported in previous Hg studies (Elliott et al. 2021, Zhang et al. 2021).

2. Methods

2.1 Sample Collection

Fieldwork was conducted at the gannet colony on Bonaventure Island in Percé, Québec, Canada during the 2017 and 2018 breeding seasons. Gannets and their nests were continuously monitored during the breeding season (June to August) and were caught routinely. During captures, blood (less than 5 mL) was drawn from the gannets' medial metatarsal vein as described in Pelletier et al. (2023). The whole blood was immediately separated into the red blood cell and plasma fractions in cryotubes and placed in liquid nitrogen and soon after stored in -80°C freezers. Only the red blood cell fraction was used for our study (henceforth referred to as "blood"). Regurgitated fish samples were collected opportunistically at the gannet colony. During captures, gannets may regurgitate due to stress, allowing for easy sampling of their stomach contents (Guillemette et al. 2018). Contents of the regurgitations were sorted and quantified by species and quantity (i.e. number of complete fish, of heads, and tails). Regurgitations were frozen in a standard freezer by the end of the same day of collection and

later stored long-term in -20°C freezers until sample processing. Seabird red blood cells have a longer half-life, representing diet over the weeks preceding the sampling date, while fish muscle has a longer turnover rate, representing months of contaminant exposure (Vander Zanden et al. 2015, Shoji et al. 2021). Together, the fish muscle and gannet red blood cells provide insight into gannet THg exposure during the breeding season, when gannets are present and foraging in the Gulf of St. Lawrence.

2.2 Sample Analysis

Regurgitations were thawed and dissected to retrieve approximately one cubic cm of dorsal muscle tissue from each species present in the sample. We collected muscle from four species of fish; Atlantic mackerel (n = 21), Atlantic herring (n = 13), capelin (n = 22), and sandlance (n = 17). The fish muscle tissue and gannet blood (n = 40) were freeze-dried using an FTS Flexi-Dry compact freeze-dryer (Triad Scientific) for 48h, powdered, and homogenized.

2.2.1 Mercury analysis

We analysed all samples (fish muscle and freeze-dried gannet blood) for THg following US EPA method 7473. Briefly, 25-40 mg of sample was combusted at 650°C, Hg was accumulated on a gold-coated sand amalgamator followed by heat desorption and quantification by CV-AAS (cold vapor atomic absorbance spectrometer; Direct Mercury Analyser, DMA-80 evo, Milestone). Certified reference materials TORT-3 (lobster hepatopancreas) and DORM-4 (fish protein), certified by the National Research Council of Canada were analysed and had mean (± sd) recoveries of 100.0 ± 2.0% (n = 22) and of 99.3 ± 2.8%, (n = 6), respectively. We assumed that around 100% of THg was methylmercury in seabird red blood cells (Lavoie et al. 2010) and in fish muscle (Carbonell et al. 2009).

2.2.2 Bulk stable isotope analysis

Fish muscle and gannet blood were analysed for bulk SIA of nitrogen ($\delta^{15}N$ values), carbon ($\delta^{13}C$ values), and sulfur ($\delta^{34}S$ values) at the Ján Veizer Stable Isotope Laboratory (Ottawa, Ontario, Canada). Stable isotopes of carbon, nitrogen, and sulfur are reported in Delta notation $\delta = ((R_x - R_{std})) / R_{std}) * 1000$ where R is the ratio of the abundance of the heavy to the light isotope, x denotes sample and std is an abbreviation for standard. $\delta^{15}N$ is the ratio of $^{15}N/^{14}N$, $\delta^{13}C$ the ratio of $^{13}C/^{12}C$, and $\delta^{34}S$ the ratio of $^{34}S/^{32}S$. The samples were combusted in a Vario EL Cube (Elementar, Germany) EA-IRMS interfaced via Conflo IV to Delta Advantage isotope ratio mass spectrometer (Thermo, Germany - (δ15N and δ13C) or the Delta Plus XP IRMS

(ThermoFinnigan, Germany). The raw isotope data were referenced to the VPDB (carbon), AIR (nitrogen), and scales using six calibration standards (IAEA-N1, IAEA-N2, USGS-40, USGS-41, NBS-22, and IAEA-CH-6). Four internal check standards were included in the analytical runs: C-51 Nicotiamide ($\delta^{13}C$: −23.0‰; $\delta^{15}N$: +0.1‰), C-52 mix of ammonium and sucrose ($\delta^{13}C$: −11.9‰; $\delta^{15}N$: +16.6‰), C-54 caffeine ($\delta^{13}C$: −16.6‰; $\delta^{15}N$: −34.5‰), and AG-2 argentite ($\delta^{34}S$: -0.6‰). The analytical error was monitored using a blind standard (C-55, glutamic acid, $\delta^{13}C$: −4.0‰; $\delta^{15}N$: −28.5‰) and was better than ±0.1‰ for carbon and nitrogen. Ten percent of the samples were randomly duplicated. Standard deviations for duplicates of gannet blood averaged 0.1‰ for $\delta^{13}C$, 0.06‰ for $\delta^{15}N$ and 0.3‰ for $\delta^{34}S$ and standard deviations for duplicates of fish muscle averaged 0.1‰ for $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$. $\delta^{13}C$ values were mathematically normalized for lipid content according to the methodology detailed by Post et al. (2007). For the mean values of bulk SIA and THg for all species, refer to Table 2.1.

2.2.3 Compound-specific stable isotope analysis

Compound-specific stable isotope analysis of amino acids was carried out for a subset of fish muscle and gannet blood (n = 39; see Table S2.1 in Supplemental Materials) at the Alaska Stable Isotope Facility at the University of Alaska Fairbanks (Alaska, USA). These methods are detailed elsewhere, by Barst et al. (2020) and (Barst et al. 2021). Briefly, samples were digested in 6 molar HCl for 20 hours at 100°C. A 6:5 mixture of hexane and dichloromethane was added to each sample and vortexed. The acidic fraction of each sample was spiked with an internal standard of norLeucine (nLeu) and evaporated under nitrogen gas. Amino acids were then methylated with an acidified methanol solution and heated at 75°C for one hour. The samples were nitrogen evaporated again, until near dryness, and an acetylation mixture of triethylamine, acetone, and acetic anhydride was added. Samples were heated again at 60°C for ten minutes and returned to the nitrogen evaporator until near dryness. A mixture of potassium phosphate buffer and chloroform was then added to the samples, and they were centrifuged to isolate the organic phase and purify the derivatized AAs. The samples were returned to the nitrogen evaporator to eliminate the chloroform, and ethyl acetate was added to the vials once dry. Samples were finally spiked with internal standards of caffeine before being capped and analysed by gas chromatography combustion isotope ratio mass spectrometry (GC-C-IRMS) to obtain $\delta^{15}N$ values of individual AAs. The instrumentation and parameters are described in detail by Barst et al. 2020 and Barst et al. 2021. The analysis yielded data for seven trophic AAs (alanine,

isoleucine (Ile), aspartic acid (Asp), proline (Pro), glutamic acid (Glu), valine (Val), leucine (Leu)) and a single source AA (phenylalanine (Phe)). All samples were run in triplicate and the mean $\delta^{15}N$ value for each AA from a sample was used in data analysis.

The average nLeu (internal standard) $\delta^{15}N$ value in all samples was 18.7 ± 0.5 ‰ (mean ± sd) which corresponds to the known value of 19.3 ‰, and the average measured for caffeine was -3.1 ± 0.2 ‰ which corresponds to the known value of -3.3 ‰. Two mixed standards of AAs with different known $\delta^{15}N$ values were derivatized and analysed with each sample batch (n = 3). The measured values (n = 12 per AA) for both standards did not significantly differ from the expected values (Pearson's correlation test: standard 1: $t = 52.6$, $p < 0.001$; standard 2: $t = 52.3$, $p < 0.001$). We also digested and derivatized subsamples of the same sample (mackerel: 17-30) with each batch which we used to verify there was no systematic bias among batches: the internal standards nLeu (18.8 ± 0.5 ‰) and caffeine (-3.0 ± 0.2 ‰) corresponded to the known values in all batches.

2.3 Statistical analysis

To compare THg within a species between 2017 and 2018 and between gannet sexes, we verified there were no significant differences in THg data by running Shapiro-Wilks tests to verify normality and conducted student t-tests (or Wilcox tests for non-normally distributed THg data: gannets and sandlance). We used linear mixed-effects models (package lme4; Bates et al. 2009) to determine which stable isotopes best predicted log-transformed THg and set species as a random effect and included year in our models. We then compared all models using Akaike's information criterion for small sample sizes (AICc) to identify the best-fitted model (Burnham and Anderson 2004).

To compare the bulk SIA and CSIA-AA derived trophic positions (see 2.4 Trophic position calculations section below), we used reduced major axis (RMA) regression because both variables were dependent and contained uncertainty (error) in their measurements (Harper 2016).

2.4 Trophic position calculations

2.4.1 Bulk stable isotopes

We calculated the trophic position (TP) for each sampled organism based on bulk $\delta^{15}N$ values, using capelin as a benchmark based on previous work in the Gulf of St. Lawrence (Lavoie et al.

2010). Trophic positions for all fish were calculated using a modified trophic level equation from Lavoie et al. (2013) and capelin $\delta^{15}N$ value:

$$TP_{\text{bulk fish}} = \frac{\delta^{15}N_{\text{fish}} - \delta^{15}N_{\text{capelin}}}{3.4} + 3.9 \quad (1)$$

Where $\delta^{15}N_{consumer}$ is the $\delta^{15}N$ value of the fish and $\delta^{15}N_{capelin}$ is the mean $\delta^{15}N$ value for capelin in this study (12.7 ± 0.4 ‰; mean ± sd; n = 22), 3.4 is the typical isotopic trophic discrimination factor (TDF) used for most organisms (Post 2002) and 3.9 is the estimated TP for our reference organism (Lavoie et al. 2010). For gannets, we used a TDF of 3.0, determined for double-crested cormorants (*Phalacrocorax auritus*) in captive studies (Craig et al. 2015):

$$TP_{\text{bulk gannet}} = \frac{\delta^{15}N_{\text{gannet}} - \delta^{15}N_{\text{capelin}} - 3.0}{3.4} + 4.9 \quad (2)$$

2.6.2 Compound-specific stable nitrogen isotopes of amino acids

To test the CSIA-AA equations used to calculate TPs for our study organisms, and compare them to TP_{bulk}, we tested multiple equations from the literature on our fish and gannet samples. The first CSIA-AA equation we tested was from Chikaraishi et al. (2009) and has been widely used in the literature:

$$TP_{\text{Glu-Phe}} = \frac{\delta^{15}N_{\text{Glu}} - \delta^{15}N_{\text{Phe}} - \beta}{\text{TDF}} + Y \quad (3)$$

Where $\delta^{15}N_{Glu}$ and $\delta^{15}N_{Phe}$ are the measured $\delta^{15}N$ values for the glutamic acid and phenylalanine AAs respectively, β is the difference between trophic and source AAs in primary producers, Y is the base TP of the primary producer, and TDF is the trophic discrimination factor, or how much the trophic AA's isotopic signature will change between a prey and its predator. Most of the TP equations we tested are modifications of Equation 3, and all β, Y, and TDF values we used can be found in Table 2.2. The second type of TP equation we tested uses the average of multiple trophic AAs which are each normalized relative to Glu. This equation was suggested by Nielsen et al. (2015), who conducted a meta-analysis of CSIA-AA nitrogen data from an array of taxa, and proposed that this method would remove bias associated with the use of a single AA:

$$TP_{Mult.AAs} = \left(\frac{\sum(\delta^{15}N_x i + \delta^{15}N_{\text{diff}} i)/X - \delta^{15}N_{\text{Phe}} - \beta_{Glu-Phe}}{TDF_{Glu-Phe}} \right) \quad (4)$$

Where $\delta^{15}N_x i$ and $\delta^{15}N_{diff} i$ are the trophic AA $\delta^{15}N$ value, and the correction term which normalizes a given trophic AA relative to Glu, respectively. All the correction terms used for this equation were from Nielsen et al. (2015) and are listed in Table 2.2. The term X is the number of trophic AAs used to calculate an organism's trophic position, and $\beta_{Glu\text{-}Phe}$ and $TDF_{Glu\text{-}Phe}$ are the specific terms used for Glu and Phe, because all trophic AAs were normalized relative to Glu. Equation 5 stems from Equation 4 and only uses Glu as the trophic AA. We also tested the use of Pro and Ile as the trophic AAs for gannets and fish, respectively (Eq. 6 and 7), due to their good performance at separating these organisms into the appropriate expected TPs when we plotted the trophic isoclines for the trophic AAs (as in Chikaraishi et al. 2014; see Table 2.1 and Figure S2.1 in the Supplemental Materials). We also identified three TP equations (Eq. 8, 9, and 10) that used seabird-derived β and TDF values and have been used previously for seabirds (see Table 2.2).

We tested combinations of fish-specific and bird-specific TP equations, based on the literature, by plotting the experimental TPs assessed using equations 3-10 with bulk TP (equations 1 and 2; using reduced major axis regression) and with log(THg) (linear regression), as both these relationships have been established in the literature (see, for example, Lavoie et al. 2013, Nielsen et al. 2015, Ohkouchi et al. 2017). The three best-performing equation combinations in terms of R^2 with log(THg) and bulk TP were selected for the biomagnification assessment. We then calculated the mean and standard deviation of the absolute difference between TP calculated using bulk SIA and using CSIA for the three equations.

2.5 Biomagnification assessment

To assess biomagnification within the fish-to-gannet food web, we plotted the three best-performing TP equations against log-transformed THg values for the four species of fish and gannets.

$$\mathrm{Log}_{10}[\mathrm{THg}] = \mathrm{b}(\mathrm{TP}) + \mathrm{a} \qquad (11)$$

Where TP was the organism's trophic position calculated using one of the three CSIA-AA or the bulk SIA TP equations, b was the slope and a was the intercept of the relationship between log(THg) and TP. The trophic magnification factor (TMF), also referred to as the food web magnification factor, is used to indicate the average change in contaminant concentrations in each step of the food web (Riget et al. 2007). We calculated the TMF for each of the four TP

equations used (three best-performing CSIA-AA equations and the bulk SIA equation) using Equation 12 from (Lavoie et al. 2013):

$$\mathrm{TMF} = 10^{b} \tag{12}$$

Where *b* is the slope from Equation 11. We also calculated the biomagnification factor (BMF) for gannets in both 2017 and 2018, while accounting for the composition of gannet diet in each year, as in Lavoie et al. (2010):

$$\mathrm{BMF} = \frac{[\mathrm{THg_{gannet}}]}{\sum_{i=1}^{n}([\mathrm{THg_{fish\ i}}] * \mathrm{f_{fish\ i}})} \tag{13}$$

The BMF is the ratio of a contaminant concentration in a consumer relative to the concentration in its diet (Riget et al. 2007). Where THg_{gannet} and THg_{fish} are the raw THg concentrations for the gannets and fish, respectively, and f_{fish} is the proportion of gannet diet that each fish species made up for the targeted year. We used diet composition proportions from two sources. First, we used regurgitation observations recorded in the field before the blood sampling efforts, that described the proportions of each of the four species of fish in gannet diet. Secondly, we used diet composition proportions obtained from stable isotope mixing models.

We ran stable isotope mixing models to determine gannet diet composition, because regurgitation observations may be biased towards the prey items gannets are providing their nestlings, and diets differing between adults and their young have been documented in seabirds (Brown and Ewins 1996, Barrett et al. 2007). We used the MixSIAR package (Stock et al. 2018), which resolves stable isotope mixing equations using Bayesian framework. We included mean and standard deviation of bulk $\delta^{15}N$, $\delta^{13}C$, $\delta^{34}S$, and nitrogen, carbon, and sulfur composition (%) for all the fish and gannet samples for 2017 and 2018 in our mixing models. We used TDFs calculated from double-crested cormorants that were fed catfish (*Ictalurus punctatus*) in controlled experiments (Craig et al. 2015) for our herring and mackerel, and TDFs calculated for Peruvian boobies (*Sula variegata*) feeding on Peruvian anchovy (*Engraulis ringens*) for our capelin and sandlance samples (Le Croizier et al. 2022). Species-specific TDFs were not available in the literature for gannets, so we used factors for genetically close species (Peruvian boobies) and for fish species constituting gannet prey (catfish substituting for herring and mackerel, and anchovy as a stand-in for smaller capelin and sandlance). We also included our regurgitation observations of diet for both studied years as a prior to inform our model. We ran

the jags model with a chain length of 100 000, burn = 50 000, thin = 50, and chains = 3. Once we calculated the dietary proportion of each of our prey species (mackerel, herring, capelin, and sandlance) in gannet diet from our isotope mixing models, we used these to calculate the BMF and compare to the BMFs calculated based on dietary observations.

All statistical analyses were run in R 4.1.2 (R Core Team 2021).

3. Results

3.1 Trophic position calculations

While testing combinations of TP equations, Equation 4 from Nielsen et al. (2015), had the highest coefficient of determination with both bulk TP and log(THg) between the fish-specific TP equations (see Table S2.2). For this reason, when we tested the bird-specific equations on the gannets, we used Equation 4 to calculate the TPs for all fish, to find the best TP equation combination for both taxa. Figure 2.1 shows the relationship between TP for all organisms calculated using bulk SIA and CSIA-AA equations. In the case of the gannet samples, bulk SIA provided much more consistent TP estimates (y-axes Fig 2.1), while CSIA-AA TP estimates were much more variable (x-axes Fig 2.1). This was not observed for the fish samples in our study. The best performing TP equation combinations based on the R^2 for reduced major axis regression were Equations 8 & 4 (henceforth "Eq. 8"), Equations 9 & 4 ("Eq. 9"), and Equations 10 & 4 ("Eq. 10"). The average difference in TP estimates calculated using bulk SIA and CSIA-AA was close to half a trophic level for fish (means ± sd : Eq. 4 = 0.65 ± 0.17) and more variable for gannets (Eq. 8 = 0.06 ± 0.40, Eq. 9 = 0.56 ± 0.26, Eq. 10 = 0.60 ± 0.23). Average TPs for the three CSIA-AA and bulk equations can be found in supplemental Table S2.3. These three equations also best correlated with log(THg), as is expected with biomagnifying contaminants such as Hg (Figure 2.2; Lavoie et al. 2013). Eq. 8, 9, and 10 were used to assess the biomagnification potential of THg in the fish-to-gannet food web.

3.2 THg concentrations in gannets

To compare our THg concentrations in gannet red blood cells to other studies and toxicity thresholds, we converted our dry-weight red blood cell concentrations to a wet-weight, whole blood equivalent following the methodology detailed in Ackerman et al. (2016). In our study, all sampled gannets had concentrations of THg which placed them in the low-risk category, after conversion from dry- to wet-weight concentrations (mean ± sd of THg in 2017 = 0.40 ± 0.05 μg/g ww, n = 20 and in 2018 = 0.28 ± 0.04 μg/g ww, n = 20; see supplemental Figure S2). THg

concentrations were significantly different between years (t = 9.0, df = 35.8, p = < 0.0001) but not between sexes (t = -1.1, df = 24.0, p = 0.3).

3.3 Biomagnification of mercury

Our linear mixed-effects models suggest that when taking the effect of species on THg concentrations into account, $\delta^{15}N$ is the most important bulk SIA ratio to consider (Table 2.3). The values of $\delta^{15}N$ (11.1 to 15.2 ‰), $\delta^{13}C$ (-21.2 to -17.9 ‰), and $\delta^{34}S$ (17.7 to 20.9 ‰) did not show a large amount of variation among our samples. Our values of $\delta^{15}N$ in AAs were more varied (Glu: 17.7 to 27.2 ‰, Ala: 13.5 to 25.2 ‰, Val: 16.8 to 27.9 ‰, Ile: 15.1 to 25.3 ‰, Asp: 13.7 to 28.4 ‰, Pro: 18.8 to 5.6 ‰) in terms of trophic AAs. Baseline values varied on a similar scale to the bulk stable isotopes (Phe: 1.3 to 5.6 ‰).

The trophic magnification slopes (*b*) and the TMF values for each of the four TP equations ranged from 10.0 to 42.7 (Table 2.4) and were all close to (Eq. 8) or greater than one (Eq. 9, 10, and bulk SIA), confirming that Hg biomagnification is occurring in our specific food web. The intercepts of these four relationships are all very low. The back-transformed intercept values for Eq. 8, 9, 10, and bulk SIA were all < 0.00006 μg/g, indicating the THg values at the base of this food web approach 0, regardless of the equation used. BMFs calculated using diet observations (proportions detailed in Supplementary Table S2.4) and stable isotope mixing models were 22.8 and 37.7 respectively for 2017, and 20.2 and 35.8, respectively for 2018.

4. Discussion

Understanding the biomagnification of Hg, a highly toxic contaminant to wildlife, is important when assessing ecosystem health. Our objective was to assess the use of compound-specific stable isotope analysis of amino acids (CSIA-AA) in biomagnification studies. To do this we compared CSIA-AA methods to calculate an organism's trophic position (TP) against the well-established bulk stable isotope analysis (bulk SIA). Then, we assessed the biomagnification level in our fish-to-gannet food web in two ways: 1) by calculating trophic magnification factors (TMFs), which indicates the extent to which total mercury (THg) is amplified over the entire food web and 2) by calculating biomagnification factors (BMFs) which inform on the extent of THg biomagnification between gannets and their prey. We found that differences between bulk SIA and CSIA-AA TMFs were stark: the true TMF value for our food web is likely somewhere in between the two extreme values and may be closer to the middle values estimated by two of the three CSIA-AA equations. Thus, bulk SIA seems to overestimate the extent of

biomagnification in the gannet food web in the Gulf of St. Lawrence, while some CSIA equations may underestimate it.

4.1 Trophic position calculations

Three CSIA-AA equations (Eq. 8, 9, 10) produced TPs that matched our studied species' expected TPs and corresponded well with TPs calculated using bulk SIA (Figure 2.1). These three equations also had positive relationships with THg (Figure 2.2). The similarity between R^2 values between the three CSIA-AA equations might be due to the fish samples, for which trophic positions were calculated the same way (using Eq. 4), leaving only the gannet samples to vary and change the slope and coefficient of determination. The gannet TPs estimated by bulk SIA were much more consistent and repeatable than those estimated by CSIA-AA, which showed more variability (see x-axes of Fig 2.1). It is difficult to determine whether the spread of the gannet TPs is due to the incorporation of more noise in the CSIA-AA data or of more nuances that bulk SIA failed to account for. It was important to thoroughly test the different trophic position equations using CSIA-AA available in the literature for our study organisms, as many different equations are available in the literature, and these may yield different results depending on the study objective.

One limitation to our trophic calculation comparison is the difficulty to validate CSIA-AA approaches independently from TPs assessed using other approaches. As discussed previously, bulk SIA has been shown to be biased due to baseline differences in some studies (McClelland and Montoya 2002). Diet observations may be biased, especially for nesting seabirds, to represent the diet of the young rather than the adult (Barrett et al. 2007). Other methods to assess TP, such as analysing stomach contents, can be biased towards organisms with harder structures, such as bones and otoliths, which are digested slowly (Buckland et al. 2017). Thus, when trying to validate TPs derived from CSIA-AA by using other, biased approaches as a benchmark, we run the risk of choosing a CSIA-AA equation that also produces biased TP estimates. However, TPs calculated using bulk SIA remains the best available tool in the literature and the most widespread practice when testing TPs calculated using CSIA-AA (see for example Wu et al. 2018, An et al. 2020, Zhang et al. 2021).

The CSIA-AA equations mainly produced lower TP estimates, especially for fish (using Eq. 4) than with bulk, which has also been observed in studies in fish and invertebrate food webs (An et al. 2020, Zhang et al. 2021). This is because when baseline $\delta^{15}N$ values are accounted for, by

subtracting source AA $\delta^{15}N$ values from trophic AA values, the overall $\delta^{15}N$ signature decreases, resulting in lower TP estimates (An et al. 2020, Zhang et al. 2021). For many gannets that were estimated to be at the same TP using bulk SIA, the CSIA equations differentiated them more due to differences in Phe values, which represent baseline $\delta^{15}N$ signatures. There is also consistent evidence for individual specialization in seabirds, including gannets (Wakefield et al. 2015), which may help in differentiating individuals when using CSIA-AA. The differences in TP estimates calculated using bulk SIA and CSIA-AA were variable (fish: 0.65, gannets: 0.06-0.60), and mostly higher than other studies that compared TPs calculated from bulk or diet, to TPs calculated from CSIA-AA (0.23 ± 0.06: Wu et al. 2018, 0.14 ± 0.08: Thébault et al. 2021) or comparable to a study in coastal fish (0.6 ± 0.32 in fish; An et al. 2020). Interestingly, Eq. 8, derived from Wu et al. (2018) produced TP estimates that were very similar to our bulk TPs in the case of gannets (see supplemental Table S2.4), yet in their own study produced CSIA-AA TPs that were lower than bulk TPs. Considering that gannets forage over extensive areas and in different regions of the Gulf of St. Lawrence (Guillemette et al. 2018), differences in baseline $\delta^{15}N$ values are expected.

4.2 THg contamination in northern gannets

Toxicity thresholds for THg in many bird taxa have been studied in the literature. We used thresholds suggested by Ackerman et al. (2016): background concentrations of THg in blood consist of concentrations < 0.2 μg/g ww, low-, moderate-, and high-risk concentrations are 0.2-1.0 μg/g ww, 1.0-3.0 μg/g ww, and 3.0-4.0 μg/g ww, respectively, and lethal-risk concentrations are >4.0 μg/g ww. In our study, all sampled gannets had concentrations of THg which placed them in the low-risk category, after conversion from dry- to wet-weight concentrations (Figure S2.2). Negative biological effects have been observed in other seabirds with similar THg concentrations. In double-crested cormorants, gene expression was shown to have been altered, which may lead to oxidative stress of genes related to cellular stress (Gibson et al. 2014), and in black-legged kittiwakes (*Rissa tridactyla*), males were unable to successfully raise two chicks compared to males with lower THg concentrations (Tartu et al. 2016). Thus, there may be small, undetected fitness impacts in our gannet population caused by THg concentrations higher than background levels.

4.3 Biomagnification of THg

Our linear mixed-effects models using three isotopes revealed that when accounting for species as a random effect, $\delta^{15}N$ values alone are best suited as an indicator of log(THg) concentrations in this food web. This result is expected, as the relationship between trophic level and Hg burden has been well-established (Cabana and Rasmussen 1994, Atwell et al. 1998, Hobson et al. 2002). However, our finding also contrasts with other biomagnification studies, such as Góngora et al. (2018) and Elliott et al. (2021), which found that combined $\delta^{15}N$ and $\delta^{34}S$ values, or simply $\delta^{34}S$ values, were the best indicators of THg in Arctic marine ecosystems. Elliott and Elliott (2016) also recommended pairing $\delta^{15}N$ values with $\delta^{34}S$ values when studying sources of Hg. In our study, $\delta^{34}S$ signatures in our samples did not show much variation (range 17.7 – 20.9‰). It is likely that because our samples were from similar mid-water habitats and, consequently, $\delta^{34}S$ values did not exert much influence in our models.

An important consideration when selecting a TP equation is the impact of the equation on the relationships that are being studied. Between the three CSIA-AA and the bulk SIA equations, the steepness of the relationship with THg (the slope, *b*) is quite different. The slope of the relationship is commonly used as a factor to calculate measures of biomagnification, such as TMF (Lavoie et al. 2010). Therefore, when conducting a biomagnification study, the choice of TP equation will affect the results and interpretations stemming from it.

4.4 Biomagnification factors

When we looked at the biomagnification of THg in this food web, the choice of CSIA-AA or bulk SIA to calculate TP yielded very different results. We chose to compare our three best CSIA-AA TP equations to the bulk SIA approach to demonstrate the range of TMFs that were estimated from the same samples. Eq. 8 produced a milder TMF estimate of 10.0, signifying that THg increases by ten times on average between each trophic level in our studied food web. Eq. 9 and 10 produced similar TMFs (17.1 and 18.8) that were steeper, but not significantly different from Eq. 8, and bulk SIA produced a higher TMF, of 42.7, than Eq. 8. This contrasts previous findings by Elliott et al. (2021) who reported that TMF for THg in an Arctic ecosystem was lower when assessed using bulk SIA and over three times greater when assessed using CSIA-AA. Further, in a lacustrine ecosystem, Zhang et al. (2021) described a higher TMF for methylmercury when assessed using CSIA-AA than with bulk SIA (9.5 compared to 5.7, respectively). Using bulk SIA to assess TP in our study produced TMFs greater than any other

previously reported in the Gulf of St. Lawrence (range 3.8 - 6.5; Lavoie et al. 2010) and elsewhere (range 0.2 - 4.3; Riget et al. 2007, Elliott et al. 2021, Vainio et al. 2022). This is a further indicator that the choice of TP equation in a biomagnification study is a critical step, and bulk SIA may be overestimating the level of biomagnification in ecosystems if the study organisms are highly mobile, as northern gannets are. Because gannets travel long distances to forage, they likely feed in many different habitats, and thus, from different food webs. It is possible the bulk approach could not account for variation in the baseline nitrogen signatures, therefore producing an exceedingly high TMF. It is also important to note, however, that other studies which calculated TMFs included organisms from lower trophic positions, such as invertebrates. The inclusion of these organisms might have decreased the slope of the TP to log(THg) relationship in our study, which in turn would cause TMFs (both bulk and CSIA-AA) to be lower.

We obtained two BMF values for each year; one was calculated from regurgitation observations (2017 = 22.8, 2018 = 20.2), and the other was calculated using diet composition proportions from stable isotope mixing models (2017 = 37.7, 2018 = 35.8). The BMFs calculated from regurgitation observations are lower than the mixing model BMFs. This may be due to fluctuations in gannet diet during the breeding season at the Bonaventure Island colony (Guillemette et al. 2018, Pelletier and Guillemette 2022). Diet observations may also be skewed to represent nestling diet (Barrett et al. 2007), or the presence of individual specialists in our sub-sampled populations (Wakefield et al. 2015) which may influence the mixing model output. Our BMFs from mixing models and our TMF derived from bulk SIA (42.7) were similar, indicating consistency between these two approaches using bulk SIA. The true value of the BMF for each year is likely somewhere in between the BMF from diet observations and the BMF from mixing models. The BMFs for our study are comparable to previously reported BMFs for THg in the Gulf of St. Lawrence: Lavoie et al. (2010) tested piscivorous seabirds and calculated BMFs ranging between 11.8 and 42.5. In the Barents Sea, BMFs for seabirds feeding on polar cod (*Boreogadus saida*) and herring yielded values between 25.4 and 107.3 (Jæger et al. 2009). Our results correspond well with these BMF estimates, although the range of reported values is wide. Our results were also slightly higher than BMFs calculated by Le Croizier et al. (2022) for Peruvian boobies, a close congener to northern gannets, in the Humboldt current ecosystem (range: 3 – 15). This may be due to differences in THg burden between the species ecology, such

as diet, and ecosystem. Overall, both of our BMF estimates produced are well within the magnitude of Hg biomagnification reported by other seabird studies.

5. Conclusion

Overall, the extent of biomagnification of THg in the Gulf of St. Lawrence when using BMFs is comparable to other seabirds and ecosystems but is very high when assessed with TMFs calculated using bulk SIA data. Our study was the first of its kind, to our knowledge, to rigorously test TP equations using CSIA-AA and bulk SIA $\delta^{15}N$ data to examine Hg biomagnification in the Gulf of St. Lawrence. Our work suggests that the use of bulk SIA may be adequate to assess trophic position in fish and seabirds on a more local scale, while CSIA-AA might be better for trophic studies over a larger (e.g. oceanic) scale. However, when assessing biomagnification, different approaches yielded largely different results for TMFs. Our results suggest a potential overestimate of Hg biomagnification extent when using bulk SIA compared to the compound-specific approach due to spatial differences in baseline $\delta^{15}N$. Thus, when assessing biomagnification, the methodology may greatly impact the outcome, and investigators should choose the appropriate method for their study organism and system. To ensure discrepancies between the two approaches are accounted for, we suggest that a subsample of the data be analysed for both bulk and CSIA-AA $\delta^{15}N$ values, so the bulk SIA data may be corrected for baseline values. A subsample of n = 3 per group should be sufficient to capture the baseline variability within the sample environment (Elliott et al. 2021). Our results are critical to understanding how contaminants of concern, such as Hg, biomagnify through marine food webs and impact wildlife health.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Table 2.1. Sample size and mean (± sd) values by species for $\delta^{15}N$, $\delta^{13}C$, $\delta^{34}S$, and total mercury concentration ([THg] dry weight) for samples of fish muscle and northern gannet red blood cells collected in 2017 and 2018 (combined means). $\delta^{13}C$ values were mathematically corrected for lipid content.

Taxa	n	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)	$\delta^{34}S$ (‰)	[THg] (μg/g)
Northern gannet (*Morus bassanus)*	40	15.0 ± 0.1	-18.6 ± 0.2	19.8 ± 0.5	2.389 ± 0.547
Atlantic mackerel (*Scomber scombrus*)	21	13.3 ± 0.7	-19.3 ± 0.7	18.7 ± 0.6	0.207 ± 0.080
Atlantic herring (*Clupea harengus*)	13	13.1 ± 0.3	-19.1 ± 0.2	19.0 ± 0.2	0.208 ± 0.081
Capelin (*Mallotus villosus*)	22	12.5 ± 0.6	-19.5 ± 0.3	18.8 ± 0.4	0.037 ± 0.019
Sandlance (*Ammodytes* spp.)	17	11.2 ± 0.5	-20.1 ± 0.7	19.3 ± 0.6	0.041 ± 0.024

Table 2.2. Compound-specific trophic position equations tested in this study for fish species and northern gannets. The listed variables include the trophic and source amino acids (AA), the trophic discrimination factor (TDF), β value, and base trophic position of primary producers (Y). An additional column includes the correction terms required when using Equation 4, to normalize individual AAs to Glutamic Acid (Glu). "Target Organisms" indicates the taxa these equations have been tested with in the literature.

Eq. #	Trophic AA	Source AA	TDF	β	Y	Target Organisms	Source Article
Eq. 3	Glu	Phe	7.6	-3.4	1	Fish, Invertebrates	Chikaraishi et al. 2009
Eq. 4	Glu, Ala, Ile, Val, Asp, Pro	Phe	6.6	-2.9	1	All taxa	Nielsen et al. 2015[1]
Eq. 5	Glu	Phe	6.6	-2.9	1	All taxa	Nielsen et al. 2015[1]
Eq. 6	Pro	Phe	5.7	-3.1	1	This study: Seabirds	Chikaraishi et al. 2009
Eq. 7	Ile	Phe	4.4	-2.9	1	This study: fish	Chikaraishi et al. 2009
Eq. 8	Glu	Phe	3.5	-3.4 -7.6	2	Seabirds	Wu et al. 2018
Eq. 9	Glu	Phe	5.39	-3.4	1	Seabirds	Hebert et al. 2016
Eq. 10	Glu	Phe	6.2	-3.4 -4	2	Seabirds	Quillfeldt and Massello 2020

[1]Correction terms($\delta^{15}N_{diff}i$) to standardize all trophic AAs relative to Glu : Ala = 0.59, Ile = 2.63, Val = -3.35, Asp = -1.78, Pro = -1.39. The correction terms were omitted in Equation 5 because Glu was the only trophic AA used in that iteration of the equation.

Table 2.3. Linear mixed effects models to determine the influence of stable isotopes of $\delta^{15}N$, $\delta^{13}C$ (mathematically corrected for lipid content), and $\delta^{34}S$ on log-transformed total mercury ($\log_{10}$(THg)) concentrations in four species of fish (Atlantic mackerel, Atlantic herring, capelin, and sandlance) and one seabird, northern gannets. Akaike's information criterion for small sample sizes (AICc) and the difference between the most supported and other models (ΔAICc) are reported.

model	df	ΔAICc	Akaike Weight
$\log_{10}(THg) \sim \delta^{15}N + (1 \mid Species)$	4	0.00	0.37
$\log_{10}(THg) \sim \delta^{15}N + \delta^{34}S + (1 + \delta 15N \mid Species)$	7	1.70	0.16
$\log_{10}(THg) \sim \delta^{15}N + \delta^{13}C + (1 + \delta^{15}N \mid Species)$	7	1.98	0.14
$\log_{10}(THg) \sim \delta^{15}N + \delta^{34}S + (1 \mid Species)$	5	2.08	0.13
$\log_{10}(THg) \sim \delta^{15}N + \delta^{13}C + (1 \mid Species)$	5	2.20	0.12
$\log_{10}(THg) \sim \delta^{15}N + \delta^{13}C + \delta^{34}S + (1 + \delta^{15}N \mid Species)$	8	4.04	0.04
$\log_{10}(THg) \sim \delta^{15}N + \delta^{13}C + \delta^{34}S + (1 \mid Species)$	6	4.33	0.04
$\log_{10}(THg) \sim Year + (1 \mid Species)$	4	35.75	0.00
$\log_{10}(THg) \sim \delta^{13}C + \delta^{34}S + (1 \mid Species)$	5	51.12	0.00
$\log_{10}(THg) \sim \delta^{15}N$	3	110.00	0.00

Table 2.4. Trophic magnification slope (TMS) and factor (TMF) for each of the tested trophic position equation combinations. Equations 4, 8, 9, and 10 are equations using the $\delta^{15}N$ values from compound-specific stable isotope analysis in amino acids (CSIA-AA) and Equations 1 and 2 use the traditional bulk stable isotope (bulk SIA, indicated by the asterisk) approach. Groups denoted by the same superscript letter are not significantly different from each other.

Equation	Slope	95% Confidence Interval	Trophic Magnification Factor
Eq. 8 & Eq. 4	1.00 ^{a}	0.78 - 1.08	10.0
Eq. 9 & Eq. 4	1.23 ^{ab}	1.01 - 1.46	17.1
Eq. 10 & Eq. 4	1.28 ^{ab}	1.04 - 1.51	18.8
Eq. 1 & Eq. 2 *	1.63 ^{b}	1.44 – 1.82	42.7

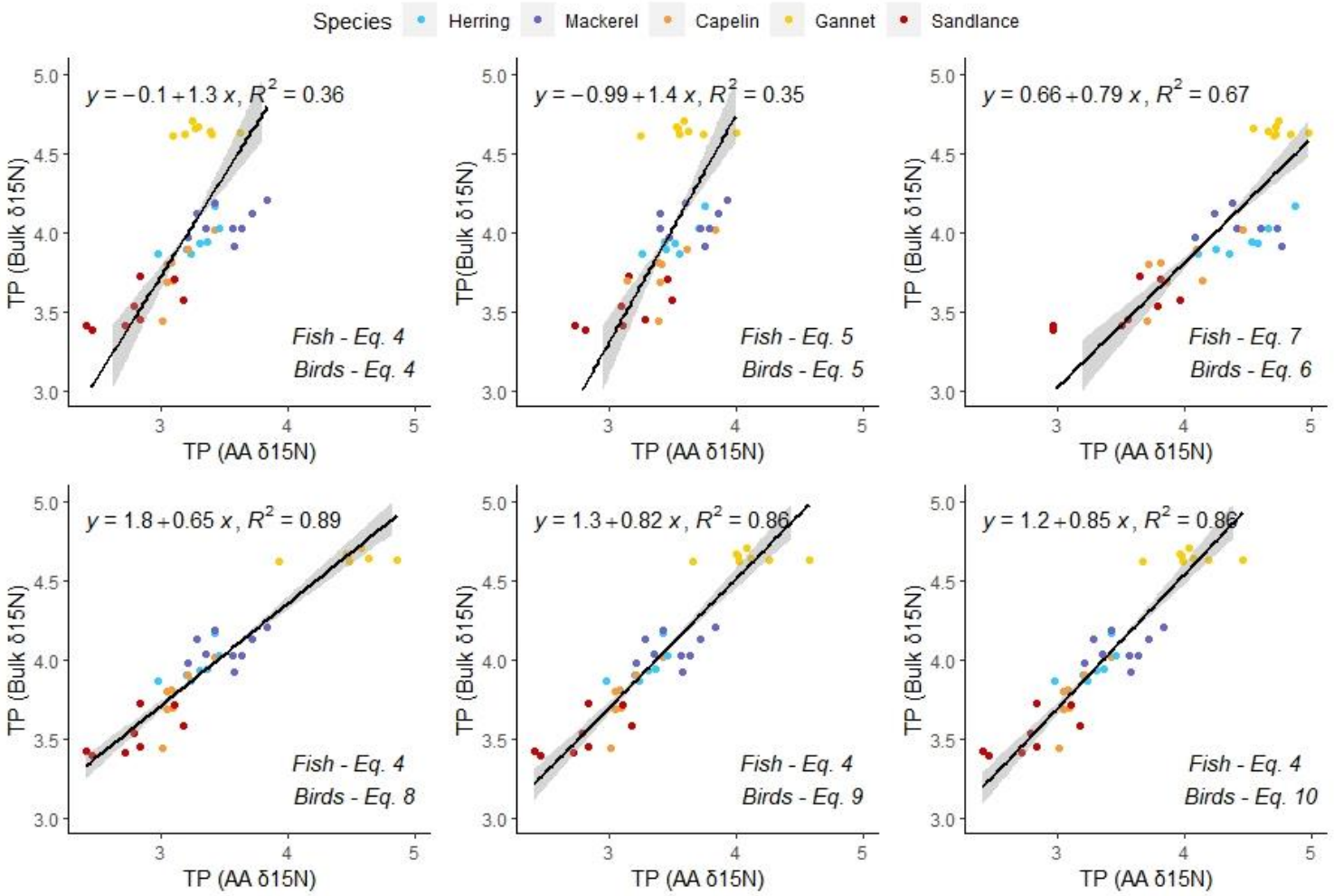


Figure 2.1. Relationship between trophic positions (TP) calculated using bulk stable nitrogen isotopes ($\delta^{15}N$ values) and TPs calculated from experimental equations using compound-specific stable isotope analysis of amino acids (AA) in northern gannets and their prey. The shaded region represents the 95% confidence interval of the slope of each relationship.

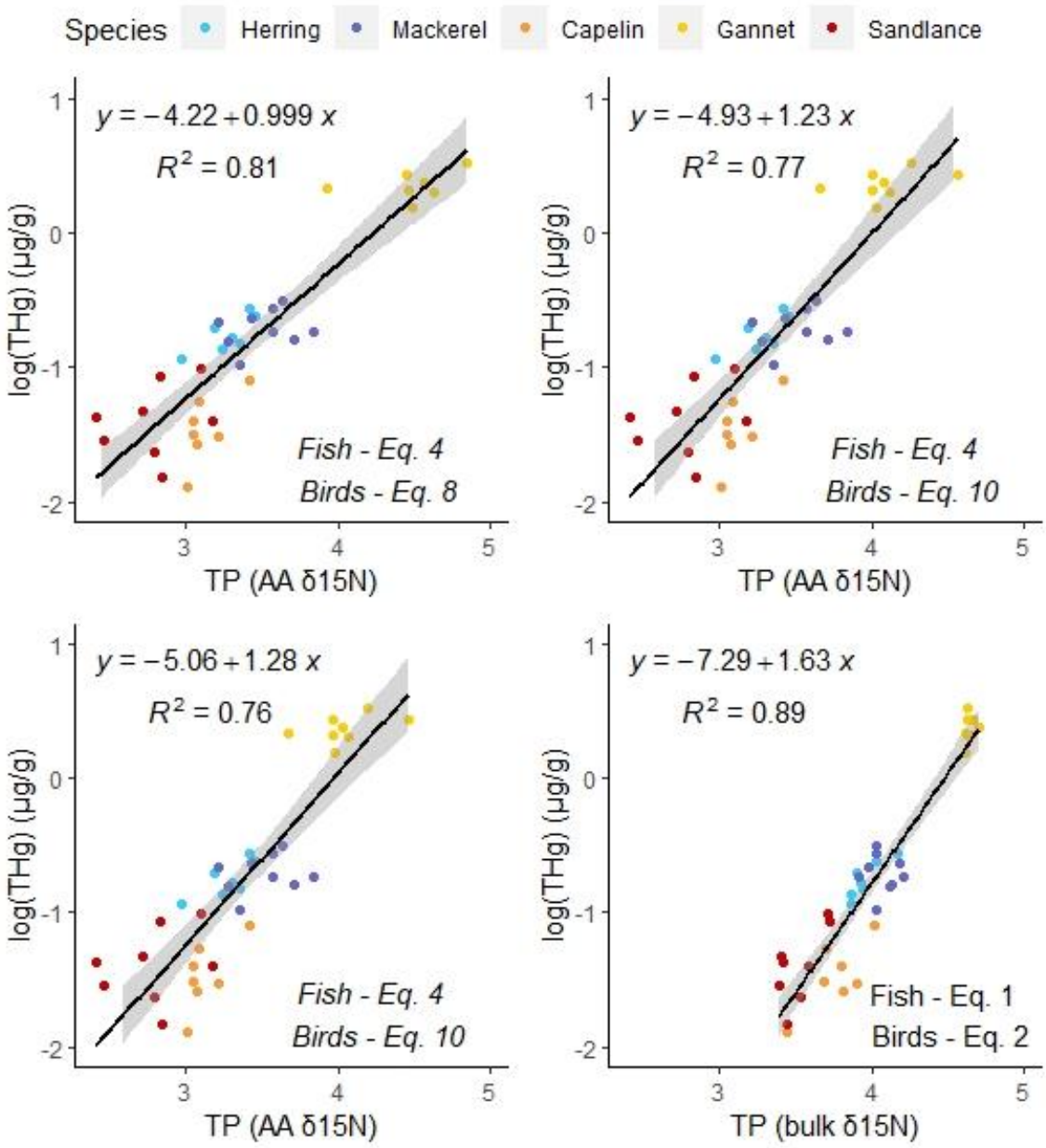


Figure 2.2. Relationship between log-transformed total mercury (log(THg)) and trophic position (TP) calculated from experimental equations using compound-specific stable isotope analysis of amino acids (AA) in northern gannets and their prey. The shaded region represents the 95% confidence interval of the slope of each relationship.

7. Supplemental Materials

Table S2.1. Compound-specific stable nitrogen isotope ($\delta^{15}N$) values (‰, mean ± sd) for each amino acid.

Species	n	Alanine	Valine	Isoleucine	Aspartic Acid	Proline	Glutamic Acid	Phenylalanine
Northern gannet	8	17.9 ± 2.1	25.2 ± 1.5	21.5 ± 1.3	22.6 ± 1.3	29.3 ± 0.6	24.9 ± 1.5	4.9 ± 0.5
Atlantic mackerel	9	22.0 ± 1.91	24.7 ± 1.8	22.5 ± 1.7	25.8 ± 1.8	23.8 ± 1.7	24.3 ± 1.8	3.8 ± 1.1
Atlantic herring	7	21.2 ± 2.4	24.0 ± 1.5	22.0 ± 1.6	19.5 ± 2.6	23.6 ± 0.8	23.4 ± 1.4	3.8 ± 1.0
Capelin	7	21.0 ± 1.7	22.2 ± 2.2	19.7 ± 1.8	18.4 ± 1.1	23.4 ± 2.1	22.9 ± 1.7	3.8 ± 1.2
Sandlance	8	17.9 ± 3.2	19.6 ± 2.0	17.5 ± 1.7	16.0 ± 1.8	21.0 ± 1.6	20.5 ± 1.9	3.5 ± 0.8

Table S2.2. Coefficients of determination for trophic position equation combinations tested for fish (rows) and gannets (columns) related to bulk stable isotope analysis (bulk) and with log-transformed total mercury (THg). Bolded values are the highest R^2 coefficients for each gannet equation tested.

Gannet Equations →	Eq.8		Eq. 9		Eq. 10	
Fish Equations ↓	R^2 Bulk	R^2 THg	R^2 Bulk	R^2 THg	R^2 Bulk	R^2 THg
Eq. 7	0.49	0.36	0.19	0.08	0.16	0.06
Eq. 3	0.84	0.77	0.84	**0.76**	0.85	**0.76**
Eq. 4	**0.89**	**0.81**	**0.86**	**0.76**	**0.86**	0.75

Table S2.3. Average trophic position by species according to the equation or method used to calculate trophic position. When using compound-specific stable isotope analysis (equations 4, 8, 9, and 10) all fish trophic positions were calculated using Eq. 4 because it had the highest coefficient of determination with both bulk TP and log(THg) between the fish-specific TP equations (see Table S2.2).

Species	Bulk	Eq. 8 & Eq. 4	Eq. 9 & Eq. 4	Eq. 10 & Eq. 4
Northern gannet	4.6	4.6	4.1	4.0
Atlantic mackerel	4.1	3.5		
Atlantic herring	4.0	3.3		
Capelin	3.8	3.1		
Sandlance	3.5	2.8		

Table S2.4. Diet composition for northern gannet diet from field observations and stable isotope mixing models (using $\delta^{15}N$ and $\delta^{13}C$) for 2017 and 2018. Mixing model results are reported for both years as mean values and the 95% confidence interval (CI).

Species	Observations		Mixing Models			
	2017 Mean (%)	2018 Mean (%)	2017		2018	
			Mean (%)	95% CI	Mean (%)	95% CI
Atlantic mackerel	46.8	35.8	8.8	0.5 – 17.6	10.2	0.8 – 17.8
Atlantic herring	9.5	2.9	9.2	0.3 – 18.9	5.2	0.3 – 12.8
Capelin	8.7	39.4	77.1	69.7 – 83.1	81.1	74.2 – 86.6
Sandlance	17.5	8.0	5.0	0.4 – 11.2	3.5	0.3 – 8.1

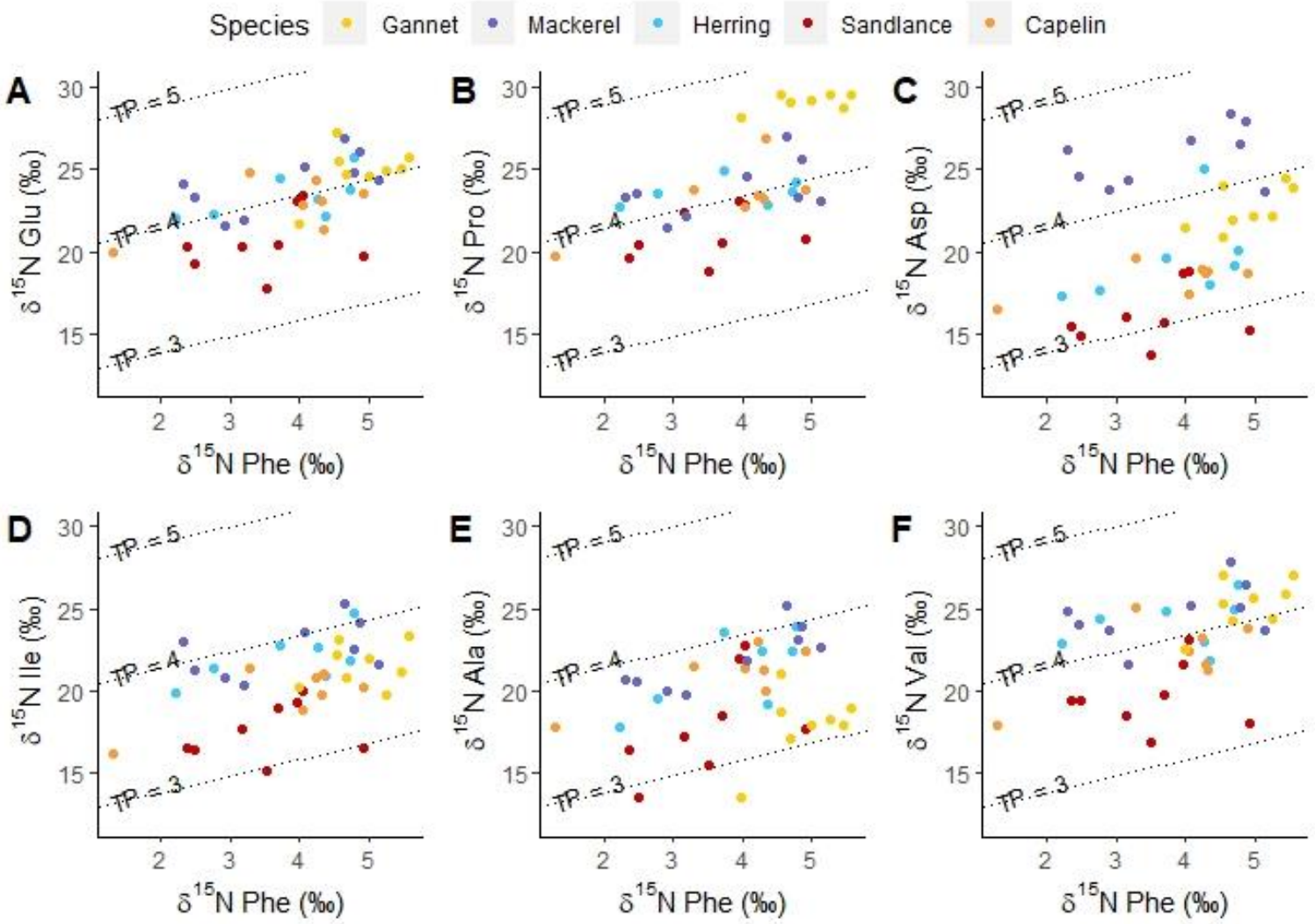


Figure S2.1. Trophic isoclines plotted for each trophic amino acid (y axes; Glutamic acid (Glu), Proline (Pro), Aspartic acid (Asp), Isoleucine (Ile), Alanine (Ala), Valine (Val)) against phenylalanine (x axes; Phe), a source amino acid. Dotted lines denote trophic position (TP) based on the trophic amino acid $\delta^{15}N$ values of each sample.

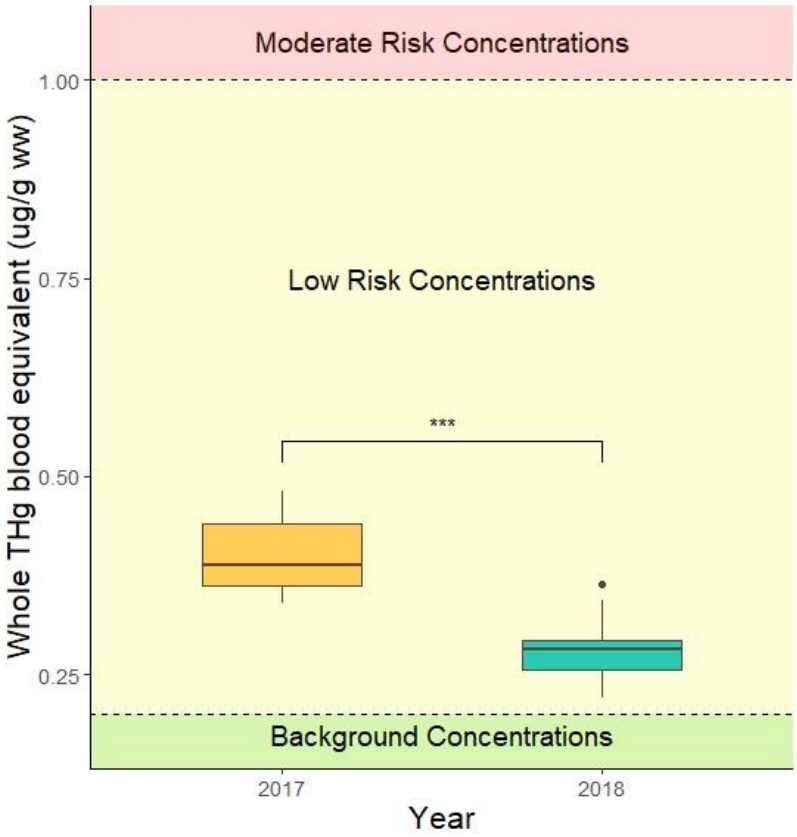


Figure S2.2. Whole blood equivalency of total mercury (THg) concentrations in northern gannets by sampling year (n = 20 for each year). THg concentrations were significantly different between years. Toxicity thresholds were used were from Ackerman et al. (2016): background concentrations consist of <0.2 µg/g ww, low, moderate and high risk are between 0.2-1.0 µg/g ww, 1.0-3.0 µg/g ww, 3.0-4.0 µg/g ww and lethal risks are > 4.0 µg/g ww. THg concentrations were not different between sexes.

BRIDGING STATEMENT

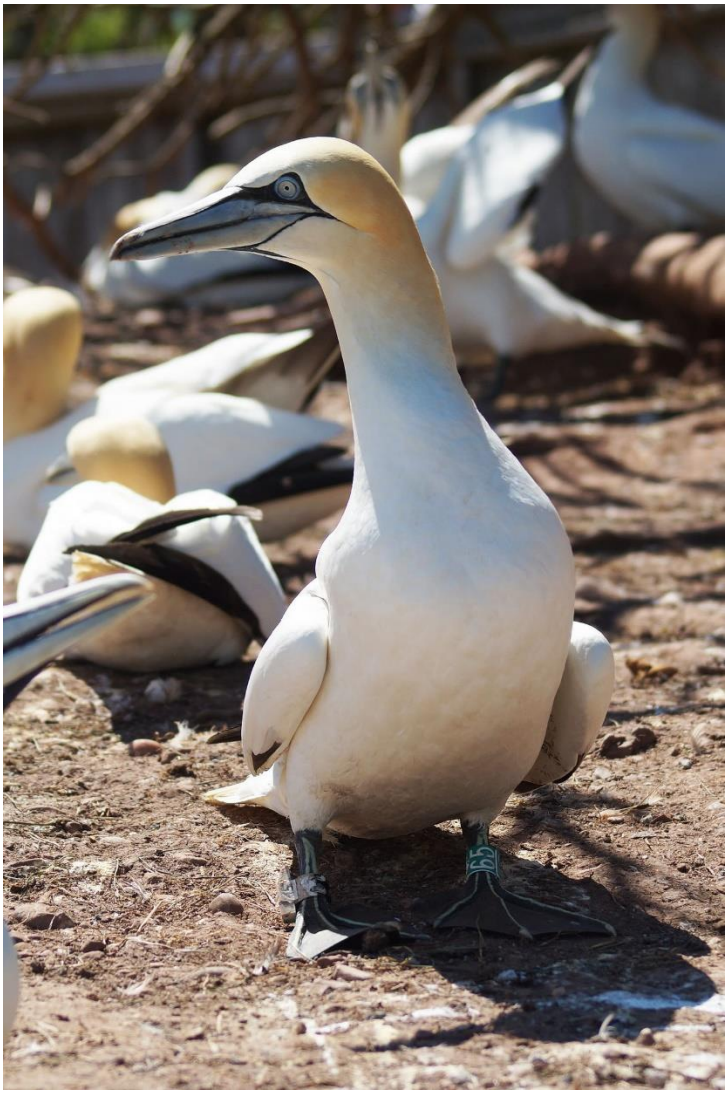
The main objective of my thesis is to better understand the factors that influence the uptake and distribution of mercury in the Gulf of St. Lawrence and to investigate the driving forces behind these mechanisms. Both chapters of my thesis address a different facet of this objective while being tightly linked by their use of novel approaches to answer questions concerning mercury contamination. As a potent neurotoxin, mercury is a highly relevant contaminant and merits the depth of the study my thesis accords it.

Chapter 2 addresses this through the lens of biomagnification, a phenomenon which greatly increases the mercury burdens from the base of the food chain to top predators. I tested the use of traditional bulk stable nitrogen isotope analysis, which has been used to evaluate the rate of biomagnification for decades, and compared it to the newer compound-specific stable nitrogen isotope analysis in amino acids. Through Chapter 2, I help to bridge an important knowledge gap concerning the bias that is likely associated with the traditional approach. Without an understanding of how the analytical methods used to assess biomagnification can impact the results, we cannot hope to effectively understand the risks posed to wildlife, humans, and entire ecosystems.

Chapter 3 seeks to understand the driving forces behind spatial differences in mercury concentrations in the Gulf of St. Lawrence. This aspect of mercury contamination is important to take into consideration when assessing health risks because a natural variation in contaminants due to spatial aspects (i.e., habitat differences, areas with higher anthropogenic disturbances, or oceanic currents) could be a critical factor determining the mercury burden in animals. In the second chapter, I investigate the driving factors that underlie spatial variation of mercury, to help further our understanding of differential risks that wildlife face. I use fish, collected from northern gannet regurgitations, as sentinels of mercury contamination in the Gulf of St. Lawrence to study the biotic and anthropogenic factors that potentially drive mercury variability.

Together, both chapters in my thesis help build on the solid foundation of knowledge we have about mercury, its behaviour in food webs and the environment, and the risks that our ecosystems face. Although studies on mercury are abundant, both chapters of my thesis address new questions in the context of the Gulf of St. Lawrence, an ecosystem of incredible ecological, economic, and cultural value. This is the first time, to the best of my knowledge, that compound-

specific stable isotope analysis of amino acids has been used to evaluate the rate of mercury biomagnification in the Gulf, to compare the results to bulk stable isotopes, that the method has been used to investigate the drivers of mercury variability in the Gulf of St. Lawrence.



A banded and tracked northern gannet, at the Bonaventure Island colony. Photo by Roxanne Turgeon © 2020

Chapter 3: Spatial variation in mercury in the Gulf of St. Lawrence, using northern gannets (*Morus bassanus*) as biological sampling units

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Abstract

Mercury (Hg) is ubiquitous and is a contaminant of concern due to its potential to cause harm for wildlife. Within ecosystems, biogeochemical processes affect the distribution of Hg concentrations in organisms, and it is important to understand what drives this variability. To examine sources of variation, we collected fish regurgitated by northern gannets (*Morus bassanus*) collected at the Bonaventure Island colony in the Gulf of St. Lawrence. We related fish total Hg (THg) concentrations to their catch location across the Gulf as well as to biotic and corresponding relevant anthropogenic factors. In small fish species, trophic position calculated from compound-specific stable nitrogen isotopes in amino acids was the best predictor for THg concentrations. In large fish species, THg was explained by $\delta^{13}C$, indicating THg concentrations were higher in fish of inshore habitats. Published THg levels in mussels sampled nearby correlated with THg levels measured in gannet prey caught along the coast, implying that spatial variation was consistent across trophic levels. We conclude that THg concentrations measured in large prey species are variable between habitats and that there is consistent spatial variation of THg across taxa in the Gulf of St. Lawrence.

1. Introduction

Concentrations of contaminants have been documented to vary within large marine ecosystems all over the world. Mercury (Hg) is ubiquitous but unevenly distributed globally (Liu et al. 2021, Médieu et al. 2022). On an oceanic scale, concentrations of Hg can increase on a gradient with latitude (Houssard et al. 2019), can differ within estuarine habitats (Eagles-Smith and Ackerman 2014), and can contaminate marine wildlife populations differently (Carravieri et al. 2017, Pomerleau et al. 2018, Albert et al. 2021b, Médieu et al. 2022). The distribution of Hg can be influenced by water currents, which can transport mercury to new habitats, and zones of upwelling have been associated with higher concentrations of Hg (Conaway et al. 2009, Cossa and Tabard 2020). In deep waters, where anoxic environments are present, sulfate-reducing bacteria and other microbes methylate Hg into MeHg, a bioavailable organic form of Hg (Ma et al. 2019), while surface waters receive Hg through atmospheric deposition (Driscoll et al. 2013). Important riverine inputs can also impact the amount of Hg in habitats, as rivers can transport Hg bound to particles and dissolved organic carbon (Cossa and Gobeil 2000, Liu et al. 2021). Thus, marine habitats are impacted by a variety of processes that bring Hg into the environment, causing spatially-diverse Hg contamination levels (Liu et al. 2021). Food webs exposed to varied levels of contamination pose different risks to marine wildlife. Therefore, it is important to understand what drives the variability of Hg, to implement conservation measures and monitor the Hg concentrations in ecologically important environments.

Marine organisms have often been used as indicators to examine the variability of Hg in marine environments. Studies using sessile primary consumers (e.g. mussels; *Mytilus* spp.) have found spatial variability in Hg from different origins on a regional scale (Vokhshoori et al. 2014, Cossa and Tabard 2020). Using mobile marine predators has also been successful in identifying spatial patterns of Hg contamination over large areas. Studies of Hg using skipjack tuna (*Katsuwonus pelamis*) found that spatial trends were related to latitude, likely in response to Hg concentrations fluctuating at the base of the food web, and to higher concentrations of Hg in surface waters due to atmospheric deposition (Médieu et al. 2022). However, in similar species (*Thunnus* spp.), the position of the thermocline was found to be the main explanatory factor (Houssard et al. 2019). Studies on the non-breeding distribution in many seabird species have shown that foraging habitat and fidelity to over-wintering sites can significantly impact individuals' Hg burdens (Fort

et al. 2014, Albert et al. 2021a). In Leach's storm petrels (*Hydrobates leucorhous*) in the North Atlantic, higher THg in blood was related to latitude, greater foraging depths, and trophic level (Pollet et al. 2022). Marine rather than terrestrial foraging was found to be an important determinant in higher Hg concentrations in gulls (*Larus* spp.; Jouanneau et al. 2022). Thus, spatial variability in Hg concentrations seems to depend on the sampled environment and can be influenced by taxa and foraging behaviours. Studying predators offers an opportunity to examine spatial trends that may go undetected at lower trophic positions (Furness and Camphuysen 1997, Hazen et al. 2019).

Stable isotopes are useful tools to study Hg contamination in wildlife organisms. $\delta^{15}N$ is commonly used as a proxy of trophic level for animals, as the $\delta^{15}N$ values increase in a stepwise manner in consumer tissues between trophic levels and are used to assess biomagnification (Cabana and Rasmussen 1994, Post 2002). However, bulk $\delta^{15}N$ can vary between habitats and over time, and thus hides baseline differences, causing spatial or temporal biases in $\delta^{15}N$ values (McClelland and Montoya 2002). Traditionally, accounting for baseline values entailed sampling of baseline organisms (e.g. mussels), which in some cases can be time-consuming, tedious, or impossible on oceanic scales. Compound-specific stable isotope analysis of nitrogen in amino acids (CSIA-AA, $\delta^{15}N$) can be used to calculate an organism's trophic position (TP_{AA}) while correcting for spatial differences in $\delta^{15}N$ values at the base of the food web (McClelland and Montoya 2002). Trophic AAs increase in a stepwise manner with trophic level, while source AAs represent primary producer $\delta^{15}N$ values and change very little as they are transferred through the food web (McClelland and Montoya 2002, Chikaraishi et al. 2009, Ohkouchi et al. 2017). Thus, by removing the baseline $\delta^{15}N$ (source AAs) from trophic $\delta^{15}N$, the resulting "spatially corrected" $\delta^{15}N$ is used to calculate trophic position (Chikaraishi et al. 2009), which is positively related to Hg in food webs (Cabana and Rasmussen 1994, Atwell et al. 1998). Stable carbon isotopes ($\delta^{13}C$) are used as indicators of habitat use, differentiating between inshore (higher values) and offshore (lower values) habitats in marine environments (Hobson and Wassenaar 1999). $\delta^{13}C$ has been positively associated with Hg contamination in a range of taxa previously (Harper et al. 2018, Elliott et al. 2021). Stable isotopes of sulfur ($\delta^{34}S$) also inform on habitat use, differentiating between inshore (lower values) and offshore (higher values) habitats, but can also provide insight into the vertical habitat use (water column – higher values vs benthic

environments – negative values) (Connolly et al. 2004). Some studies on fish have found that Hg was negatively related to $\delta^{34}S$ values (Góngora et al. 2018, Harper et al. 2018), but others report positive relationships (Elliott and Elliott 2016, Elliott et al. 2021). The use of stable isotopes is helpful in the study of the spatial distribution of Hg.

Northern gannets (*Morus bassanus*; hereafter, gannets) are large, piscivorous seabirds that breed in the North Atlantic Ocean. The largest North American colony is located on Bonaventure Island in Québec, Canada in the Gulf of St. Lawrence. Gannets travel long distances across the Gulf to forage for themselves and to provide for their nestlings (Garthe et al. 2007, Montevecchi et al. 2012, Guillemette et al. 2018). At the Bonaventure Island colony, they feed on commercially relevant fish such as Atlantic mackerel (*Scomber scombrus*), Atlantic herring (*Clupea harengus*), as well as capelin (*Mallotus villosus*), sandlance (*Ammodytes* spp.), and more recently, rockfish (*Sebastes* spp.; Pelletier and Guillemette 2022). When disturbed, gannets will spontaneously regurgitate (Guillemette et al. 2018), allowing investigators to easily study diet and sample consumed fish. Regurgitations can then be analyzed for chemical tracers such as contaminants and stable isotopes. Gannets forage over large areas (Guillemette et al. 2018) and can therefore be excellent sentinels of large ecosystems. Previous ecotoxicological studies on the Bonaventure Island colony have investigated decreasing temporal contaminant trends (Hg, organochlorines, polychlorinated biphenyls, and brominated flame retardants), demonstrating that gannets are useful contaminant monitors (Elliott et al. 1988, Champoux et al. 2015, Champoux et al. 2017).

Many studies using GPS-tracked birds have been conducted in urban environments in the St. Lawrence system and demonstrated the importance of examining the contribution of different habitats to wildlife contaminant burdens (Gentes et al. 2015, Brown et al. 2019, Sorais et al. 2020, Kerric et al. 2021). Our study is the first to use GPS tracking and piscivorous seabirds to study contaminants in the Gulf of St. Lawrence.

In this study, we relate Hg concentrations from regurgitated fish, collected at the Bonaventure Island gannet colony, to the location of the most recent foraging area in the trip, and to relevant biotic and anthropogenic factors that have the potential to influence Hg concentrations in the Gulf of St. Lawrence. Our objective is to determine what primarily drives total Hg (THg; the

sum of both inorganic and organic methylmercury) concentrations in fish from the Gulf. Our hypotheses are 1) spatial variability of THg is due to biotic differences such as foraging habitats ($\delta^{13}C$ and $\delta^{34}S$), 2) anthropogenic activities (coastal development, shipping traffic, and pollution) are determining THg variability, and 3) THg in fish varies according to biological factors, rather than varying spatially (size class or trophic position).

2. Methods

2.1 Sample Collection

During the 2021 breeding season (June to September), 38 gannets were captured with noose poles and equipped with GPS devices (i-gotU GT-120, Mobile Action Technology Inc., Taiwan, or CatLog Gen 2, Catnip Technologies Ltd., Hong Kong) attached to the three central rectrices using a strip of Tesa #4651 tape. GPS deployments lasted on average 10 days and individuals were recaptured once before removing the device, following the return of their first foraging trip. The GPS devices were set to record the bird's geographical position (using latitude and longitude) every five minutes.

All fish samples were collected opportunistically during gannet captures at the Bonaventure Island colony in the Parc national de l'Île-Bonaventure-et-du-Rocher-Percé near Percé, Québec, Canada during the breeding season. Gannets regurgitate spontaneously when stressed, which makes for easy sampling of their stomach contents (Guillemette et al. 2018). Only regurgitations that came from a gannet equipped with a GPS tracker were considered in our analyses. Contents of the regurgitations were sorted and quantified by species and digestion state. Regurgitations were frozen in a -20°C freezer at the end of the day and later stored long-term in -20°C freezers until dissections. The fish regurgitations were thawed and ~1 cm^3 of dorsal muscle was collected from the regurgitated prey. Given that the digestion of samples affected the integrity of the fish, we could not measure the size of the fish with confidence. In instances where two species were present, we sampled both species. We collected muscle from five groups of fish: Atlantic mackerel, Atlantic herring, capelin, sandlance, and rockfish. In total, 46 fish were sampled from regurgitations; 36 single-species occurrences and five regurgitations containing two fish species.

2.2 GPS tracking and spatial analysis

We extracted and analysed the GPS tracks using Hidden Markov Models (HMMs) to categorize each point in the track as one of four possible behaviours: colony, travelling, foraging, or resting on water using the R package "momentuHMM" (McClintock and Michelot 2018). Our model integrated speed, distance from the colony, and angle concentration (i.e., directed or undirected movements) to infer the behavioural state of gannets. We applied a buffer of 1 km around the center of our colony, chosen to represent the middle point between the nests for all our tracked gannets. A description of distributions and starting values used to inform our model can be found in Table 3.1. We used the Viterbi algorithm (McClintock and Michelot 2018) to determine the most likely behavioural states from the model. Then, we divided each GPS track into foraging trips and analysed the trip directly preceding the date and time of the regurgitation. We identified the last foraging behaviour in the tracks (i.e., "foraging bout") and calculated the mean longitudinal and latitudinal points (i.e., "mean foraging location"). This location represents the area we estimate the fish in each regurgitation was caught. If the last identified foraging location had a time stamp older than six hours, we considered it unlikely that the regurgitation came from that area due to digestion rates (Jackson et al. 1987) and excluded that point and sample from our analyses. In the case of regurgitations containing two species, we also analyzed the next-to-last foraging bout and assigned the last foraging point to the least digested species and the next-to-last foraging point to the more digested species if both foraging location time stamps were within our six-hour window. From the 38 GPS tracks and 46 regurgitations, we isolated 36 mean foraging locations: 32 points related to single-species regurgitations and four points from regurgitations containing two species (Table 3.2).

2.4 Mercury analysis

We analysed all samples for total mercury (THg) using a Direct Mercury Analyser (DMA-80 evo, Milestone), using TORT-3 (lobster hepatopancreas) and DORM-4 (fish protein) certified reference materials (National Research Council of Canada). These had recovery values (mean ± sd) of 100.0 ± 2.0% (n = 22) and 99.3 ± 2.8%, (n = 6), respectively. We assumed that 100% of THg was methylmercury in the fish muscle (Carbonell et al. 2009).

2.5 Bulk stable isotope analysis

Samples were analysed for bulk SIA of carbon ($\delta^{13}C$ values) and sulfur ($\delta^{34}S$ values) at the Ján Veizer Stable Isotope Laboratory (Ottawa, Ontario, Canada). Stable isotopes values are reported in Delta notation $\delta = ((R_x - R_{std})) / R_{std}) * 1000$ where R is the ratio of the abundance of the heavy to the light isotope, x denotes the sample and std is an abbreviation for standard. $\delta^{13}C$ the ratio of $^{13}C/^{12}C$, and $\delta^{34}S$ the ratio of $^{34}S/^{32}S$. The samples were combusted in a Vario EL Cube (Elementar, Germany) EA-IRMS interfaced via Conflo IV to Delta Advantage isotope ratio mass spectrometer (Thermo, Germany - ($\delta^{15}N$ and $\delta^{13}C$) or the Delta Plus XP IRMS (ThermoFinnigan, Germany). The raw isotope data were referenced to the VPDB (carbon) and scales using six calibration standards (IAEA-N1, IAEA-N2, USGS-40, USGS-41, NBS-22, and IAEA-CH-6). Four internal check standards were included in the analytical runs: C-51 Nicotiamide ($\delta^{13}C$: −23.0‰), C-52 mix of ammonium and sucrose ($\delta^{13}C$: −11.9‰), C-54 caffeine ($\delta^{13}C$: −16.6), and AG-2 argentite ($\delta^{34}S$: -0.62‰). The analytical error was monitored using a blind standard (C-55, glutamic acid, −4.0‰) and was better than ±0.1‰ for carbon and sulfur. Ten percent of the samples were randomly duplicated. Standard deviations for duplicates of fish muscle averaged 0.1‰ for $\delta^{13}C$ and $\delta^{34}S$. $\delta^{13}C$ values were mathematically normalized for lipid content according to the methodology detailed by Post et al. (2007). For the mean values of stable isotope analysis and THg for all species, refer to Table 3.2.

2.6 Compound-specific stable isotope analysis

Samples were additionally analysed for compound-specific stable isotope analysis of amino acids (CSIA-AA) at the Alaska Stable Isotope Facility at the University of Alaska Fairbanks (Alaska, USA). This method is detailed elsewhere in Barst et al. (2020) and Chapter 2. The analysis yielded data for seven trophic AAs (alanine (Ala), isoleucine (Ile), aspartic acid (Asp), proline (Pro), glutamic acid (Glu), valine (Val), leucine (Leu)) and a single source AA (phenylalanine (Phe)). All samples were run in triplicate and the mean $\delta^{15}N$ for each sample was used in data analysis (Table 3.2).

The average nLeu (internal standard) value in all samples was 18.7 ± 0.5 ‰ (mean ± sd), corresponding to the known value of 19.3 ‰, while the average measured for caffeine was -3.1 ± 0.2 ‰, corresponding to the known value of -3.3 ‰. Two pre-made standards of amino acids with known values were included in each batch. The measured values for both standards did not

significantly differ from the expected values (Pearson's correlation test: standard 1: $t = 52.6$, $R^2 = 1.00$, $p < 0.0001$, standard 2: $t = 52.3$, $R^2 = 1.00$, $p < 0.0001$). We also digested and derivatized subsamples of the same sample (mackerel: "17-30") with each batch which we used to verify there was no systematic bias among batches: the internal standards nLeu (18.8 ± 0.5 ‰) and caffeine (-3.0 ± 0.2 ‰) corresponded to the known values in all batches.

We calculated the trophic positions for all fish in our study using the CSIA-AA equation detailed in Nielsen et al. (2015):

$$TP_{AA} = \left(\frac{(\delta^{15}N_{Glu} + (\delta^{15}N_{Ala} + 0.59) + (\delta^{15}N_{Ile} + 2.63) + (\delta^{15}N_{Val} - 3.35) + (\delta^{15}N_{Asp} - 1.78) + (\delta^{15}N_{Pro} - 1.39)) / 6 - \delta^{15}N_{Phe} - \beta)}{TDF} \right) + 1$$

Where $\delta^{15}N_x$ represents the $\delta^{15}N$ value for each amino acid (AA), the constants added to each AA, other than Glu and Phe, are correction terms meant to standardize the AA $\delta^{15}N$ values relative to Glu, β represents the difference between trophic and source AAs in primary producers, and TDF is the trophic discrimination factor, or how much the trophic AA's isotopic signature will change between prey and its predator.

2.7 Statistical analysis

We used the mean foraging location identified for every sample (n = 36) to map the variability of THg, $\delta^{13}C$, and $\delta^{34}S$ in the Gulf of St. Lawrence, sampled in gannet regurgitations. To facilitate our interpretation of how anthropogenic and biotic factors drive THg contamination in the Gulf of St. Lawrence, we classified mean foraging points as either coastal (i.e., less than 5 km from shore as in Beauchesne et al. 2020) or pelagic. We used linear models to determine whether stable isotopes of $\delta^{13}C$ and $\delta^{34}S$ (proxies for biotic factors), a suite of anthropogenic factors in the Gulf of St. Lawrence, or trophic level calculated using CSIA-AA $\delta^{15}N$ (indicating null spatial effect) were the main drivers of THg concentrations in our samples. Based on the results, which indicated size class was the main influence on THg, we used linear mixed-effects models to test the same explanatory factors on all samples pooled together, while specifying "size class" as a random effect. In the pooled samples, size class also emerged as the best predictor of THg, so we split the samples by size class and tested the factors again using linear models. The anthropogenic factors we chose to use were coastal development (the presence and extent of

coastal infrastructure development), shipping traffic (frequency and location of shipping vessels), pollution (organic originating mainly from agricultural areas near the coast, inorganic originating from human coastal structures such as roads, and marine pollution as a consequence of shipping and fisheries traffic) as characterized in Beauchesne et al. (2020). One mackerel sample was removed for having extreme values for two variables (Cook's distance was greater than three times the mean; TP_{AA} = 0.19, Shipping = 0.11), and coastal development was only used when testing coastal samples, due to the lack of data for pelagic samples.

To determine which models were the best predictors of THg in our samples, we used Akaike's Information Criterion corrected for small sample sizes (AICc). We specified a threshold ΔAICc = 2 and calculated the weight for each model, which shows the strength of evidence for each model, and the evidence ratio (the weight of the first-ranked model divided by the weight of another model) for the second-best and null models (Burnham and Anderson 2004).

Differences in THg among the fish species were tested using a one-way ANOVA. The Atlantic herring sample (n = 1) was excluded from our analyses being the only sample of that species. Species that did not significantly differ in their THg concentrations were then pooled. We then compared the THg concentrations in fish to the THg concentrations of mussels sampled at coastal stations around the Gulf of Saint Lawrence in 2017 and 2018 by Cossa and Tabard (2020), using correlation analysis to investigate consistency in THg concentrations between the two datasets from similar locations. Comparisons were done using the closest sampling station to each mean foraging location and pelagic points (greater than 5km from shore) were excluded due to greater distances from shore.

We tested for differences in $\delta^{13}C$ and $\delta^{34}S$ between species size classes using a Wilcoxon rank sum test due to non-normally distributed data. We also used linear regressions to examine the relationship between $\delta^{13}C$ and $\delta^{34}S$, respectively, and the distance to shore for each species of fish separately, excluding sandlance due to low sample size (n = 3).

All statistical analyses were run in R 4.1.2 (R Core Team 2021).

3. Results

3.1 Determinants of THg in the Gulf of St. Lawrence

We mapped the foraging locations for each of the regurgitated fish samples and assigned THg values to these points (see Figure 3.1) for small and large fish species. No visual trends in THg variability were apparent throughout the Gulf as a whole, or within or between different areas.

Linear models for coastal and pelagic fish suggested that size class (large vs small) and trophic position derived from amino acid-specific $\delta^{15}N$ (TP_{AA}) were the main drivers of THg concentrations in coastal samples (p-value < 0.0001, R^2 = 0.91) but a second model ranked within ΔAICc = 2 (Size Class * TP_{AA}, ΔAICc = 0.3), while size class alone was the best predictor of THg in pelagic samples (p-value = 0.03, R^2 = 0.34, Table S3.1). Size class in pelagic samples explained THg variability three times better than the second-best model (the null model, ΔAICc = 2.43). We then tested all samples together, regardless of coastal or pelagic classification, using linear mixed effects models and specifying "Size Class" as a random factor (Table S3.2). The size class and TP_{AA} emerged as the main influences on THg concentrations (p-value < 0.0001, R^2 = 0.57) but the second-best model was also ranked within ΔAICc = 2 (Size Class, ΔAICc = 0.84). Based on this, we pooled species according to THg concentrations (small: capelin and sandlance, large: mackerel and rockfish; THg did not significantly differ within a size class, Table 3.3). After running linear models for each size class, TP_{AA} was the best predictor of THg in small species (p-value = 0.005, R^2 = 0.44) and $\delta^{13}C$ was the best predictor of THg in large species (p-value = 0.08, R^2 = 0.19, Table S3.3). TP_{AA} explained THg variability in small species ten times better than our second-best model ($\delta^{34}S$, ΔAICc = 4.49) and 26 times better than the null model (ΔAICc = 6.32). $\delta^{13}C$ explained THg variability in large species just as well as the second-best model (Shipping, ΔAICc = 0.23), and the null model (ΔAICc = 0.58).

The THg concentrations of fish foraged by gannets were positively but not significantly correlated with THg concentrations of mussels sampled at the station nearest the fish catch location (p = 0.06, R^2 = 0.46; see Figure 3.2). The comparison between THg in regurgitated fish samples (this study) and mussels collected at coastal sampling stations by Cossa and Tabard (2020) shows there is spatial consistency in THg concentrations across taxa.

3.3 Stable isotope variation in the Gulf of St. Lawrence

We mapped out $\delta^{13}C$ and $\delta^{34}S$ signatures (Figure 3.3) as we did with THg, but because these two isotopes did not differ significantly among size classes ($\delta^{13}C$; W = 172, p-value = 0.2 and $\delta^{34}S$; W = 192, p-value = 0.1), fish of both size classes were pooled. $\delta^{34}S$ correlated strongly (R^2 = 0.86), but non-linearly with distance to shore in rockfish samples, and correlated linearly with distance to shore in mackerel (R^2 = 0.36). $\delta^{13}C$ in rockfish and mackerel and both isotopes in capelin did not significantly relate to distance to shore (Figure 3.4).

4. Discussion

4.1 Determinants of THg in the Gulf of St. Lawrence

Our mapped THg concentrations in regurgitated fish across the Gulf did not show any clear pattern in the studied area (Figure 3.1). Multiple factors likely influenced THg concentrations across this vast area and among species, making it difficult to visualize different THg trends. However, our linear models identified important trophic and spatial factors that were strongly related to the THg concentrations in fish samples.

In small species, TP_{AA} was the best explanatory factor for THg variability. TP_{AA} was calculated using CSIA-AA, which allowed us to exclude the effect of spatial differences in d15N at the base of the food web (Chikaraishi et al. 2009, Ohkouchi et al. 2017). Therefore, there are no underlying spatial differences incorporated into the TP_{AA} calculated. In our samples of capelin and sandlance, biomagnification was the determining factor of THg concentrations, as is commonly reported (Cabana and Rasmussen 1994, Lavoie et al. 2010, Lavoie et al. 2013). These smaller fish may not accumulate enough THg in their tissues to properly reflect any spatial variability. THg concentrations ranged from 0.02 to 0.06 μg/g in capelin and sandlance tissues, similarly to previous studies of THg for these small species (Lavoie et al. 2010, Pedro et al. 2017).

Within the large species group, $\delta^{13}C$ was the best predictor of THg, indicating higher levels of THg are linked to more inshore habitats. This is consistent with previous studies across many taxa, including fish (Le Croizier et al. 2019, see meta-analysis in Elliott et al. 2021). Inshore habitats are exposed to more inland and allochthonous influences, such as riverine inputs that can transport Hg from anthropogenic sources (Amos et al. 2014, Liu et al. 2021). Inshore benthic

sediments are also more disturbed by coastal development, keeping Hg present in the water column and available for biotic uptake (Mayer et al. 1991, Amos et al. 2014). Further, disturbances to benthic sediments can increase the presence of anaerobic bacteria, consequently increasing Hg methylation (Mayer et al. 1991). Thus, large fish caught closer to shore likely experience processes that promote exposure to Hg. However, three other models ranked within ΔAICc = 2, including the null model, indicating our results should be interpreted with caution. Shipping and $\delta^{34}S$ were the second and fourth-ranked models for the large fish samples, further giving weight to a spatial influence on THg concentrations.

Further, it is possible that higher, and varied concentrations of THg in large fish species allowed us to detect spatial differences more easily than in small species. Mackerel THg concentrations ranged from 0.02 to 0.28 μg/g, a ten-fold increase between the lowest and highest concentrations, which were higher than those reported in most other Northern Atlantic studies (Azad et al. 2019, Costa et al. 2020, Ulusoy and Sühendan 2020). These differences may reflect temporal fluxes, due to changing oceanic conditions (Cossa and Gobeil 2000, Conaway et al. 2009) or spatial distribution in THg within the Atlantic, or differences in trophic level (trophic levels were unreported in Azad et al. 2019, Costa et al. 2020, Ulusoy and Sühendan 2020). Rockfish THg concentrations (0.025 - 0.19 μg/g, mean = 0.11 μg/g), on the other hand, were comparable to published values (Azad et al. 2019). In large species, it was likely easier to identify a spatial influence on THg values due to higher concentrations and differences between individuals.

We found a positive, although not significant, relationship between the THg concentrations in marine mussels (*Mytilus* spp.) sampled by Cossa and Tabard (2020) and the regurgitated fish caught close to the mussel sampling stations (see Figure 3.2). While many of our samples could not be related to sampling locations from Cossa and Tabard (2020) because they were too pelagic, our costal fish THg concentrations were correlated with coastal mercury burdens in mussels. Concentrations in fish and mussels in the Gaspesian peninsula (stations 16, 19, 21; Figure 3.2) had lower THg concentrations, which (Cossa and Tabard 2020) attributed to a dilution effect on THg once waters from the St. Lawrence Estuary enter the highly-dynamic Gulf. Our study did not have access to fish caught in the Estuary, but future studies should sample the Estuary and St. Lawrence River to further investigate their contribution of THg in the

Gulf. The relationship between fish and mussel THg further provides evidence of a spatial effect on marine wildlife THg concentrations across different sampling locations along the coasts of the Gulf of St. Lawrence.

4.2 Stable Isotopes

Strong patterns are also difficult to discern in our maps of the variability of $\delta^{13}C$ and $\delta^{34}S$ among our sampled points in the Gulf of St. Lawrence (Figure 3.3). The isotopic signatures of $\delta^{34}S$ were significantly related to the distance of the sampled point to shore (non-linear relationship) when examining rockfish signatures (Figure 3.4). Mackerel $\delta^{34}S$ values were positively and linearly related to distance from shore (Figure 3.4). In rockfish, $\delta^{34}S$ signatures decrease until the inflection point at ~23 km from shore and increase once again. The distance of this inflection point may explain the lack of a significant relationship between the isotopes and distance from shore for capelin, as the range of their distances from shore in our study is much smaller (0 - 11 km). This corresponds with previous literature describing the isotopic trends of $\delta^{34}S$, and its common use as an indicator of an organism's foraging habitat (Post 2002, Connolly et al. 2004). Previous work on tropical fish did not find the distance to shore to be an important determinant in $\delta^{13}C$ composition (Trystram et al. 2015). To our knowledge, no published studies have investigated the relationship between $\delta^{34}S$ and distance to shore in higher trophic-level organisms such as fish.

4.3 Recommendations

This study was the first to use a marine predator, northern gannets, to monitor THg concentrations in different areas of the Gulf of St. Lawrence and was an important first step to refining this approach. As such, we offer recommendations that should minimize bias and confounding factors from future studies, while addressing our limitations.

When planning to use marine predators as sampling units for biological specimens (e.g. dietary items or biological samples), we recommend using organisms and technologies which can efficiently describe that animal's behaviours. Our tracked gannets were equipped with GPS devices that offered a geographical fix every few minutes, which may have led to some missed foraging bouts in our HMMs. The addition of a time-depth recorder or an accelerometer, to the

GPS device, would help log behaviours more accurately while removing the inherent uncertainty incorporated into the HMMs used to classify behaviour.

Standardizing $\delta^{15}N$ for baseline effect is crucial in trophodynamic studies, but sampling baseline organisms (e.g., primary consumers) is impossible in large ecosystems like the Gulf, given the spatial scale. We strongly recommend the use of CSIA-AA to ensure that baseline variability is not influencing the calculated trophic positions of sampled species.

Further, choosing predators which forage in constrained ranges could be advantageous to sample a larger set of points covering a smaller area. For example, our 36 regurgitations were insufficient to cover the very large Gulf of St. Lawrence and interpolate between points to map a complete chemical landscape. Thus, the larger area accessible to gannets led to a higher spread of our sampled points. Finally, a shorter sampling period would help the obtention of temporally comparable samples and data, to maximize the likelihood of collecting prey items of the same species, and to avoid changes in THg or isotopic signatures. For example, capelin landings are dominant during the capelin spawning season from May to June, when capelin flood the shores to spawn and become a highly available forage fish in the Gulf of St. Lawrence (McQuinn et al. 2012). Fish life history traits such as these should be taken advantage of when possible.

5. Conclusion

This is the first study in the Gulf of St. Lawrence that attempted to map out and determine the variability and drivers of THg in fish using northern gannets as sampling units. We found evidence of higher THg concentrations in inshore habitats in Atlantic mackerel and rockfish, and consistent spatial variability of THg across mussels and fish in the Gulf of St. Lawrence. We tested a novel method using piscivorous seabirds to sample fish to use in an ecotoxicology study. Our study could have many potential uses in the future, including the identification of vulnerability hotspots for fish populations and their predators, or assessing risk factors for the predators themselves by using biologically relevant prey. Using seabirds, or other marine predators, as samplers could help identify ecologically valuable areas for further study of contamination. Our methodology of using biological samplers is less costly and time-consuming than trawling surveys and is worth developing further. Our recommendations for future work

should help investigators create a more robust sampling design, leading to stronger analyses and conclusions.

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Table 3.1. Mean values and distributions input into the Hidden Markov Models to classify northern gannet behaviour based on GPS tracks of foraging trips. Values in the colony, travelling, foraging, and resting columns represent the mean starting values used in the models and are the four targeted behaviours classified by these.

Variable	Distribution	Parameter	Colony	Travelling	Foraging	Resting
Speed	Gamma	Mean	0.01	30	7	0.05
		SD	0.01	0.5	0.1	0.01
		Zero-mass	0.9	0	0.01	0.9
Angle	von Mises	Concentration	3	0.5	0.01	0.5
Distance from colony	Bernouilli	Probability	0.99	0.5	0.01	0.9

Table 3.2. Sample sizes and values (mean ± standard deviation) of total mercury (THg) stable isotopes of $\delta^{15}N$, $\delta^{13}C$, and $\delta^{34}S$ and the five species sampled through regurgitation collections from northern gannets. $\delta^{13}C$ values were mathematically corrected for lipid content.

Species	n	THg (µg/g)	$\delta^{13}C$ (‰)	$\delta^{34}S$ (‰)	Ala (‰)	Val (‰)	Ile (‰)	Asp (‰)	Pro (‰)	Glu (‰)	Phe (‰)
Atlantic herring (*Clupea harengus*)	1	0.53	-19.5	19.0	22.9	24.6	22.1	19.5	23.7	23.9	4.3
Atlantic mackerel (*Scomber scombrus*)	12	0.16 ± 0.07	-19.6 ± 0.9	19.5 ± 0.9	21.1 ± 3.0	24.1 ± 2.4	22.1 ± 2.6	24.1 ± 3.2	23.1 ± 1.7	23.9 ± 1.7	3.8 ± 0.9
Capelin (*Mallotus villosus*)	13	0.04 ± 0.01	-19.3 ± 0.2	18.7 ± 0.5	21.7 ± 1.8	22.9 ± 1.2	20.7 ± 0.8	18.5 ± 0.9	23.6 ± 1.0	23.4 ± 0.9	4.7 ± 0.6
Sandlance (*Ammodytes* spp.)	3	0.03 ± 0.01	-20.2 ± 1.0	19.6 ± 1.0	17.1 ± 2.2	18.9 ± 2.2	17.0 ± 2.3	15.0 ± 2.0	20.3 ± 1.8	19.7 ± 2.1	3.4 ± 0.4
Rockfish (*Sebastes* spp.)	6	0.11 ± 0.06	-19.1 ± 0.2	19.0 ± 0.3	18.2 ± 2.4	22.5 ± 2.2	18.3 ± 1.4	16.7 ± 1.7	27.6 ± 1.3	21.5 ± 1.6	3.9 ± 0.8

Table 3.3. Our ANOVA test indicated significant differences in log-transformed total mercury between species (F value = 14.0, df = 3, P-value < 0.001). P-values from Tukey's Honestly Significant Differences test for each species-pairing when testing the differences in total mercury (THg) concentrations between the four species of regurgitated fish. Based on these results, species were pooled by size: small species (capelin and sandlance) and large species (Atlantic mackerel and rockfish).

	Capelin	Sandlance	Atlantic mackerel
Sandlance	0.61		
Atlantic mackerel	<0.001	<0.001	
Rockfish	0.02	0.01	0.52

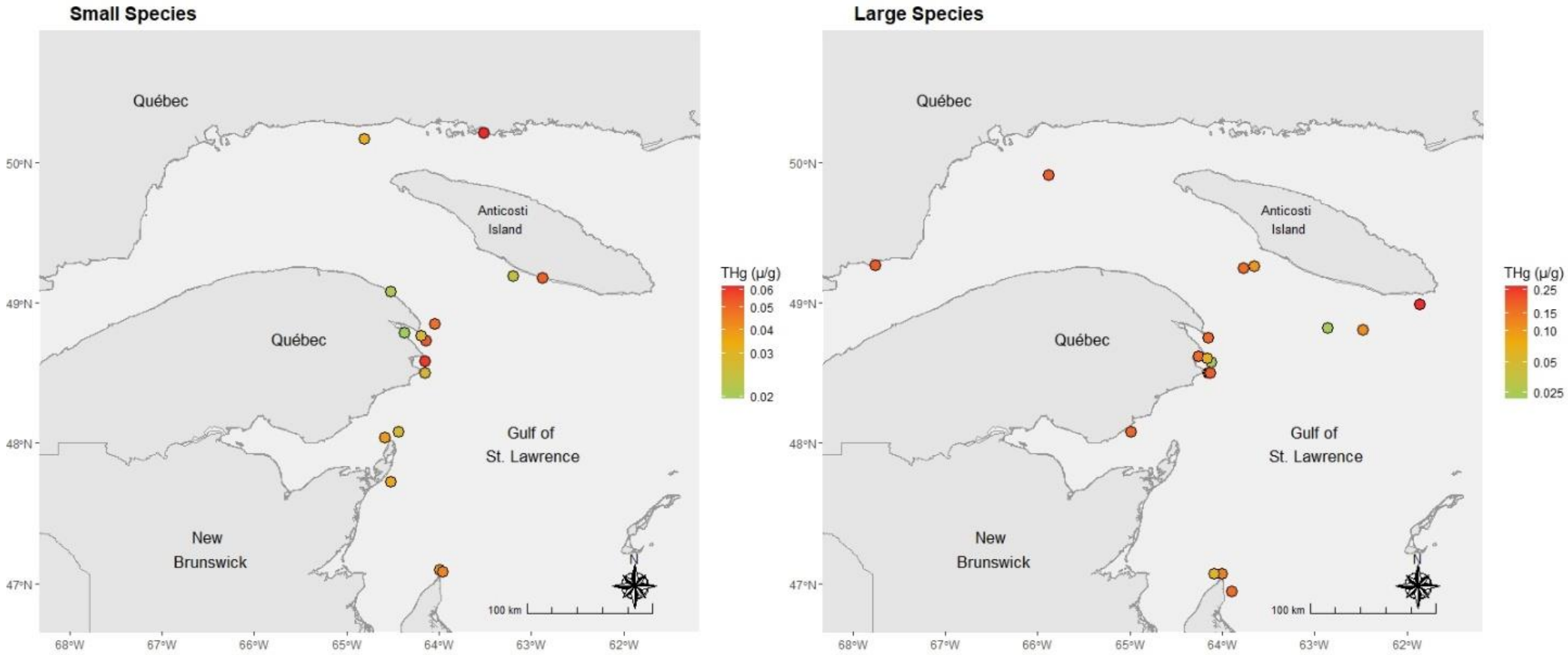

Figure 3.1. Total mercury (THg, reported in µg/g) for each regurgitated fish sample according to the mean foraging location of northern gannets in the trip directly preceding the regurgitation. Small species consist of sandlance (*Ammodytes* spp.) and capelin (*Mallotus villosus*), while large species consist of Atlantic mackerel (*Clupea harengus*) and rockfish (*Sebastes* spp.).

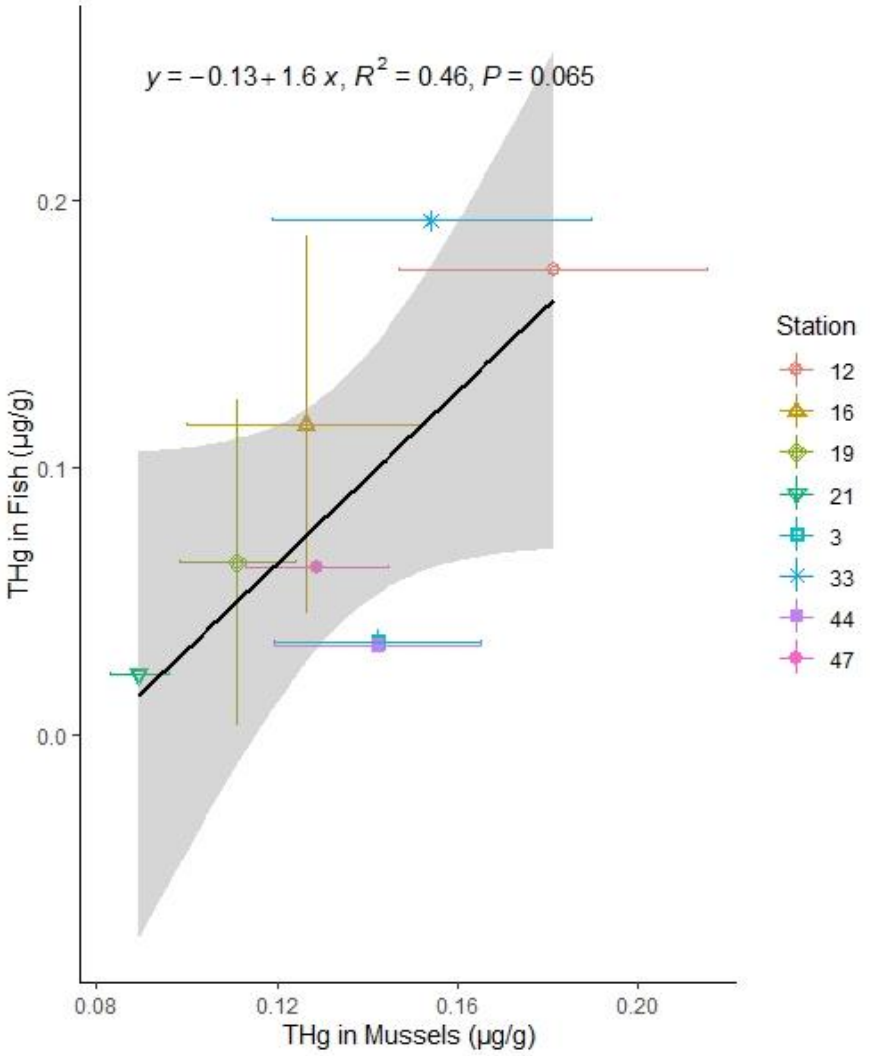

Figure 3.2. The relationship between mussel total mercury (THg) from coastal sampling locations (data from Cossa and Tabard (2020)) and THg in regurgitated fish samples caught close to those stations (this study). The 95% confidence interval is shaded in grey. Four species of fish are included in our study: sandlance (*Ammodytes* spp.), capelin (*Mallotus villosus*), Atlantic mackerel (*Scomber scombrus*), and rockfish (*Sebastes* spp.). Pelagic samples (>5km from the coast) were excluded from these analyses due to the coastal nature of the mussel data.

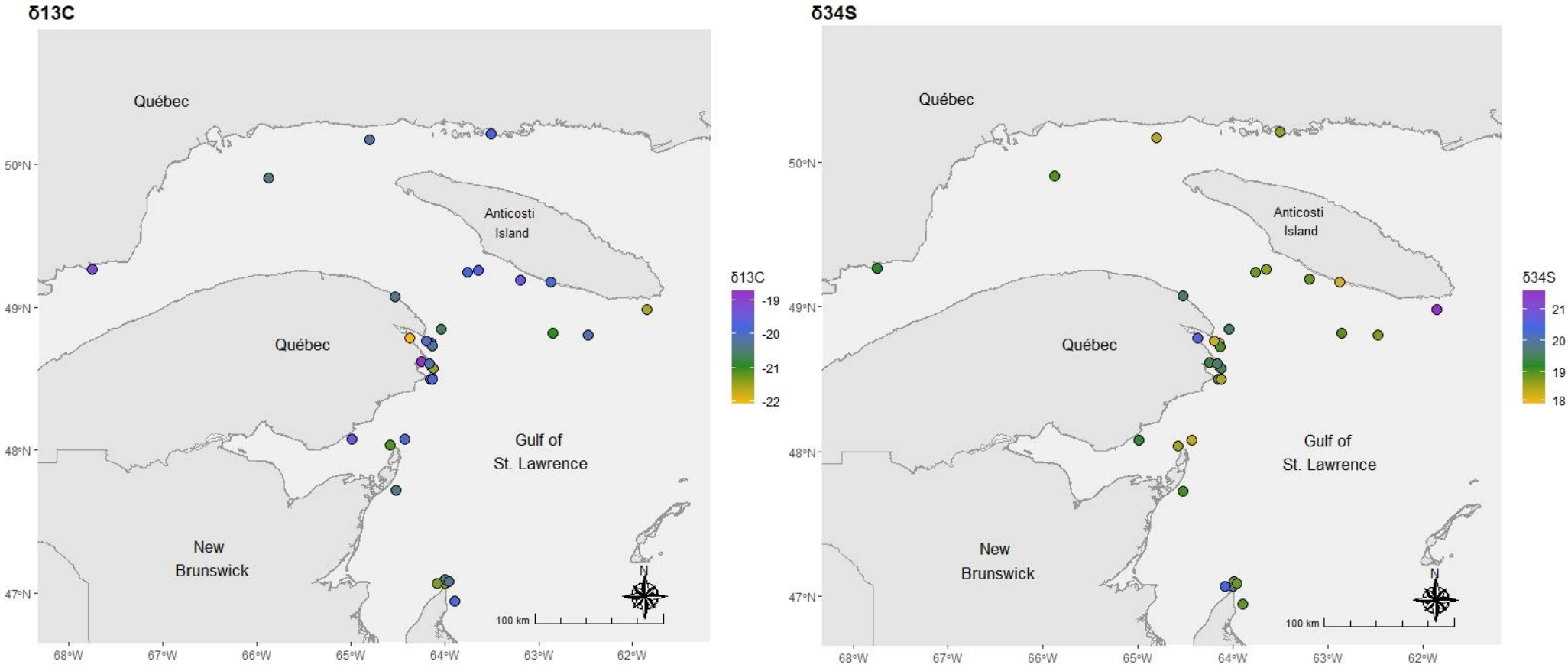

Figure 3.3. Values of $\delta^{13}C$ and $\delta^{34}C$ for each regurgitated fish sample according to the mean foraging location of northern gannets in the trip directly preceding the regurgitation. Species of fish include sandlance (*Ammodytes* spp.), capelin (*Mallotus villosus*), Atlantic mackerel (*Clupea harengus*), and rockfish (*Sebastes* spp.). Atlantic Herring (n = 1) was not included due to small sample size.

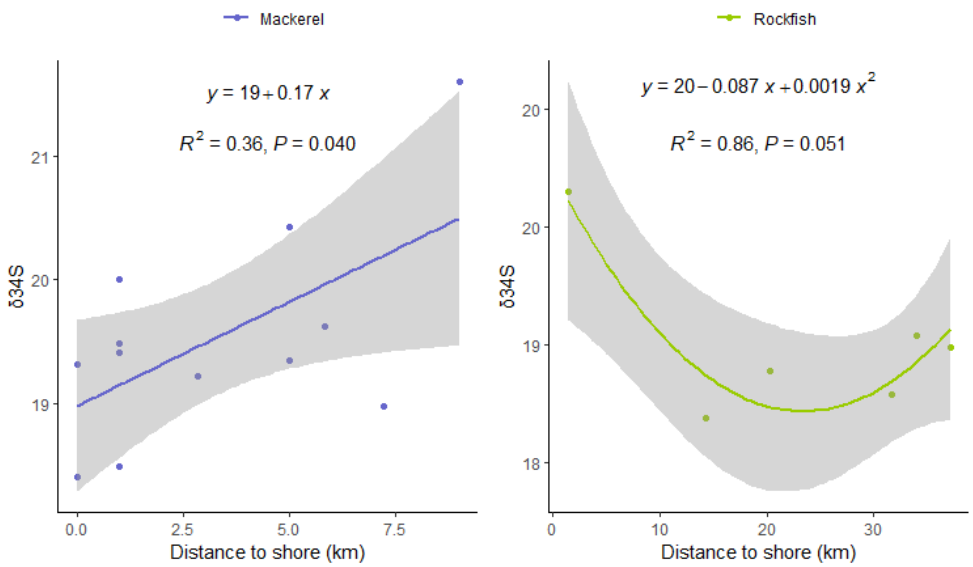

Figure 3.4. $\delta^{34}S$ signatures in fish muscle in relation to the distance of their catch location from shore. Note the different axes. Significant relationships are shown by the trendlines and 95% confidence interval (shaded in grey) for Atlantic mackerel (*Scomber scombrus*) and rockfish (*Sebastes spp.*) Capelin are not shown as no significant relationships were observed. Sandlance (n = 3) and Atlantic herring (n = 1) were excluded due to low sample sizes.

7. Supplemental Materials

Table S3.1. Akaike Information Criterion for small sample sizes (AICc) linear model selection for samples grouped into Coastal (<5 km from shore) and Pelagic. Models within ΔAIC ≤ 2 are bolded.

Model	AICc	ΔAIC	AICc Weight	Cumulative Weight
Coastal Samples				
log(THg) ~ Size Class + TP_{AA}	**-19.5**	**0.00**	**0.52**	**0.52**
log(THg) ~ Size Class * TP_{AA}	**-19.2**	**0.30**	**0.44**	**0.97**
log(THg) ~ Size Class	-13.9	5.60	0.03	1.00
log(THg) ~ TP_{AA}	-7.3	12.2	0.00	1.00
log(THg) ~ TP_{AA} + $\delta^{13}C$ + $\delta^{34}S$	-1.0	18.5	0.00	1.00
log(THg) ~ Coastal Dev.	11.8	31.3	0.00	1.00
log(THg) ~ $\delta^{13}C$	16.1	35.6	0.00	1.00
log(THg) ~ 1	16.7	36.2	0.00	1.00
log(THg) ~ Shipping * Coastal Dev.	17.9	37.4	0.00	1.00
log(THg) ~ $\delta^{34}S$	19.2	38.7	0.00	1.00
log(THg) ~ Shipping	19.6	39.1	0.00	1.00
log(THg) ~ $\delta^{34}S$ * $\delta^{13}C$	23.1	42.6	0.00	1.00
log(THg) ~ Organic Poll. + Inorganic Poll. + Marine Poll. + Shipping	28.7	48.2	0.00	1.00
log(THg) ~ Coastal Dev. + Organic Poll. + Inorganic Poll. + Marine Poll. + Shipping	42.5	62.0	0.00	1.00
log(THg) ~ Organic Poll. + Inorganic Poll. + Marine Poll.	47.1	66.5	0.00	1.00
Pelagic Samples				
log(THg) ~ Size Class	**0.00**	**0.00**	**0.50**	**0.50**
log(THg) ~ 1	2.43	2.43	0.15	0.65
log(THg) ~ Marine Poll.	3.39	3.39	0.09	0.74
log(THg) ~ Size Class + TP_{AA}	3.57	3.57	0.08	0.83
log(THg) ~ Shipping	4.71	4.71	0.05	0.87
log(THg) ~ $\delta^{13}C$	4.95	4.95	0.04	0.92
log(THg) ~ $\delta^{34}S$	5.74	5.74	0.03	0.94
log(THg) ~ TP_{AA}	5.74	5.74	0.03	0.97
log(THg) ~ Size Class * TP_{AA}	7.19	7.19	0.01	0.99
log(THg) ~ Marine Poll. + Shipping	7.57	7.57	0.01	1.00
log(THg) ~ TP_{AA} + $\delta^{13}C$ + $\delta^{34}S$	13.14	13.14	0.00	1.00
log(THg) ~ $\delta^{34}S$ * $\delta^{13}C$	13.14	13.14	0.00	1.00
log(THg) ~ TP_{AA} + $\delta^{13}C$ + $\delta^{34}S$ + Marine Poll. + Shipping	29.27	29.27	0.00	1.00

Table S3.2. Akaike Information Criterion for small sample sizes (AICc) linear mixed effects model selection for all samples. Models within ΔAIC ≤ 2 are bolded.

Model	AICc	ΔAIC	AICc Weight	Cumulative Weight
log(THg) ~ Size Class + TP_{AA}	**2.80**	**0.00**	**0.48**	**0.48**
log(THg) ~ Size Class	**3.65**	**0.84**	**0.32**	**0.80**
log(THg) ~ Size Class * TP_{AA}	5.03	2.22	0.16	0.96
log(THg) ~ TP_{AA} + (1| Size Class)	9.95	7.14	0.01	0.97
log(THg) ~ Shipping + (1 | Size Class)	10.29	7.49	0.01	0.98
log(THg) ~ $\delta^{13}C$ + $\delta^{34}S$ + (1 | Size Class)	10.40	7.59	0.01	0.99
log(THg) ~ TP_{AA} + $\delta^{13}C$ + $\delta^{34}S$ + (1 | Size Class)	11.36	8.55	0.01	1.00
log(THg) ~ Region + (1 | Size Class)	23.40	20.60	0.00	1.00
log(THg) ~ 1	25.83	23.02	0.00	1.00
log(THg) ~ Shipping + Region + Marine Poll. + (1 | Size Class)	27.10	24.29	0.00	1.00
log(THg) ~ Shipping * Region + (1 | Size Class)	36.91	34.10	0.00	1.00

Table S3.3. Akaike Information Criterion for small sample sizes (AICc) model selection for samples grouped into small (capelin and sandlance) and large (Atlantic mackerel and Sebastes) species classes. Models within $\Delta AIC \leq 2$ are bolded.

Model	AICc	ΔAICc	AICc Weight	Cumulative Weight
Small Species				
log(THg) ~ TP_{AA}	**-17.42**	**0.00**	**0.78**	**0.78**
log(THg) ~ $\delta^{34}S$	-12.93	4.49	0.08	0.86
log(THg) ~ TP_{AA} + $\delta^{13}C$ + $\delta^{34}S$	-12.83	4.60	0.08	0.94
log(THg) ~ 1	-11.10	6.32	0.03	0.97
log(THg) ~ $\delta^{13}C$	-9.77	7.65	0.02	0.99
log(THg) ~ Shipping	-8.51	8.91	0.01	1.00
log(THg) ~ Longitude + Latitude	-6.42	11.00	0.00	1.00
log(THg) ~ $\delta^{13}C$ * $\delta^{34}S$	-5.11	12.31	0.00	1.00
log(THg) ~ Organic Poll. + Inorganic Poll. + Marine Poll. + Shipping	2.35	19.77	0.00	1.00
log(THg) ~ Distance to shore + Region	10.56	27.98	0.00	1.00
log(THg) ~ Organic Poll. + Inorganic Poll. + Marine Poll.	72.04	89.46	0.00	1.00
Large Species				
log(THg) ~ $\delta^{13}C$	**10.20**	**0.00**	**0.26**	**0.26**
log(THg) ~ Shipping	**10.43**	**0.23**	**0.24**	**0.50**
log(THg) ~ 1	**10.78**	**0.58**	**0.20**	**0.70**
log(THg) ~ $\delta^{34}S$	**11.20**	**1.00**	**0.16**	**0.86**
log(THg) ~ TP_{AA}	13.18	2.98	0.06	0.92
log(THg) ~ Organic Poll. + Inorganic Poll. + Marine Poll. + Shipping	14.34	4.14	0.03	0.95
log(THg) ~ Longitude + Latitude	14.41	4.21	0.03	0.98
log(THg) ~ TP_{AA} + $\delta^{13}C$ + $\delta^{34}S$	16.75	6.55	0.01	0.99
log(THg) ~ $\delta^{13}C$ * $\delta^{34}S$	17.40	7.19	0.01	1.00
log(THg) ~ Distance to shore + Region	30.82	20.62	0.00	1.00
log(THg) ~ Organic Poll. + Inorganic Poll. + Marine Poll.	76.91	66.71	0.00	1.00

GENERAL DISCUSSION

The Gulf of St. Lawrence is a large and valuable ecosystem in Atlantic Canada due to it's ecological, cultural, and economic value. The Gulf is rich in biodiversity, but it also experiences heavy anthropogenic activities, such as shipping traffic, which can introduce contaminants into the environment. Contaminants, such as mercury (Hg), pose risks to the health of wildlife and can have long residency times in aquatic ecosystems (Gworek et al. 2016). The effects of Hg contamination can have far-reaching consequences for wildlife populations (Wolfe et al. 1998, Scheuhammer et al. 2007, Zheng et al. 2019). It is, therefore, crucial to monitor and go further to understand how Hg behaves in the environment, is being transported, and biomagnifies through food webs to contaminate organisms.

The main objective of this thesis was to understand how the food-web dynamics and spatial distribution of mercury influences the contamination of wildlife in the Gulf of St. Lawrence. In Chapter 2, I evaluated and compared biomagnification metrics describing the transfer of mercury through food webs by using traditional and new methods of stable isotope analyses (SIA) in the fish-to-gannet food web. This chapter highlighted the important discrepancies in trophic magnification factors obtained using bulk and compound-specific methods. Discrepancies of that magnitude have not yet been reported in the literature. In Chapter 3, I investigated the drivers of spatial variability of mercury concentrations across the Gulf of St. Laurence using regurgitated fish samples collected from GPS-tracked gannets. I found evidence for consistent spatial variation in THg concentrations across trophic levels in the Gulf, and that THg in large fish species was higher in coastal than pelagic environments.

In Chapter 2, I demonstrated the discrepancies that came from using different analytical methods to evaluate the extent of biomagnification of Hg through the fish-to-northern gannet (*Morus bassanus*) food web in the Gulf of St. Lawrence. The traditional method (bulk SIA) produced an estimated trophic magnification factor (TMF) of 42.7, one of the highest values ever reported in the literature. Further, the bulk TMF was nine-fold the value of the only other TMF reported for fish and seabirds in the Gulf of St. Lawrence (4.55; Lavoie et al. 2010). The compound-specific stable isotope analysis in amino acids (CSIA-AA) equations produced much milder estimates of trophic biomagnification, with the values of three tested equations ranging from 10.0 to 18.8. TMFs provided insight into the rate at which Hg concentrations multiply between each trophic step. It is important to note that because Hg concentrations are multiplied by the TMF at each

trophic step, Hg is magnified exponentially. Thus, such a large discrepancy between TMFs produced from the two analytical approaches (bulk SIA and CSIA-AA) entail drastically different build-ups of Hg burdens in top predators, the end members of the food web. Using hypothetical baseline values of 0.01 μg/g in primary producers in a five trophic level food web, the bulk SIA TMF would produce top-level concentrations of 31 117 μg/g, while the lowest CSIA-AA TMF would produce concentrations of 100 μg/g, a 311-fold difference.

One potential explanation of these major discrepancies is that because multiple food webs were sampled in my study, as gannets travel far throughout the gulf to forage, the bulk SIA method could not account for differences in baselines. Varying baselines could artificially increase trophic position estimates of our species, thus changing the slope of the relationship, and consequently inflating the TMF estimate. Thus, my study suggests that CSIA-AA may be essential to studying large, dynamic ecosystems like the Gulf of St. Lawrence.

One important consideration is that our TMF estimates only included the few top levels of consumers in the gannet food web. Had we been able to incorporate organisms from lower trophic levels (phytoplankton, zooplankton, crustaceans, etc.), the slope of the relationship, a determining factor of the TMF, may not have been so steep. This is likely because of the exponential nature of biomagnification. By only testing the top few trophic levels, we may have sampled from the steeper end of the exponential curve of the food web. For example, when looking at the entire food web in the Gulf of St. Lawrence, Lavoie et al. (2010) reported a TMF for THg of 3.81. However, when we remove all organisms from that study other than fish and seabirds, and recalculate the TMF, we obtain a TMF equal to 4.55. The inclusion of lower trophic levels is necessary to get a more accurate estimate of trophic magnification factors (Foster et al. 2012). However, there is evidence of primary consumers (zooplankton) having highly variable THg concentrations, and accounting for baseline variability is essential, either by using CSIA-AA or by sampling primary consumers in different locations and times.

These considerations are important when studying the transfer of mercury through the environment, to fully understand how wildlife are exposed to Hg. It is also relevant to consider these contaminant transfers when we also factor in the spatial differences in THg burdens at the base of the food chain. A habitat with higher baseline THg values would ultimately have exponentially higher THg burdens in top predators, through the process of biomagnification. The

existence of such “hotspots” has been suggested in the past and identified as a potential determinant of THg in fish (Fry and Chumchal 2012). Chapter 3 focused on the differences in THg concentrations between fish while considering species size but relating these spatial differences in baseline THg values is important to obtain a more holistic view of Hg dynamics in the Gulf of St. Lawrence.

I found that habitat was a predictor of THg concentrations in large fish. Inshore fish had higher THg burdens than offshore ones when using $\delta^{13}C$ as an indicator of fish foraging habitat. This is unsurprising considering how disturbed inshore habitats are by anthropogenic activities (Mayer et al. 1991, Amos et al. 2014). Further, inshore habitats are more exposed to river effluents which can carry important quantities of mercury into the environment (Liu et al. 2021). It is interesting that when I classified fish as either coastal (<5km from the coast) or pelagic, based on the distance to shore, the best explanatory factor of THg concentrations was size class. This was also the case when I pooled all samples together to see if there was an overarching trend, which indicated that to gain insight into spatial trends of contamination, the sampled individuals should be from a similar trophic position.

I also found evidence of consistent spatial variability when the fish THg concentrations were compared to published concentrations of THg in marine mussels (*Mytilus* spp.) from Cossa and Tabard (2020). The positive relationship between the two datasets supports the idea that spatial differences in THg are not necessarily consistent within a region. Rather, the consistency was present when coastal fish catch locations were paired with the nearest mussel sampling location. Mine and Cossa and Tabard’s study are the only two to examine the distribution of THg in the Gulf of St. Lawrence and the correspondence between our results is compelling evidence that there is indeed a spatial variation of mercury within the Gulf.

Another important aspect of my thesis is the use of northern gannets as sampling units to collect fish. Equipping wildlife with biologging technologies is common, but rarely have studies used regurgitated fish samples to analyze contaminants. The only other study I am aware of that has done this was recently conducted on Peruvian boobies (*Sula variegate*) and tested the regurgitated Peruvian anchovy samples for THg and stable isotopes of $\delta^{15}N$ and $\delta^{13}C$, as I did (Le Croizier et al. 2022). The sampling of regurgitations does require a study species that can do so. Sulids are suited for this, as they regurgitate to feed their nestling or offload weight when

feeling threatened (Barrett et al. 2007). Further, the regurgitations are an efficient way to sample fish and are often easily identified and dissected, provided they are not too digested.

Gannets from Bonaventure Island offer up a unique opportunity to sample a diverse set of habitats, over their large foraging range, which encompasses more than half of the Gulf of St. Lawrence (Guillemette et al. 2018). However, the tools we use to study these seabirds must be appropriate to the research question. This much was evident in Chapter 3, where some foraging locations were likely missed due to a longer GPS fix, and the lack of time-depth recorders decreased the resolution of foraging locations. Our methods for Chapter 3 were opportunistic, and I used samples that were previously collected at the Bonaventure Island gannet colony, meaning that the sampling design could not be modified. For studies wishing to use or improve upon this methodology, I recommend using settings on the biologging devices that can record behaviours on a finer scale. In addition, the pairing of accelerometers or time depth recorders could identify catch locations more easily and strengthen studies by removing inherent uncertainty incorporated into behavioural classification models.

Another way I believe my research could have been improved would have been by sampling gannet blood and relating the THg concentrations in the plasma to stable isotope values (representing a short-term diet Hobson and Clark 1993). This would have offered insight into another taxa, to see if the trends we observed in the fish also occurred in gannets. Further, because we know more about each GPS-tracked gannet's foraging behaviour than we do the fish's, we may have been able to give more weight to our findings. A similar study to mine conducted on Leach's storm petrels (*Hydrobates leucorhous*) in the North Atlantic tracked the seabirds and was able to relate foraging habits (latitude and depth) to the THg concentrations in whole blood across many breeding colonies (Pollet et al. 2022). The use of plasma samples in Chapter 3 would have added an interesting second taxon to the study and provided more insight into an important indicator species in the Gulf.

Future studies that wish to use seabirds as samplers could expand on my methodology and adapt it to their study species. Other fish-carrying species may offer an even better opportunity to sample intact, relatively fresh fish. For example, single-prey and bill-loading species, such as guillemots (*Uria* spp.; Barrett et al. 2007), equipped with an accelerometer or time-depth recorder and GPS would be excellent samplers, seeing as the single prey item would originate

from the last foraging area, and even likely from the last dive. A previous study effectively used thick-billed murres (*Uria lomvia*) to sample prey items and study determinants of THg in Arctic ecosystems (Góngora et al. 2018). Further, species with smaller foraging ranges would also help sample more points within a constrained area. For example, razorbills (*Alca torda*) or common murres (*Uria aalge*) studied in the Gulf of St. Lawrence had foraging ranges that were much more constrained than those of northern gannets; 5.3 km on average for razorbills and 18.4 km for common murres (Petalas et al. 2021). Seabirds such as these, equipped with appropriate biologging devices could efficiently sample a larger portion of their foraging grounds, likely in a shorter time frame than gannets.

Finally, analytical techniques used to study mercury contamination are just as important as the experimental design. CSIA-AA is relatively new, and most studies continue to use bulk SIA because of cost and accessibility barriers, as few labs have the expertise to do CSIA-AA. However, my thesis has demonstrated that the use of CSIA-AA is essential to conducting spatial analyses of contaminants and may be important to biomagnification studies conducted in large ecosystems. However, much is still unknown about the method. Future research on this approach should conduct more controlled studies on food webs. For example, controlled studies of biomagnification of contaminants in laboratories should be conducted to help determine which method, bulk SIA or CSIA-AA more accurately describes biomagnification. Some crucial studies have already been performed to determine how $\delta^{15}N$ values in amino acids change between trophic levels (Chikaraishi et al. 2009, Ohkouchi et al. 2017), but more studies are needed on a variety of taxa, especially on predatory animals. To date, most CSIA-AA studies focus on low-trophic positioned animals (McClelland and Montoya 2002, Chikaraishi et al. 2009, Chikaraishi et al. 2014, Ohkouchi et al. 2017). Further, studies seeking to validate the use of CSIA-AA with different sampling techniques (e.g. Barst et al. 2021) should continue to be conducted. Overall, CSIA-AA opens many possibilities for new studies and should continue to be developed.

GENERAL CONCLUSION & SUMMARY

My thesis aimed to understand how the food-web dynamics and spatial distribution of mercury influences the contamination of wildlife in the Gulf of St. Lawrence. Chapter 2 examined the influence of the analytical method (bulk versus compound-specific stable isotope analysis in amino acids) on the outcome of calculated biomagnification metrics. The difference between the two approaches was four-fold in my study, which is enormous considering that biomagnification factors describe exponentially increasing contaminant burdens in food webs, leading to 311 times the concentrations in top-level organisms in a hypothetical five trophic level food web. This has important implications for other biomagnification studies, which may be improperly assessing the rate of biomagnification when only using bulk stable isotope analysis. Chapter 3 investigated the determinants of spatial variability of THg concentrations at different locations across the Gulf of St. Lawrence using regurgitated fish sampled from GPS-tracked northern gannets. I found that within large fish species, THg was related to the fish's foraging habitat: THg increased in inshore habitats. I also found a correlation between THg concentrations in marine mussels and the gannet regurgitations that were sampled closest to the mussel sampling stations. These results indicate that spatial variation in THg across taxa is present in the Gulf of St. Lawrence and should be accounted for in future studies by sampling baseline values, either by using compound-specific stable isotope analysis in amino acids or by sampling primary consumers in different locations. The latter may, however, be impossible given the spatial scale of the Gulf. Combined, these two chapters demonstrate the importance of accounting for baseline differences in stable isotopes when studying the biomagnification and spatial distribution of mercury. Overall, I combined ecological methods, such as biologging and the use of seabirds as sampling units, and ecotoxicological methods, CSIA-AA and bulk SIA to answer important questions about mercury contamination in the Gulf of St. Lawrence. My thesis contributes important information that is critical in bridging the knowledge gap surrounding the accumulation of mercury in wildlife.

Northern gannets at the Bonaventure Island colony. Photo by Roxane Turgeon © 2020

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