Conservation physiology of Arctic cetaceans: Using endocrine techniques to measure stress in beluga and bowhead whales in the Canadian Arctic

By

Justine M. Hudson

A Thesis submitted to the Faculty of Graduate Studies of

The University of Manitoba

in partial fulfillment of the requirement of the degree of

MASTER OF SCIENCE

Department of Biology Sciences

University of Manitoba

Winnipeg, Manitoba, Canada

2019

ABSTRACT

Conservation physiology is a field of study that focuses on physiological changes in response to human activity and environmental change, including elevations in stress hormones, changes in metabolism, and declines in reproductive hormones, body condition and immune function. Physiological changes such as these can indicate environmental pressures and be used to monitor the health of wildlife populations. In this study, I analyzed endocrine data of two Arctic cetaceans, the Western Hudson Bay beluga population and the Eastern Canada-West Greenland bowhead whale population to better understand how they may respond to climate change. In Chapter 2, I demonstrated, for the first time, that it was possible to collect blow samples from free-swimming beluga whales and that cortisol and urea concentrations could be reliably measured in beluga blow samples. Urea was found to be an unsuitable dilution marker to normalize blow samples. Absolute cortisol concentrations were influenced by sample device, quantity rating, and age class, while urea concentrations were only influenced by sample device. In Chapter 3, I determined baseline triiodothyronine (T3) and corticosterone concentrations measured from baleen plates collected from bowhead whales and conservatively concluded that bowhead whales foraged year-round, based on a lack of observed seasonal variation in T3 and a lack of correlation between T3 and stable isotope values. Interestingly, we found a strong positive correlation between T3 and corticosterone in each whale, which requires further investigation. This research provides important baseline information about Arctic marine mammal endocrinology and establishes key recommendations for carrying out physiological studies.

ACKNOWLEDGEMENTS

I would like to thank my supervisor Dr. Marianne Marcoux for her unwavering support and guidance throughout this project. I cannot thank you enough for your amazing mentorship and the incredible opportunities that you have given me throughout this process. I would also like to thank my committee members Dr. Gary Anderson and Dr. Sabine Kuss for their support and expertise. I am very grateful to Dr. Cory Matthews for the countless hours of mentorship and feedback, Dr. Saman Muthukumarana for taking the time to answer all of my statistical questions, and Dr. Steve Ferguson for financial support that allowed me to attend conferences.

This project could not have been completed without support from the community of Churchill, Manitoba. I would like to thank the Churchill Northern Studies Centre, not only for their financial support, but also for allowing me to conduct my research out of such an amazing facility. Thank you to all of the guides from SeaNorth Tours – Remi, Jocelyn, Tiffany, Alex, and Julie, you are some of the hardest working people that I have ever met and my summers in Churchill would not have been the same without you.

I would like thank funders that provided financial support for this project, including Fisheries and Oceans Canada (DFO), the University of Manitoba, the Churchill Northern Studies Centre, the International Society of Wildlife Endocrinology, NSERC, and ArcticNet.

I feel extremely lucky to have been surrounded by such an amazing group of people during my master's degree. To my friends at Fisheries and Oceans Canada – Claire, Kate, Karyn, Cass, Wesley, Kevin, Kyle, Lauren, Cody, Elizabeth, and Shu, thank you for making my graduate experience so enjoyable. I would also like to personally thank Karyn Booy for being the best field assistant anyone could ask for. Thank you for forcing me to get out of bed every Saturday

for trivia night and making sure that I worked on my thesis instead of binging shows on Netflix. I would also like to thank Denise Tenkula, Dr. Valeria Vergara, and Dr. Lianne Postma for their support in the field.

Lastly, thank you to my family: Mom, Dad, Mercedes, and Nana – thank you for the emotional and financial support, I could not have done this without you. To my partner Carlos, thank you for always being my biggest cheerleader and making the last year of my master's so memorable.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES AND ILLUSTRATIONS

CHAPTER 1: GENERAL INTRODUCTION

1.1 BACKGROUND

Over the last 100 years, surface temperatures in the Arctic have increased at almost double the rate of the global average and are predicted to increase by 2-9°C by 2100 (Solomon et al. 2007). As a result, Arctic sea ice has decreased by 3.5 to 4.1% or the equivalent of 0.45 to 0.51 million km^2 per decade and the Arctic Ocean is predicted to be nearly ice-free by 2050 (Allen et al. 2013). Other consequences of climate warming include changes in sea level, ocean circulation patterns, pH, precipitation, and salinity, and increases in storm frequency and intensity (Tynan and Demaster 1997; Learmonth et al. 2006; IPCC 2007). Although temperatures in the Arctic are increasing at twice the rate of the global average (Solomon et al. 2007), little is known as to how the ecosystem will be impacted.

The consequences of climate change may have direct or indirect effects on Arctic organisms (Tynan and Demaster 1997; Forchhammer and Post 2004; Learmonth et al. 2006; Laidre et al. 2008). Direct effects are defined as conditions that directly impact an individual (i.e. a single trophic level), in terms of survival and fitness; whereas, indirect effects impact an individual's environment (i.e. multiple trophic levels), which make them more complex and difficult to study (Tynan and Demaster 1997; Forchhammer and Post 2004; Laidre et al. 2008). For example, climate change has directly caused the advancement of caterpillar phenology, which in turn has indirectly caused a reduction in pied flycatcher (*Ficedula hypoleuca*) populations, as the timing of the pied flycatcher migration no longer coincides with peak caterpillar abundance (Both et al. 2006).

In the Arctic, loss of sea ice is correlated with an increase in primary production (Arrigo et al. 2008; Mundy et al. 2009) and northward migrations of temporal species (Fleischer et al. 2007). This has altered the diet of thick-billed murres (*Uria lomvia*) in the Hudson Bay region as there has been a steady decrease of Arctic cod (*Boreogadus saida*) and an increase of capelin (*Mallotus villosus*) and sandlance (*Ammodytes* spp.) (Gaston et al. 2012). In the Brunnich's guillemot (*Uria lomvia L.*), reductions of summer sea ice has resulted in the advancement of egg-laying and a reduction in chick growth rates and low body condition in adults (Gaston et al. 2005). In marine mammals, earlier break-up of Arctic sea ice is associated with poorer body condition of polar bears (*Ursus maritimus*) (Stirling et al. 1999). While, warm and wet weather (a consequence of climate change) is associated with weakened ringed seal (*Pusa hispida*) subnivean lairs, leaving pups exposed to the elements and increased predation by polar bears (Stirling and Smith 2004). Arctic marine mammals are also expected to experience changes in prey distribution, higher incidences of disease, and increased pollution, shipping, and human activity as a result of climate change (Tynan and Demaster 1997; Learmonth et al. 2006; Anisimov et al. 2007; Burek et al. 2008).

Although Arctic marine mammals are adapted to an environment that experiences high seasonal and inter-annual variation, long-term unidirectional change that leads to permanent alterations in habitat would make it challenging for some marine mammals to adapt (Laidre et al. 2008). The behaviours, life histories, and foraging patterns of Arctic marine mammals are synced to ecological conditions occurring during specific times of the year (Laidre et al. 2008) and alterations of these conditions can have dire impacts on individuals and populations.

There are a number of different ways researchers can study the impacts of climate change on wildlife and one field that is gaining popularity is conservation physiology. Conservation physiology is a field of study that focuses on the physiological impacts of environmental change and human activity on wildlife populations (Wikelski and Cooke 2006; Cooke et al. 2013). Changes in physiology, such as elevations in stress hormones, declines in reproductive hormones and body condition, changes in metabolism, and decreased immune function, can all be used as indicators to monitor the occurrence and severity of environmental change (Wikelski and Cooke 2006; Cooke and O'Connor 2010; Hunt et al. 2013). Measuring glucocorticoids is one of the most common tools to assess environmental stressors (Wikelski and Cooke 2006). When an animal encounters a stressor, the hypothalamic-pituitary-adrenal (HPA) axis becomes activated, resulting in the release of glucocorticoids (GCs), such as cortisol and corticosterone (Wikelski and Cooke 2006; Sheriff et al. 2011). The HPA axis allows animals to cope with biological pressures (i.e. mating, migrating, and foraging) and environmental pressures (i.e. predation, weather, climate, relocation, and habitat disturbance) (Boonstra et al. 2001; Sheriff et al. 2011). Acute elevations in GCs (hours to days) are considered to be an adaptive trait, allowing an animal to survive life-threatening circumstances, while chronic stress (days to weeks), which results in prolonged activation of the HPA axis, can have detrimental effects, including infertility, inhibited growth, heart disease, and decreased immune function (Wingfield and Kitaysky 2002; Sheriff et al. 2011).

Thyroid hormones (THs; triiodothyronine, T3 and thyroxine, T4) play an important role in vertebrate metabolism, thermoregulation, reproduction, and growth (Wasser et al. 2010; Behringer et al. 2018). Many studies have focused on the role that THs play in metabolism, as

THs decrease when some organisms experience nutritional stress, which slows metabolism and allows the organism to conserve energy (Wasser et al. 2010). In humans, for example, acute and chronic fasting (i.e. low caloric intake) resulted in decreased TH levels and reduced metabolic rate (Fontana et al. 2006), whereas obesity (i.e. high caloric intake) resulted in increased TH levels (Reinehr and Andler 2002; Michalaki et al. 2006). The same pattern has been observed in non-human vertebrates (Harris et al. 1987; Ayres et al. 2012; Gobush et al. 2014; Schaebs et al. 2016).

Several studies have highlighted the importance of conservation physiology, for example, following the events of 9/11, there was a reduction in shipping traffic in the Bay of Fundy, Canada, which resulted in a significant decrease in underwater noise (Rolland et al. 2012). This reduction in noise pollution was associated with a decrease in baseline glucocorticoid levels (elevated levels of glucocorticoids indicate stress) in North Atlantic right whales (*Eubalaena glacialis*; Rolland et al. 2012). This result indicated that ship noise may cause long-term stress in baleen whales. Scheuerlein *et al.* (2001) found that stonechat (*Saxicola torquata axillaris*) males in territories where shrikes (*Lanius collaris*; a predator of stonechats) were present had higher baseline corticosterone levels and lower body condition than male stonechats in areas without shrikes, indicating that predators can act as stressors (Scheuerlein et al. 2001). By measuring glucocorticoids and thyroid hormones, Ayres *et al.* (2012) found that a decrease in salmon availability was having a physiological impact on the southern resident killer whale population (*Orcinus orca*; Ayres et al. 2012).

Understanding the relationship between physiology and fitness can help conservation managers monitor and predict future issues (Wikelski and Cooke 2006). For example, a minor

oil spill in the Galapagos Islands resulted in an increase in corticosterone levels in Galapagos marine iguanas and was predicted to cause a major mortality event, which did eventually occur (Wikelski et al. 2002). Researchers were able to predict a mortality event because they had access to baseline corticosterone data before and after the oil spill; however in many cases, baseline data is not available or is insufficient to predict and monitor potential issues (Wikelski and Cooke 2006). The aim of this research was to gather baseline physiological data for two Arctic cetaceans to better understand the impacts of environmental change and human activity.

1.2 STUDY SPECIES AND LOCATIONS

Western Hudson Bay beluga population

Belugas have a circumpolar distribution in Arctic and sub-Arctic waters (COSEWIC 2016). There are 8 genetically distinct beluga populations in Canada: the Eastern Beaufort Sea; Eastern High Arctic-Baffin Bay; Cumberland Sound; Ungava Bay; Western Hudson Bay; Eastern Hudson Bay; St. Lawrence Estuary; and James Bay populations (Figure 1; COSEWIC 2016). The Western Hudson Bay (WHB) beluga population summers in the estuaries lining the Western coast of Hudson Bay, including the Churchill, Seal, and Nelson River estuaries (Matthews et al. 2016). During this time, the WHB beluga population makes up the largest aggregation of belugas in the world, with an estimated population of 54,000 individuals (Matthews et al. 2016). It is thought that belugas spend time in estuaries to calf, moult, feed, and for protection from predators (COSEWIC 2016). In the fall, the population migrates through Hudson Bay and overwinters in Hudson Strait (Matthews et al. 2016).

All populations of beluga are listed as endangered except for the Eastern Beaufort Sea population (COSEWIC 2016). The WHB beluga population is listed as a species of special concern by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) due to hunting pressures, and threats from shipping and development (COSEWIC 2004). The WHB population is harvested by Inuit throughout its range and between 2000-2015, on average, 300 whales were harvested annually (Matthews et al. 2016). The Port of Churchill, Canada's largest deep-water Arctic port also resides in Western Hudson Bay, and it is predicted that more ships will travel through the region as the ice-free season increases (Andrews et al. 2017). Beluga are also an important part of the economy in Churchill, drawing in tourists from all over the world to whale watch, swim, and kayak with the whales.

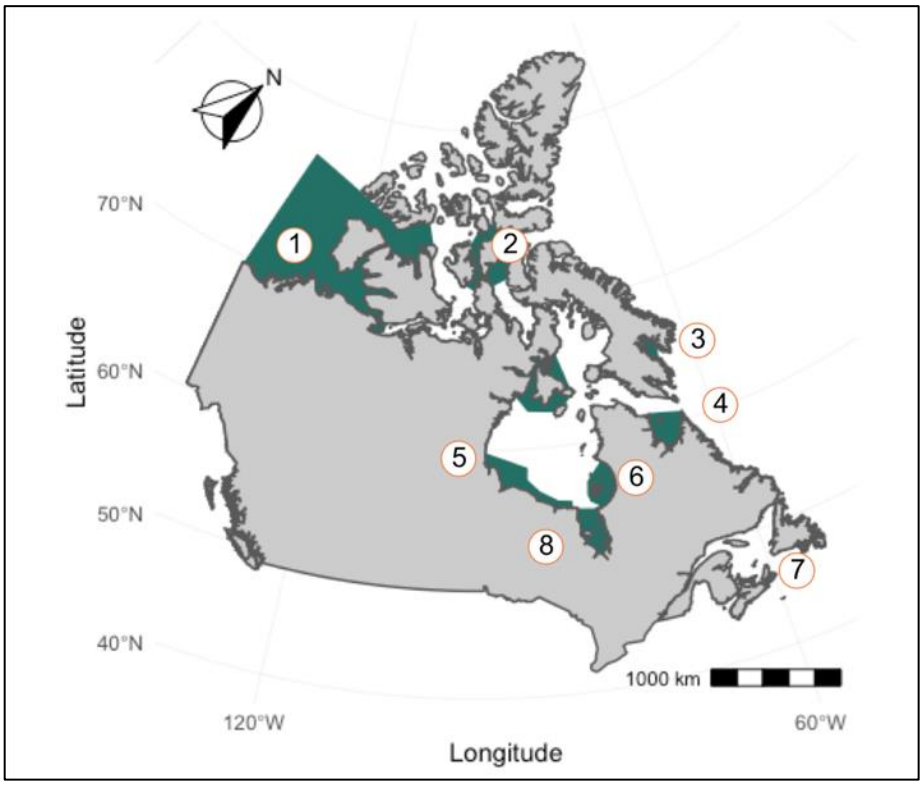

Figure 1.1. Map of eight genetically distinct beluga populations across the Canadian Arctic. (1) Eastern Beaufort Sea; (2) Eastern High Arctic-Baffin Bay; (3) Cumberland Sound; (4) Ungava Bay; (5) Western Hudson Bay; (6) Eastern Hudson Bay; (7) St. Lawrence Estuary; and (8) James Bay (COSEWIC 2016).

Eastern Canada- West Greenland bowhead whale population

The bowhead whale (*Balaena mysticetus*) has a nearly circumpolar distribution and is the only mysticete whale to spend the entire year in Arctic waters (COSEWIC 2009). There are two populations of bowhead whales in Canada: the Bering-Chukchi-Beaufort Sea (BCB) population and the Eastern Canada-West Greenland (EC-WG) population (COSEWIC 2009). The EC-WG population summers in Baffin Bay and the waters of the Canadian High Arctic, Foxe Basin, and northwestern Hudson Bay before migrating to their wintering grounds in northern Hudson Bay, Hudson and Davis Strait, southern Baffin Bay, and Disko Bay (Figure 2; Reeves et al. 1983; Postma et al. 2006; COSEWIC 2009).

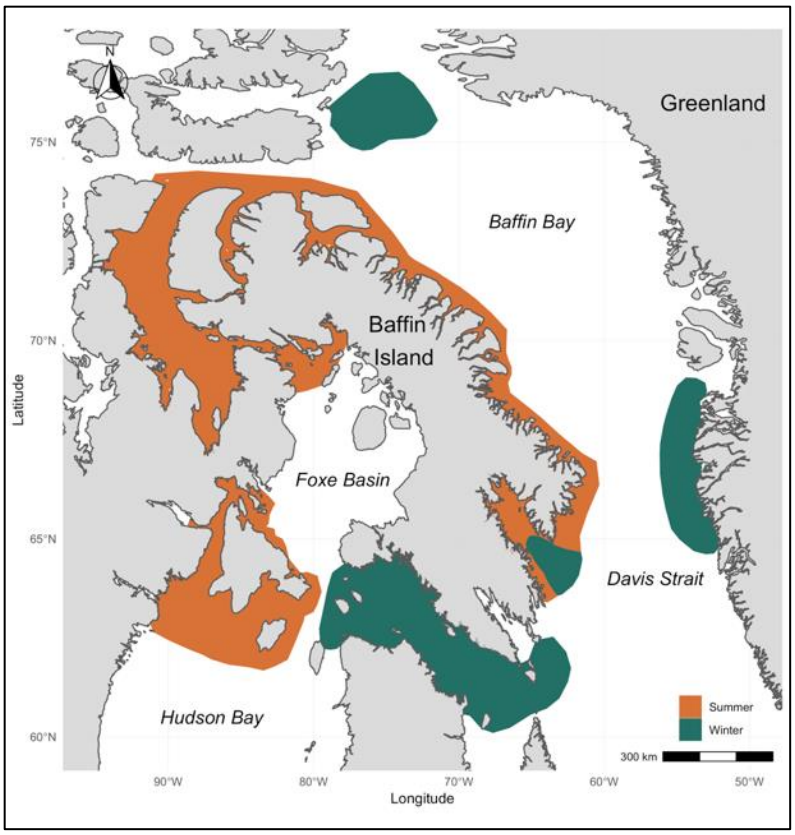

Figure 1.2. Map of summer and winter ranges of the Eastern Canada-West Greenland bowhead whale population (Matthews and Ferguson 2015).

Due to their large size and thick blubber layer, bowhead whales were a main target for commercial whaling in the 18th-20th centuries, which resulted in large population declines and eventually a population collapse (Reeves et al. 1983; Postma et al. 2006). The EC-WG population is currently listed as a species of special concern by COSEWIC due to threats from shipping, offshore development, and commercial fishing (COSEWIC 2009). It is not well understood how bowhead whales will respond to climate change. Previous studies have linked reductions in sea ice to higher body condition, most likely due to increased primary production (Laidre et al. 2008); however, other studies predict an increase in killer whale abundance in the Arctic, which are a predator of bowhead whales (George et al. 1994; Higdon et al. 2016).

Physiological studies of beluga and bowhead whales are limited. Diet (Kelley et al. 2010; Matthews and Ferguson 2015), genetic structure (De March and Postma 2003; Postma et al. 2006), and distribution (Reeves et al. 1983; Smith et al. 2017) have been studied in both populations; however, there is a lack of physiological data available. With growing concerns over climate change and anthropogenic activity within the Canadian Arctic, determining the impacts of environmental change on populations remains a priority.

1.3 THESIS OBJECTIVES

The proposed research aims to use a physiological approach to study two Arctic cetacean populations, the Western Hudson Bay beluga whale and the Eastern Canada-West Greenland bowhead whale. My thesis is divided into 2 studies with the following objectives:

1) Determine the feasibility of blow collection as a method to measure cortisol levels in the Western Hudson Bay beluga population
2) Assess the foraging ecology of Eastern Canada-West Greenland bowhead whales by measuring thyroid hormones along baleen plates

Study 1 aims to investigate the feasibility of blow collection as a method to measure cortisol in free-swimming beluga whales in the WHB population. The findings from this study will provide important information regarding the best methods to collect blow from free-swimming cetaceans and factors that influence cortisol levels. As a baseline study, this project will establish a foundation for future physiological studies and will be beneficial for researchers looking to collect blow samples in the future. Study 2 aims to assess the foraging ecology of the

EC-WG bowhead population, using an endocrine approach. The findings from this study will provide critical information regarding the foraging ecology of bowhead whales and can be used to identify and protect important foraging habitat.

1.4 THESIS STRUCTURE

This thesis is comprised of four chapters. Chapters 2 and Chapter 3 are written in manuscript style and include an Abstract, Introduction, Methods, Results, Discussion, Conclusion, Acknowledgements, and Literature Cited sections. Chapter Two addresses objective 1), which examines the feasibility of blow collection to measure baseline cortisol levels in the Western Hudson Bay beluga population. Chapter three addresses objective 2), which examines the foraging ecology of Eastern Canada-West Greenland bowhead whales by measuring thyroid hormones along baleen plates. Chapter 4 summarizes the main findings of both chapters. Furthermore, I discuss their implications to our understanding of Arctic whale physiology and how they can be used for conservation.

LITERATURE CITED

Allen SK, Bindoff NL, France FB, Cubasch U, Uk MRA, France OB, Hesselbjerg J, Denmark C, France PC, Uk MC, et al. 2013. Working Group I 2013: The Physical Science Basis.

Andrews J, Babb D, Barber DG. 2017. Climate change and sea ice: Shipping accessibility on the marine transportation corridor through Hudson Bay and Hudson Strait (1980-2014). Elementa 5:1–17.

Anisimov OA, Vaughan DG, Callaghan T, Furgal C, Marchant H, Prowse TD. 2007. Chapter 15. Polar regions (Arctic and Antarctic): Climate Change 2007: Impacts, Adaptation and

Vulnerability. Contrib. Work. Gr. II to Fourth Assessment Rep. Intergov. Panel Clim. Chang.:653–685.

Arrigo KR, van Dijken G, Pabi S. 2008. Impact of a shrinking Arctic ice cover on marine primary production. Geophys. Res. Lett. 35.

Ayres KL, Booth RK, Hempelmann JA, Koski KL, Emmons CK, Baird RW, Balcomb-Bartok K, Hanson MB, Ford MJ, Wasser SK. 2012. Distinguishing the Impacts of Inadequate Prey and Vessel Traffic on an Endangered Killer Whale (*Orcinus orca*) Population. PLoS One 7:e36842.

Behringer V, Deimel C, Hohmann G, Negrey J, Schaebs FS, Deschner T. 2018. Applications for non-invasive thyroid hormone measurements in mammalian ecology, growth, and maintenance. Horm. Behav. 105:66–85.

Boonstra R, Hubbs AH, Lacey EA, McColl CJ. 2001. Seasonal changes in glucocorticoid and testosterone concentrations in free-living arctic ground squirrels from the boreal forest of the Yukon. Can. J. Zool. 79:49–58.

Both C, Bouwhuis S, Lessells CM, Visser ME. 2006. Climate change and population declines in a long-distance migratory bird. Nature 441:81–83.

Burek KA, Gulland FMD, O'Hara TM. 2008. Effects of climate change on arctic marine mammal health. Ecol. Appl. 18:S126–S134.

Cooke SJ, O'Connor CM. 2010. Making conservation physiology relevant to policy makers and conservation practitioners. Conserv. Lett. 3:159–166.

Cooke SJ, Sack L, Franklin CE, Farrell AP, Beardall J, Wikelski M, Chown SL. 2013. What is conservation physiology? Perspectives on an increasingly integrated and essential science. Conserv. Physiol. 1

COSEWIC. 2004. COSEWIC assessment and update status report on the beluga whale *Delphinapterus leucas* in Canada. Comm. Status Endanger. Wildl. Canada Ottawa:ix + 70 pp.

COSEWIC. 2009. COSEWIC Assessment and Update Status Report Bowhead Whale *Balaena mysticetus,* Bering-Chukchi-Beaufort population and Eastern Canada-West Greenland population, in Canada. Comm. Status Endanger. Wildl. Canada. Ottawa. vii:49 pp.

COSEWIC. 2016. Designatable Units for Beluga Whales (*Delphinapterus leucas*) in Canada. Committee on the Status of Endangered Wildlife in Canada. Ottawa. 73 pp.

Fleischer D, Schaber M, Piepenburg D. 2007. Atlantic snake pipefish (*Entelurus aequoreus*) extends its northward distribution range to Svalbard (Arctic Ocean). Polar Biol. 30:1359–1362.

Fontana L, Klein S, Holloszy JO, Premachandra BN. 2006. Effect of Long-Term Calorie Restriction with Adequate Protein and Micronutrients on Thyroid Hormones. J. Clin. Endocrinol. Metab. 91:3232–3235.

Forchhammer MC, Post E. 2004. Using large-scale climate indices in climate change ecology studies. Popul. Ecol. 46:1–12.

Gaston AJ, Gilchrist HG, Hipfner JM. 2005. Climate change, ice conditions and reproduction in an Arctic nesting marine bird: Brunnich's guillemot (*Uria lomvia L.*). J. Anim. Ecol. 74:832–841.

Gaston AJ, Woo K, Hipfner JM, Stirling B. 2012. in Northern in Forage Hudson Trends Fish Populations Bay since 1981 , from Murres the Diet of Nestling *Uria lomvia* as Determined. 56:227–233.

George JC, Philo M, Hazard K, Withrow D, Carroll GM, Suydam R. 1994. Frequency of Killer Whale (*Orcinus orca)* attacks and ship collisions based on scarring on bowhead whales (*Balaena mysticetus*) of the Bering-Chukchi-Beaufort Seas stock. Science (80-.). 47:247–255.

Gobush KS, Booth RK, Wasser SK. 2014. Validation and application of non-invasive glucocorticoid and thyroid hormone measures in free-ranging Hawaiian monk seals. Gen. Comp. Endocrinol. 195:174–182.

Harris ARC, Fang S-L, Azizi F, Lipworth L, Vagenakis AG, Braverman LE. 1987. Effect of starvation on biochemical indices of renal function in the rat. Br. J. Exp. Pathol. 68:767–775.

Higdon JW, Ferguson SH, Higdon JW, Ferguson SH. 2016. Loss of Arctic Sea Ice Causing Punctuated Change in Sightings of Killer Whales (*Orcinus Orca*) over the Past Century. Ecol. Appl. 19:1365–1375.

Hunt KE, Moore MJ, Rolland RM, Kellar NM, Hall AJ, Kershaw J, Raverty SA, Davis CE, Yeates LC, Fauquier DA, et al. 2013. Overcoming the challenges of studying conservation physiology in large whales: a review of available methods. Conserv. Physiol. 1.

Kelley TC, Loseto LL, Stewart REA, Yurkowski M, Ferguson SH. 2010. Importance of Eating Capelin: Unique Dietary Habits of Hudson Bay Beluga. In: A Little Less Arctic. Dordrecht: Springer Netherlands. p. 53–70.

Laidre KL, Stirling I, Lowry LF, Wiig Ø, Heide-Jørgensen MP, Ferguson SH. 2008. Quantifying the sensitivity of arctic marine mammals to climate-induced habitat change. Ecol. Appl. 18:S97–S125.

Learmonth JA, MacLeod CD, Santos MB, Pierce GJ, Crick HQP, Robinson RA. 2006. Potential effects of climate change on marine mammals. Oceanogr. Mar. Biol. - an Annu. Rev. Vol 44 44:431–464.

De March BGE, Postma LD. 2003. Molecular Genetic Stock Discrimination of Belugas (*Delphinapterus leucas*) Hunted in Eastern Hudson Bay, Northern Quebec, Hudson Strait, and Sanikiluaq (Belcher Islands), Canada, and Comparisons to Adjacent Populations. Arctic 56:111–124.

Matthews CJD, Ferguson SH. 2015. Seasonal foraging behaviour of eastern Canada-West Greenland bowhead whales: An assessment of isotopic cycles along baleen. Mar. Ecol. Prog. Ser. 522:269–286.

Matthews CJD, Watt CA, Asselin NC, Dunn JB, Young BG, Montsion LM, Westdal KH, Hall PA, Orr JR, Ferguson SH, et al. 2016. Estimated abundance of the Western Hudson Bay beluga stock from the 2015 visual and photographic aerial survey. Fish. Ocean. Canada, Can. Sci. Advis. Secr. Res. Doc.

Michalaki MA, Vagenakis AG, Leonardou AS, Argentou MN, Habeos IG, Makri MG, Psyrogiannis AI, Kalfarentzos FE, Kyriazopoulou VE. 2006. Thyroid Function in Humans with Morbid Obesity. Thyroid 16:73–78.

Mundy CJ, Gosselin M, Ehn J, Gratton Y, Rossnagel A, Barber DG, Martin J, Tremblay JÉ, Palmer M, Arrigo KR, et al. 2009. Contribution of under-ice primary production to an ice-edge upwelling phytoplankton bloom in the Canadian Beaufort Sea. Geophys. Res. Lett. 36.

Postma LD, Dueck LP, Heide-Jorgensen MP, Cosens SE. 2006. Molecular genetic support of a single population of bowhead whales (*Balaena mysticetus*) in eastern Canadian Arctic and western Greenland waters. Can. Sci. Advis. Secr. Res. Doc. 2006/051. 19pp. 2006. 3848:22.

Reeves R, Mitchell E, Mansfield A, Mclaughlin M. 1983. Distribution and migration of the bowhead whale, *Balaena mysticetus*, in the eastern North American Arctic. Arctic, 36(1):5–64. Deep Sea Res. Part B. Oceanogr. Lit. Rev. 36:5–64.

Reinehr T, Andler W. 2002. Thyroid hormones before and after weight loss in obesity. Arch. Dis. Child. 87:320–323.

Rolland RM, Parks SE, Hunt KE, Castellote M, Corkeron PJ, Nowacek DP, Wasser SK, Kraus SD. 2012. Evidence that ship noise increases stress in right whales. Proceedings. Biol. Sci. 279:2363–8.

Scheuerlein A, Van't Hof TJ, Gwinner E. 2001. Predators as stressors? Physiological and reproductive consequences of predation risk in tropical stonechats (*Saxicola torquata axillaris*). Proc. R. Soc. B Biol. Sci. 268:1575–1582.

Sheriff MJ, Dantzer B, Delehanty B, Palme R, Boonstra R. 2011. Measuring stress in wildlife: Techniques for quantifying glucocorticoids. Oecologia 166:869–887.

Smith AJ, Higdon JW, Richard P, Orr J, Bernhardt W, Ferguson SH. 2017. Beluga whale summer habitat associations in the Nelson River estuary, western Hudson Bay, Canada. PLoS One 12:1–19.

Solomon S, Qin D, Manning M, Chen Z, Marquis M, Averyt KB, Tignor M, Miller HL. 2007. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge Univ. Press. Cambridge, United Kingdom New York, NY, USA.

Stirling I, Lunn NJ, Iacozza J. 1999. Long-term trends in the population ecology of polar bears in western Hudson Bay in relation to climatic change. Arctic 52:294–306.

Stirling I, Smith TG. 2004. Implications of warm temperatures and an unusual rain event for the survival of ringed seals on the Coast of southeastern Baffin Island. Arctic 57:59–67.

Tynan CT, Demaster DP. 1997. Observations and Predictions of Arctic Climatic Change: Potential Effects on Marine. Source Arct. 50:308–322.

Wasser SK, Azkarate JC, Booth RK, Hayward L, Hunt K, Ayres K, Vynne C, Gobush K, Canales-Espinosa D, Rodríguez-Luna E. 2010. Non-invasive measurement of thyroid hormone in feces of a diverse array of avian and mammalian species. Gen. Comp. Endocrinol. 168:1–7.

Wikelski M, Cooke SJ. 2006. Conservation physiology. Trends Ecol. Evol. 21:38–46.

Wikelski M, Wong V, Chevalier B, Rattenborg N, Snell HL. 2002. Marine iguanas die from trace oil pollution. Nature 417:607–608.

Wingfield JC, Kitaysky AS. 2002. Endocrine Responses to Unpredictable Environmental Events: Stress or Anti-Stress Hormones? Integr. Comp. Biol. 42:600–609.

CHAPTER 2: SNOT FOR SCIENCE: A NON-INVASIVE TECHNIQUE FOR MEASURING CORTISOL IN FREE-SWIMMING BELUGA WHALES (*DELPHINAPTERUS LEUCAS*)

Justine M. Hudson*[1,2] and Marianne Marcoux[2]

* Corresponding Author

[1]Univerisity of Manitoba, 66 Chancellors Circle, Winnipeg, Manitoba R3T 2N2

[2]Fisheries and Oceans Canada, Freshwater Institute, 501 University Crescent, Winnipeg, Manitoba R3T 2N6

ABSTRACT

Climate change has caused significant warming in the Arctic, which has led to a decrease in sea-ice extent and duration, an increase in predators and human activity, changes in prey distribution, and the emergence of infectious disease. These factors have the potential to cause stress in marine mammals, which can ultimately impact the health and fitness of populations. Cortisol is often used as an indicator of health, as elevated levels can indicate environmental stressors. Blow collection is a non-invasive technique for monitoring the physiology of cetaceans and although this technique shows promise, a major challenge to blow collection is the inability to quantify blow samples due to variable amounts of seawater contamination and evaporation. In this study, we aimed to (1) determine whether blow samples could be collected from free-swimming beluga whales; (2) determine which factors influenced cortisol and urea concentrations, including sample device used, quantity rating, number of exhalations, time of day, age class, and ship presence; and (3) assess whether urea could be used as a dilution marker to normalize blow samples. Blow samples were collected from belugas in the Churchill River by placing a collection device over the blowhole of belugas as they surfaced for air. We collected a total of 252 blow samples from free-swimming belugas, making it the first time that it had been done. Cortisol was detected in 65.87% of samples and concentrations ranged from 6.6 pg/ml to 963.17 pg/ml. Urea was detected in 90.48% of samples and ranged from 0.17-19.05 mg/l. To determine whether urea could be used as a dilution marker to normalize blow samples, we calculated the difference between cortisol levels of paired blow samples as either absolute cortisol values or normalized cortisol values. We found that urea was not an appropriate dilution marker to normalize blow samples collected from free-swimming belugas.

We also found that cortisol was influenced by sample device, quantity rating, and age class, while urea was only influenced by sample device. Although we demonstrated that blow can be successfully collected from free-swimming belugas, further refinement of the technique is needed before it can be used as a reliable method for physiological assessments.

2.1 INTRODUCTION

The Arctic is warming at twice the rate of the global average (Gagnon and Gough 2005; Anisimov et al. 2007) exposing Arctic marine mammals to a number of threats, including a loss of sea-ice habitat, changes in prey distribution, higher incidences of disease, and increased pollution, shipping, and human activity (Tynan and Demaster 1997; Learmonth et al. 2006; Anisimov et al. 2007; Burek et al. 2008). These threats have the potential to cause acute or chronic physiological responses in individual organisms, which overtime can have population-level consequences (Wikelski and Cooke 2006; Hunt et al. 2013). Conservation physiology is a field of study that focuses on the effects of human activity on animal physiology, including elevations in stress hormones, declines in reproductive hormones and body condition, changes in metabolism, and decreases in immune function (Wikelski and Cooke 2006). Changes such as these can indicate the occurrence and severity of environmental pressures and can be used to monitor the health of wildlife populations (Wikelski and Cooke 2006; Cooke and O'Connor 2010; Hunt et al. 2013).

Measuring glucocorticoids is one of the most useful tools for assessing environmental stressors (Wikelski and Cooke 2006). When an animal encounters a stressor, the hypothalamic-pituitary-adrenal (HPA) axis becomes activated, resulting in the release of glucocorticoids (GCs) (Wikelski and Cooke 2006; Sheriff et al. 2011). The HPA axis allows animals to cope with biological pressures (i.e. mating, migrating, and foraging) and environmental pressures (i.e. predation, weather, climate, relocation, and habitat disturbance) (Boonstra et al. 2001; Sheriff et al. 2011). Short-term elevations in GCs (hours to days) are considered to be an adaptive trait, as it allows an animal to survive life-threatening circumstances, while long-term stress (days to

weeks), which results in prolonged activation of the HPA axis, can have detrimental effects, including infertility, heart disease, inhibited growth, and decreased immune function (Wingfield and Kitaysky 2002; Sheriff et al. 2011). GCs are often used as indicators of health in ecological and conservation focused studies since the duration and extent of the stress response is directly linked to the overall health of the individual (Wikelski and Cooke 2006; Sheriff et al. 2011).

Blood sampling is considered the gold standard for physiological assessments and is frequently used to study animals in zoos and aquaria (Thompson et al. 2014). However, the process of collecting blood samples requires capturing and restraining an animal, which often elicits a stress response, making it difficult to interpret any results (Thompson et al. 2014; Hunt et al. 2013). Blood can be collected from small cetaceans but is nearly impossible to collect from large whales without causing harm to the animal (Hunt et al. 2013; Thompson et al. 2014). Minimally-invasive and non-invasive techniques, such as blubber and skin biopsies, and faecal samples are also often used to study cetacean physiology; however, there is a need for a non-invasive technique that would allow researchers to collect repeated samples from targeted individuals, over time, seasons, and life histories (Hunt et al. 2013; Burgess et al. 2018).

Respiratory vapour analysis is a relatively new technique that was initially developed to study human health (Kim et al. 2012). Respiratory vapour or "blow" samples have been collected to study reproductive hormones (Hogg et al. 2009; Hunt et al. 2014; Thompson et al. 2014; Richard et al. 2017; Burgess et al. 2018), stress hormones (Hunt et al. 2014; Thompson et al. 2014; Burgess et al. 2018), genetics (Frère et al. 2010), and disease (Geoghegan et al. 2006; Acevedo-Whitehouse et al. 2010) in a number of whale species. A key benefit to blow collection

is the non-invasive nature of the technique, which does not harm the animal and results in minimal behavioural disruptions (Hunt et al. 2013).

Although several studies have used blow to examine endocrine function in cetaceans, one challenge of this technique is the inability to measure the total volume of blow in a sample due to unknown amounts of seawater contamination and evaporation (Burgess et al. 2018). In human breath studies, urea is often used as a dilution marker because it's evenly dispersed throughout the body, remains relatively constant within an individual, and is relatively stable (Dwyer 2004; Esther et al. 2009; Pitiranggon et al. 2014). Burgess et al. (2018) investigated the use of urea as a way to normalize hormone concentrations in blow collected from North Atlantic right whales (*Eubalaena glacialis*) and found that after using urea to normalize samples, hormone concentrations became more biologically relevant and varied depending on sex, age, and reproduction (i.e. a pregnant female had the highest concentration of progesterone; Burgess et al. 2018). However, other studies have been unable to determine the suitability of urea as a dilution marker to normalize blow samples (Thompson et al. 2014); or found that urea was not an appropriate dilution marker for normalizing blow hormone concentrations (Mingramm et al. 2019).

To date, no studies have determined whether it is possible to collect blow from free-swimming beluga whales. Furthermore, only a few studies have examined potential dilution markers to normalize blow samples from free-swimming cetaceans. In this study, we aim to (1) determine whether it is possible to collect blow from free-swimming beluga whales; (2) determine which factors influence cortisol and urea concentrations, including sample device used, quantity rating, number of exhalations, age class, time, and ship presence, and (3) assess

whether urea can be used as a dilution marker to normalize blow samples as an alternative to absolute hormone concentrations.

2.2 METHODS

2.2.1 Study species

The Western Hudson Bay (WHB) beluga population summers in the Churchill, Seal, and Nelson river estuaries along the Western coast of Hudson Bay. It is thought that belugas spend time in estuaries to calf, moult, feed, and for protection from predators (COSEWIC 2016). The WHB population is listed as a species of special concern by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC), due to threats caused by hydroelectric dams and shipping (COSEWIC 2004). There is growing concern regarding the impacts of shipping on the population, as Canada's only deep-water Arctic port, the Port of Churchill, is situated within their summering range.

2.2.2 Sample collection

Blow samples were collected from beluga in the Churchill River estuary (58°47'23.77"N, 94°12'37.90"W) from a 4.88 m (16 ft.) zodiac. Samples were collected between July 18th-August 10th, 2018 for 4 hours during high tide each day. We collected samples for 24 consecutive days with the exception of 2 rain days.

We tested two collection devices to evaluate their feasibility in the field and to determine if either material caused any assay interference. The first was a 150 mm polystyrene petri dish (MilliporeSigma, Oakville, ON, Canada; P5981) and the second was a 150 mm polystyrene petri dish with a 20 x 20 cm piece of nitex membrane (Elko Filtering, Miami, Fl,

USA; NC0123567) secured to it using a rubber band. Any excess nitex surrounding the dish was removed to minimize the chance of contamination between samples. Prior to sample collection, the nitex membrane was washed in soapy water, rinsed twice with tap water, rinsed with distilled water, and then soaked in 95% ethanol before air drying overnight (Thompson et al. 2014). The collection devices were attached to a piece of plexi-glass using Velcro, which was secured to a 0.91-1.83 m (3-6 ft.) extendable painter's pole.

To collect samples, we approached a group of whales from a distance, turned the engine to idle, and waited for the belugas to approach the rear of the boat. The collection device was positioned over the blowhole of belugas as they surfaced for air. For each sample, we recorded the time, date, location, number of exhalations, rating, estimated age class (Table 1), and if ships were present. Each sample was rated on a 1-3 scale based on the amount of blow on the collection device. A sample was rated as a 1 if there were very few, fine drops of blow on the device, a 2 if there were many, fine drops or few, large drops of blow on the collection device, and a 3 if there were many, large drops of blow on the collection device. A single person rated the samples for the entire field season for consistency. We also noted if samples were collected from the same individual, which could only be done on the same day (i.e. not over multiple days) and when the individual had a unique identifying mark. Samples were stored in a cooler, on ice, for no more than 6 hours and were then put into a -20°C freezer for the duration of the field season. Water samples were also collected during our field season to test whether hormones could be detected. Samples were shipped on ice from Churchill, Manitoba, Canada to the University of Manitoba, Winnipeg, Manitoba, Canada, and stored in a -20°C freezer until the

extraction process. This project was approved by Fisheries and Oceans Canada; Animal Use Protocol number ACC-2018-20.

Table 2.1. Estimated age class of beluga whales based on size, colouration, and behaviour (Brodie 1971; Suydam 2010; Michaud 2014).

Age Class	Characteristics
Adult	Large (304-427 cm) Pure white in colour
Subadult	2/3 to almost the full length of an adult Whiter in colour than a juvenile
Juvenile	2/3 to almost the full length of an adult Grey in colour May or may not be associated with an adult
Yearling	1/2 to 2/3 the length of an adult (209-225 cm) Marbled skin Bulkier than calves Closely associated with an adult
Calf	1/3 to 1/2 the length of an adult (mean 159.6 cm) Peachy-grey in colour Dark circle around eyes Closely associated with an adult

2.2.3 Sample analysis

2.2.3.1 Extraction

It was not possible to determine a true volumetric measurement of blow samples due to unknown quantities of seawater contamination and evaporation (Burgess et al. 2018); therefore, we extracted samples using a modified technique developed by Burgess *et al.* (2016). Samples collected on petri dishes were rinsed with 25 ml of 100% EtOH and agitated on a plate shaker for 1 hour. The sample was then pipetted into a 40 ml glass centrifuge tube and dried down under nitrogen for approximately 24 hours. Samples collected on nitex were extracted by

placing the nitex membrane into a 40 ml glass centrifuge tube and rinsed with 15 ml of 100% EtOH. The petri dish that the nitex was secured to was rinsed with 25 ml of 100% EtOH. The nitex and petri dish were then both agitated on a plate shaker for 1 hour. The nitex membrane was centrifuged at 5000 g for 10 minutes to remove excess extract and then decanted into the original 40 ml centrifuge tube, along with the extract from the petri dish. The extract was dried down under nitrogen for ~24-36 hours. All samples were reconstituted in 500 ul of ultra-pure water (total extract volume) and kept frozen at -20°C until analysis. We performed the same extraction protocol on sterile collection devices to determine if either device resulted in assay interference.

2.2.3.2 Hormone and metabolite assays and Validation

To measure cortisol, we assayed all samples using a commercially available cortisol enzyme-linked immunosorbent assay (ELISA) kit (Cayman Chemical; catalogue #500360; Ann Arbor, Michigan USA), following the manufacturer's protocol. The manufacturers reported the following performance characteristics for the cortisol assay: range=6.6-4,000 pg/ml; sensitivity (defined as 80% B/B_0)=35 pg/ml; cross-reactivities: prednisolone=4.0%, cortexolone=1.6%, 11-deoxycorticosterone=0.23%, dexamethasone=15%, 17-hydroxyprogesterone=0.23%, cortisol glucuronide=0.15%, corticosterone=0.14%, cortisone=0.13%, androstenedione=<0.01%, enterolactone=<0.01%, 17-hydroxypregnenolone=<0.01%, pregnenolone=<0.01%, and testosterone=<0.01%). To measure total urea in blow samples, we used a colorimetric diacetyl monoxime assay developed by Rahmatullah and Boyde (1980) and a standard curve was created using 0, 20, 40, 60, 80, 100, 120 μmol/l urea standards.

Thompson et al. (2014) validated the Cayman Chemical ELISA kit using blow collected from captive and wild-captured belugas, which showed good parallelism, accuracy, and intra- and inter-assay variability. To ensure that samples collected from wild beluga also showed good parallelism and accuracy, we tested both using a pool of blow extract from 3 individuals. For parallelism, we tested 6 dilutions, 1:1, 1:2, 1:4, 1:8, 1:16, 1:32, which were compared to the standard curve. To test accuracy, we spiked 4 standards (41, 102.4, 256, 640 pg/ml) with equal amounts of pooled blow and plotted the expected versus observed cortisol concentrations. Intra-assay variability was determined by averaging the % CVs for all duplicates, while inter-assay variability was tested by calculating the % CVs of the same sample assayed over multiple plates. We followed the same procedure for the urea assay. We tested extraction efficiency by extracting and assaying 1 ml of a 400 pg/ml cortisol and a 15 mg/l urea solution to determine what percentage of the solution was recovered. To test whether cortisol and urea were present in the water, we extracted and assayed 1 ml of seawater collected throughout our field season.

We followed the recommended QA/QC protocols, including running standards, non-specific binding wells, and maximum binding wells on each plate and running samples in duplicate. Samples were assayed undiluted (500 μl of extract volume). Absolute cortisol concentrations were expressed as pg/ml of extract volume and total urea was expressed as mg/l of extract volume.

2.2.3.3 Normalization

To assess whether we could use urea as a dilution marker to normalize blow samples, we examined the difference in cortisol concentrations between pairs of blow samples collected

on different collection devices from the same individual, on the same day, within minutes, using absolute cortisol values (i.e. pg/ml extract volume) versus normalized values (i.e. ng cortisol/mg urea). If urea was an acceptable dilution marker, we expected to see less variation in cortisol concentrations between normalized samples than non-normalized samples.

2.2.4 Data Analysis

GraphPad Prism version 8.0.0 for Mac OS X (GraphPad Software, San Diego, California USA) was used to calculate cortisol measurements and to determine parallelism. RStudio Version 1.1.463 (RStudio Inc. Boston, Massachusetts USA) was used to analyze all data. Parallelism was tested, using F statistics, by comparing the linear areas of a serially diluted pool of blow extract to the assay standard curve. Accuracy was assessed using linear regression to determine the line of best fit. Results were considered acceptable if the slope was between 0.7-1.3 and the R^2 was greater than 0.95. We used a paired t-test to determine whether the difference between normalized paired blow samples were significantly different than non-normalized paired samples and we used a Kruskal-Wallis test to determine differences in cortisol and urea concentrations based on sample device, rating, number of exhalations, age class, time of day, and ship presence.

2.3 RESULTS

A total of 252 blow samples were collected from free-swimming beluga in Churchill, MB (Figure 1). Samples were predominantly collected on petri dishes (n=193) but were also collected on nitex (n=59; Table 2). Blow samples were collected from all age classes, including

calves (n=2), yearlings (n=3), juveniles (n=69), subadults (n=94), and adults (n=84). Due to small samples sizes, calves and yearlings were combined for subsequent analyses. We collected mostly 1-rated samples (n=113) but also collected 2-rated samples (n=86) and 3-rated samples (n=53). We collected mostly single exhalations (n=163) but also collected 2 (n=43), 3 (n=20), 4 (n=3), and 5 (n=2) exhalations from the same individual on the same device. We were also able to collect repeated samples from the same individual on separate collection devices on 25 occasions. We did not record the number of exhalations for 21 samples.

Table 2.2. Number of blow samples collected from free-swimming beluga whales for each variable.

Category	Details	Number of Samples (n)
Collection Device	Petri	193
	Nitex	59
Number of Exhalations	1	163
	2	43
	3	20
	4	3
	5	2
	NA	21
Quantity Rating	1	113
	2	86
	3	53
Age Class	Adult	84
	Subadult	94
	Juvenile	69
	Yearling	3
	Calf	2

2.3.1 Cortisol analysis

2.3.1.1 Cortisol validations

Parallelism was tested using serially diluted pools of beluga blow extract. Pooled blow samples produced a curve parallel to that of the standard curve (Figure 2a; $F_{1,10}$= 0.97; *P*=0.35). All six dilutions yielded detectable cortisol in the parallelism test. Accuracy was determined using an undiluted pooled blow sample (Figure 2b). The linear relationship between observed and expected cortisol concentrations indicated an acceptable level of accuracy with an R^2=0.973 and a slope=0.854.

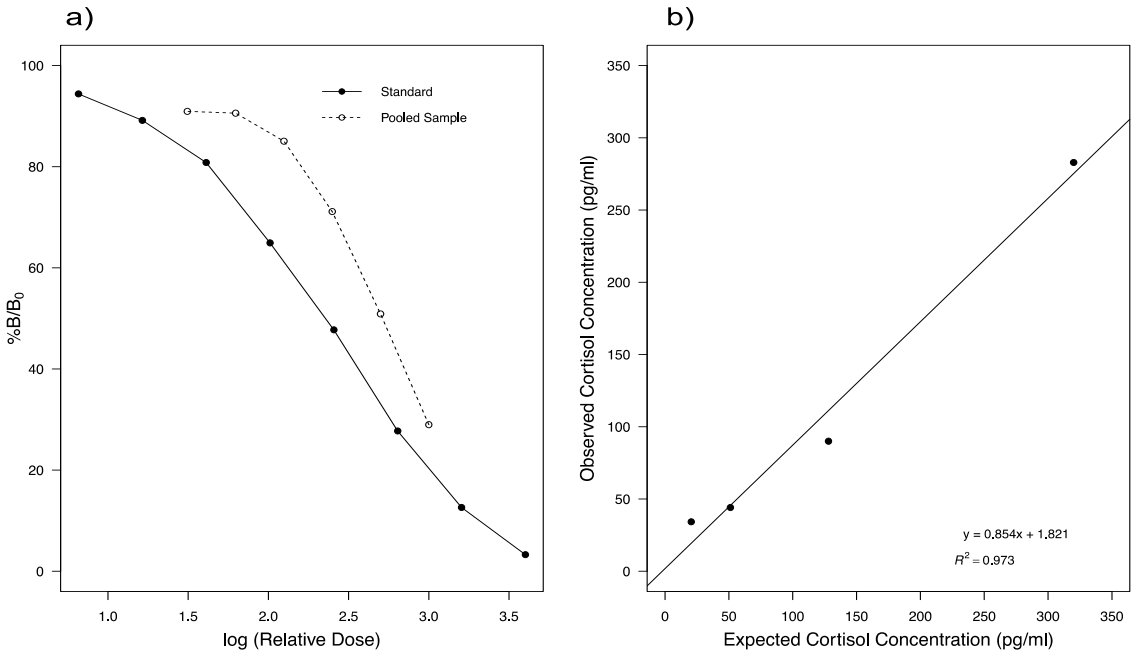

Figure 2.1. Validation plots for measuring cortisol in beluga whale blow samples, using Cayman Chemical cortisol enzyme immunoassay kits. (a) Parallelism results for a serially diluted pool of blow extract (1:1-1:32; open circles) compared to the standard curve or a cortisol assay kit (6.6-4,000 pg/ml; circles; $F_{1,10}$=0.97; P=0.35). (b) Accuracy results showing a positive linear relationship between observed cortisol concentrations and expected cortisol concentrations (y=0.854x + 1.821; R^2=0.973).

Average intra-assay coefficient of variation (CV) was 2.29% (n=252 duplicates) and inter-assay CV was 4.64% (n=10). The cortisol assay detected no interference for petri dishes (n=8), while the nitex sampler (n=14) resulted in an average of 25.86±15.90 pg/ml. Extraction efficiency was 80.74%±1.75% for petri dishes and 94.00% ±17.32% for nitex. Cortisol concentrations for seawater samples (n=6) all fell below the limit of detection (LOD) except for 1 sample that measured 123.84 pg/ml. This sample was collected after we observed what we think was a birth event and may have contained afterbirth.

2.3.1.2 Variation in cortisol concentration

Cortisol was detected in 65.87% of samples and absolute cortisol concentrations ranged from 6.6 (LOD)- 963.17 pg/ml. Samples that fell below the LOD were assigned a value of 6.6 pg/ml, which was the LOD for the assay kit. We compared cortisol concentrations from samples collected on nitex (n=59) to those collected on petri dishes (n=193) and found that samples collected on nitex had significantly higher cortisol concentrations (159.63±144.84 pg/ml; X^2=119.99; df= 1; P<2.2e-16) than those collected on petri dishes (12.58±10.11 pg/ml; Figure 3). Due to the significant difference between cortisol values collected on petri dishes and nitex and assay interference for samples collected on nitex, we removed all samples collected on nitex for subsequent analyses. Cortisol concentrations varied significantly based on the quantity rating (X^2=11.53; df=2, P=0.0031) with 3-rated samples having significantly higher cortisol levels than 1 and 2-rated samples ($P_{1,3}$=0.0021; $P_{2,3}$=0.0129; Figure 3). Cortisol concentrations were not influenced by the number of exhalations (X^2=1.13; df = 2; P=0.5678; Figure 3). We excluded samples with 5 exhalations due to a low sample size. Cortisol concentrations differed

significantly based on age class (X^2=15.17; df=3; P=0.0017) with adults having higher cortisol levels than subadults and juveniles ($P_{Adult, Subadult}$=0.0018; $P_{Adult, Juvenile}$=0.0230; Figure 3). We did not observe any differences in cortisol concentrations based on the time of day (morning=8-11 am; afternoon=12-14 pm; evening 5-8 pm; X^2=3.13; df = 2; P=0.2089) or when ships were present (X^2=0.01; df=1; P=0.9584; Figure 3).

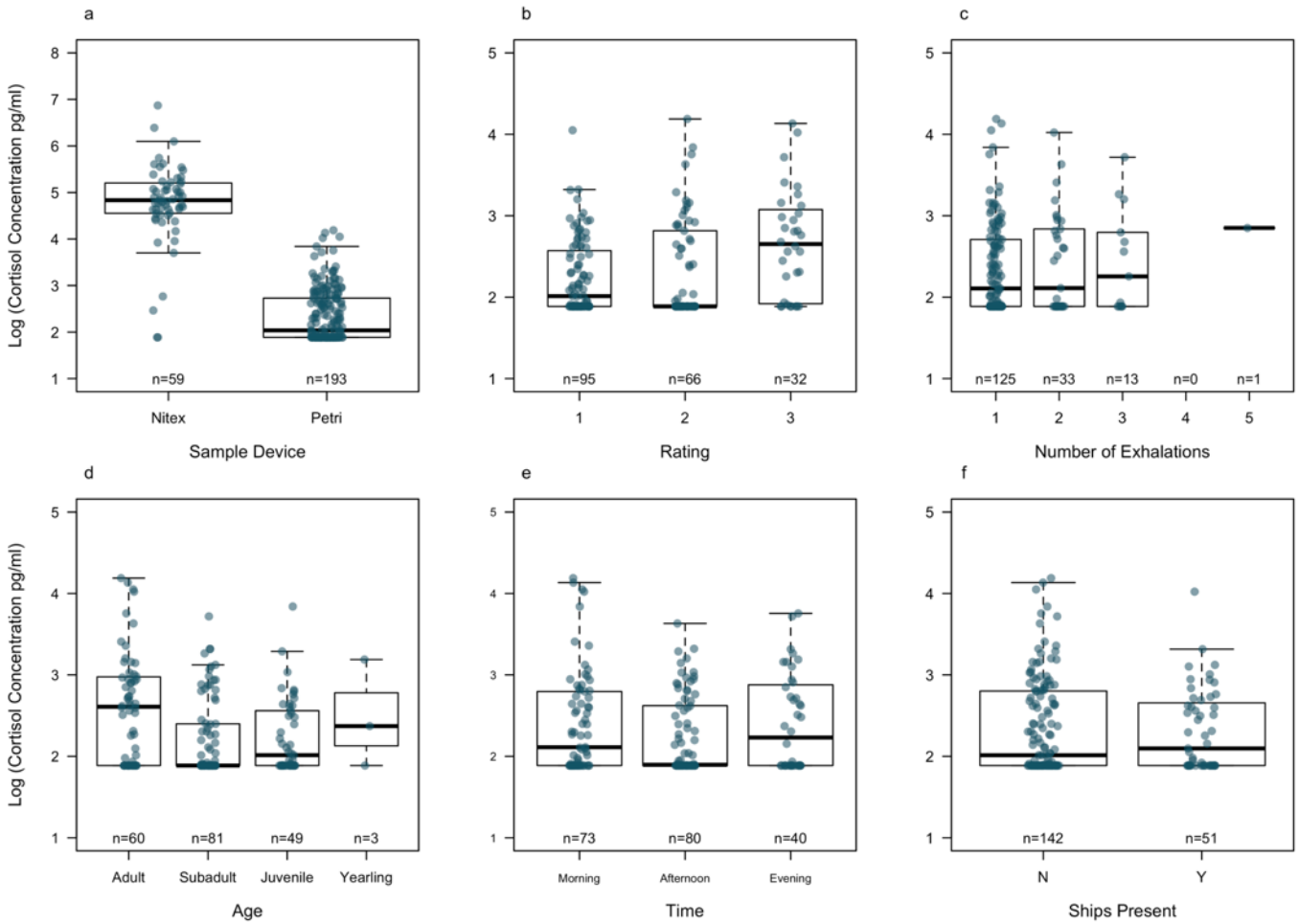

Figure 2.2. Boxplot comparison of cortisol concentrations (pg/ml) in relation to (a) sample device, (b) quantity rating, (c) number of exhalations, (d) age class, (e) time of day, and (f) ship presence. The top and bottom of the box represent 1st and 3rd quartiles (Q1 and

Q3), the bolded line in the middle represents the median, and the vertical lines represent 1.5 times the interquartile range from the box and the points represent individual samples.

2.3.2 Urea analysis

2.3.2.1 Urea validations

Parallelism was tested using a serially diluted pool of beluga blow extract. The pooled blow sample produced a curve parallel to that of the standard curve ($F_{1,8}$=2.46; *P*=0.16; Figure 5). Accuracy was not tested due to an insufficient amount of pooled blow extract. The urea assay detected a small level of interference from negative nitex controls (0.57±0.38 mg/l; n=8), whereas the petri dish resulted in no interference (n=8). Intra-assay coefficient of variation (CV) was 3.69% (n=252) and inter-assay CV was 15.00% (n=10). Extraction efficiency was 81.94% for the petri dish and 80.76% for the nitex membrane. Urea was detected in 60% of water samples (n=5; afterbirth sample removed) and averaged 1.70±0.28 mg/l. The afterbirth water sample had a urea concentration of 4.72 mg/l.

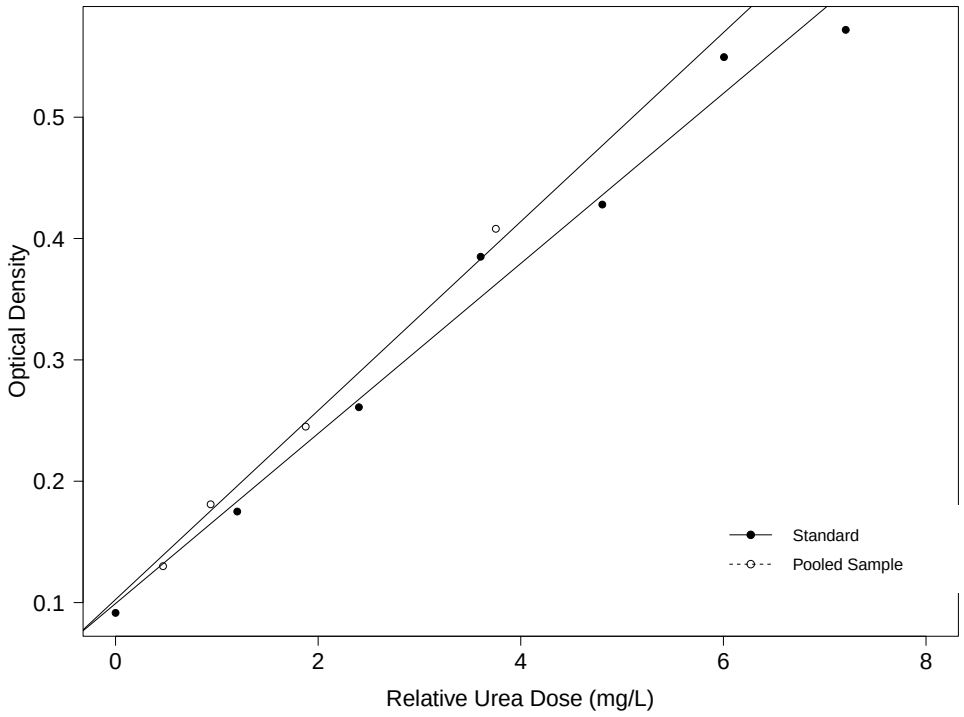

Figure 2.3. Parallelism results for a serially diluted pool of blow extract (1:1-1:16; open circles) compared to the standard curve of a colorimetric diacetyl monoxime assay (0-7.20 mg/l; closed circles; $F_{1,8}$=2.46; P=0.16).

Urea was detected in 90.48% of samples. Absolute urea concentrations averaged 2.58±2.22 mg/l of blow extract and ranged from 0.17-19.05 mg/l. The approximate LOD was 0.17 mg/l and was determined by measuring the absorbance of zero standards (n=10) and calculating the mean+3SD (Shrivastava and Gupta 2011). Samples that fell below the LOD were given the value of 0.17 mg/l. Samples collected on petri dishes had significantly higher urea concentrations (2.69± 2.14; X^2= 11.24; df = 1; P = 0.0008) than those collected on nitex (2.21± 2.43 mg/l; Figure 4). Due to the difference in urea concentrations between sampling devices, we removed nitex samples from the following analyses. Urea concentrations were not influenced by rating (X^2= 0.78; df = 2; P = 0.6761), number of exhalations (X^2= 0.21, df = 1; P =

0.6528), age class (X^2= 0.65, df = 3, P = 0.8854), or based on time of day (X^2= 0.87; df = 2; P = 0.6472; Figure 4). For the number of exhalations, we removed the sample that had 5 exhalations due to small sample size.

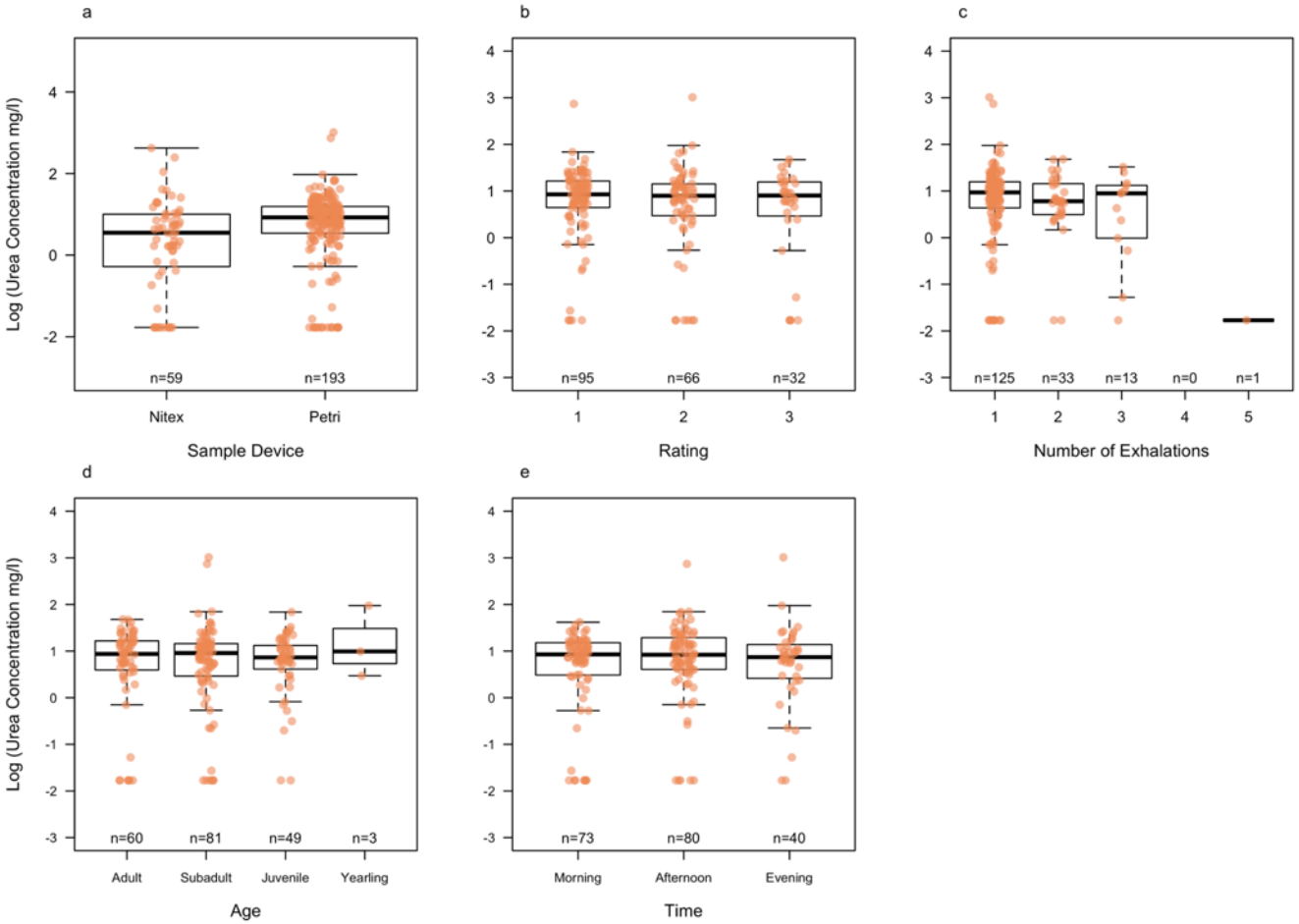
a
b
c
d
e
Log (Urea Concentration mg/l)
n=59
n=193
Nitex
Petri
Sample Device
n=95
n=66
n=32
1
2
3
Rating
n=125
n=33
n=13
n=0
n=1
1
2
3
4
5
Number of Exhalations
n=60
n=81
n=49
n=3
Adult
Subadult
Juvenile
Yearling
Age
n=73
n=80
n=40
Morning
Afternoon
Evening
Time

Figure 2.4. Urea concentrations in relation to (a) sample device, (b) quantity rating, (c) number of exhalations, (d) age class, and (e) time of day. The top and bottom of the box represent 1st and 3rd quartiles (Q1 and Q3), the bolded line in the middle represents the median, and the vertical lines represent 1.5 times the interquartile range from the box and the points represent individual samples.

2.3.3 Sample Normalization

One of the key objectives of this study was to assess whether we could use urea as a dilution marker to normalize cortisol concentrations in beluga blow samples. To do so, we calculated the difference in cortisol concentrations between 11 paired blow samples collected from the same individual using urea as a dilution marker and compared the results to non-normalized samples. Paired samples were collected from the same individual, on the same day, and on the same type of collection device. The timing between sample collection ranged from 0-5 minutes. Both sets of samples were standardized to remove units to allow for comparisons. We found that normalized samples did not differ from non-normalized samples ($t=-1.45$; $df=10$; $P=0.1788$; Figure 5), suggesting that urea is not an appropriate dilution marker for beluga blow samples.

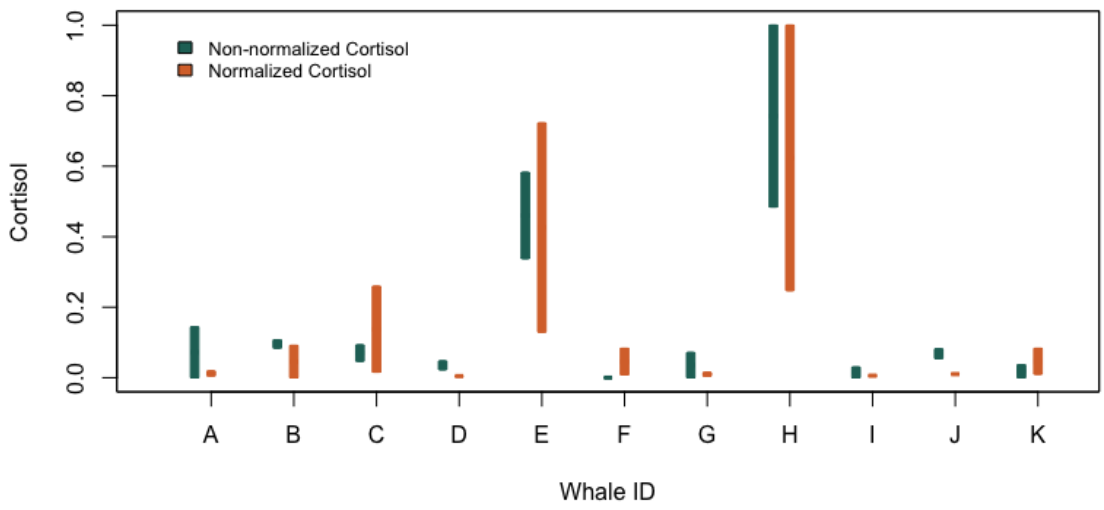

Figure 2.5.Difference between cortisol measurements from paired blow samples collected from the same individual using urea as a dilution marker (i.e. normalized; orange) vs. absolute cortisol concentrations (i.e. non-normalized; teal). Both sets of samples were standardized to allow for comparisons. The length of the bars represents the difference between paired blow samples as normalized or non-normalized cortisol concentrations.

2.4 DISCUSSION

Blow monitoring is a promising approach to studying the physiology of free-swimming whales; however, a major challenge to using this technique is the inability to quantify blow volumes due to seawater contamination and evaporation. Our results demonstrate that it is possible to measure cortisol and urea from blow samples collected from wild, free-swimming beluga and to our knowledge, this is the first study to do so. We also demonstrated that it is possible to collect repeated samples from the same individual from a wild population. We found that cortisol measurements were influenced by sample device, rating, and age, while urea was only influenced by sample device. We also determined that urea was not a suitable dilution marker for normalizing cortisol concentrations in beluga blow samples.

A commercially available cortisol enzyme immunoassay kit validated in this study was sensitive enough to measure cortisol in two thirds of beluga blow samples. Close parallelism with cortisol kit standards, good accuracy results, and a low limit of detection suggest that this protocol is sensitive enough to analyze blow samples from free-swimming beluga whales. Cortisol concentrations in blow of wild-captured beluga whales in Bristol Bay, Alaska, USA, ranged from 300-2300 pg/ml (pre-health assessment) and 57-5900 pg/ml (post-health assessment; Thompson et al. 2014). Our results fall within a similar range (6.6-963.17 pg/ml); however because Thompson et al. (2014) collected blow from wild-captured and captive belugas, their samples are likely to contain smaller volumes of seawater contamination and therefore less diluted samples.

We validated a diacetyl monoxime urea assay that was sensitive enough to measure urea in most beluga blow samples. Close parallelism with assay standards and a low limit of detection suggests that this assay is sensitive enough to analyze urea in blow samples from free-swimming beluga. In mammals, urea is the end product of nitrogen metabolism (Knepper and Roch-Ramel 1987), and remains relatively constant in the body (Effros et al. 1992; Dwyer 2004). In this study, we found that urea concentrations ranged from 0.17-19.05 mg/l in beluga blow samples, which were comparable to urea nitrogen concentrations in blow from North Atlantic right whales (*Eubalaena glacialis*; 0.02-10.41 mg/l; Burgess et al. 2018) and blow from captive bottlenose dolphins (Tursiops spp.; 0.70-200 mg/l; Mingramm et al. 2019).

Burgess et al. (2018) found that normalizing hormones using urea made hormone profiles more biologically relevant than non-normalized hormones (i.e. pregnant females had higher progesterone than non-pregnant females). Thompson et al. (2014) were unable to

conclusively say whether urea was a suitable normalizing factor for blow samples collected from captive belugas. Mingramm et al. (2019) found urea was not a suitable marker for normalizing hormone values from blow samples collected from captive bottlenose dolphins, as it did not improve biological relevance. We collected pairs of blow samples from the same individual, on the same day, using the same collection device and compared the difference between absolute values of cortisol (i.e. pg/ml blow extract) and normalized cortisol values (i.e. ng cortisol/mg urea). We hypothesized that paired samples should have similar cortisol concentrations but would have varying amounts of seawater contamination and therefore, normalizing the samples using urea as a dilution marker should result in less variation between paired samples when compared to non-normalized samples. Our results showed that there was no difference between normalized and non-normalized cortisol concentrations; and therefore, we concluded that urea was not an effective dilution marker to normalize blow samples from free-swimming belugas.

Although we were unable to normalize our blow samples, we explored which factors influenced absolute cortisol concentrations. Our study found that samples collected on nitex, 3-rated samples (i.e. large quantity samples), and samples collected from adults had higher cortisol concentrations, which was most likely due to increased sample volume. Nitex and veil-like materials have been used in a number of studies (Hogg et al. 2009; Hunt et al. 2014; Thompson et al. 2014), as they are thought to retain more sample than other collection devices like petri dishes (Burgess et al. 2016). However, these materials have been shown to exhibit high and variable levels of assay interference, which could lead to inaccurate hormone concentrations (Burgess et al. 2016; Mingramm et al. 2019). In this study, we found that nitex

blanks had highly variable assay interference for the cortisol assay and to a lesser extent, the urea assay, which could potentially mask biological factors. We suggest that nitex and other veil-like sample devices be avoided in order to achieve more accurate measurements. This finding also highlights the importance of immunoassay validations and of evaluating new collection devices to ensure the results are accurately interpreted. Adult beluga whales tended to have higher cortisol levels than subadults and juveniles; however, because we were unable to normalize the samples, higher cortisol concentrations may just be a reflection of larger exhalations. Surprisingly, we did not see a pattern in cortisol levels in relation to the number of exhalations. We expected to see a positive relationship between the number of exhalations and cortisol measurements as we predicted more exhalations would result in higher sample volumes; however, in the field, we noticed that when multiple exhalations were collected, the force of the exhalations would blow out any liquid that was already in the collection device. This could explain why we did not see a correlation between the number of exhalations and cortisol levels.

Absolute cortisol levels measured in blow samples were not influenced by time of day, or shipping activity. This could be due to our inability to normalize blow samples; therefore, any patterns that were present could have been masked by seawater contamination. We also might have missed any increases in cortisol caused by shipping activity because we only sampled for 4 hours per day and often missed when ships arrived at the port. In captive belugas, cortisol peaked after 30 minutes during an out of water examination and then started to decline and return to baseline within 24 hours (Thompson et al. 2014). It is possible that we simply missed

an increase in cortisol, if there was one, due to the amount of time between ship arrival and departures, and our sampling.

Interestingly, cortisol was undetectable in all water samples with the exception of 1 sample that was collected in an area where a potential birth had just occurred. This highlights the potential for collecting environmental hormones as a matrix to monitor population physiology, which has been used to study fish and amphibians in a laboratory setting (Kidd et al. 2010; Bosch et al. 2013; Gabor et al. 2016).

Urea levels were only influenced by sample device, with petri dishes having significantly higher urea concentrations than those collected on nitex. Unlike Burgess et al. (2018) who found that higher rated samples resulted in higher urea concentrations, we found no significant difference between quantity rating. It should be noted; however, that the rating scale used in our study was based on the visible quantity of blow in the petri dish; whereas, the rating scale used by Burgess et al. 2018 was based on proximity of the sample device to the blowhole of the animal. Interestingly, in our study, higher rated samples resulted in higher cortisol concentrations but did not influence urea concentrations. This could explain why urea was an ineffective dilution marker, as we expected urea concentrations to fluctuate based on the volume of blow and seawater contamination in the sample. Biological factors, such as age class and time of day had no effect on urea concentrations, which again strengthens the hypothesis that urea remains relatively constant in an individual's body (Dwyer 2004; Esther et al. 2009; Pitiranggon et al. 2014).

2.5 CONCLUSION

Here we have demonstrated that blow collection is possible from wild, free-swimming beluga whales in Western Hudson Bay and that we can successfully measure cortisol and urea from blow samples. Cortisol concentrations were influenced by sample device, rating, and age class, while urea concentrations were only influenced by sample device. In order for blow collection to be used as a reliable method for monitoring free-swimming beluga whales, we must develop a technique to quantify blow volumes or determine a method to normalize the samples. In this study, we conclude that urea was not an effective dilution marker and suggest that other dilution markers be explored, including total protein and lipid, and specific gravity (Burgess et al. 2018). We suggest that future studies avoid nitex for sample collection and recommend validation studies for all new materials. Future studies should also explore the use of abiotic substances in the water, such as pollutants or microplastics, as a method to normalize the amount of seawater contamination in blow samples. Continued exploration and development of blow collection, particularly from known individuals, could also be used to further develop this technique. We also recommend that future studies examine other hormones, including progesterone, testosterone, and corticosterone. Overall, this work demonstrates that blow collection has the potential to be a promising tool for studying the endocrinology of free-swimming beluga whales and highlights factors that influence absolute cortisol and urea concentrations.

ACKNOWLEDGEMENTS

The authors thank SeaNorth Tours for their support in this project. We also thank Fisheries and Oceans Canada, the University of Manitoba, ArcticNet, NSERC, the Churchill Northern Studies Centre, and the International Society of Wildlife Endocrinology for providing funding and resources that made this project possible.

LITERATURE CITED

Acevedo-Whitehouse K, Rocha-Gosselin A, Gendron D. 2010. A novel non-invasive tool for disease surveillance of free-ranging whales and its relevance to conservation programs. Anim. Conserv. 13:217–225.

Anisimov OA, Vaughan DG, Callaghan T, Furgal C, Marchant H, Prowse TD. 2007. Chapter 15. Polar regions (Arctic and Antarctic) : Climate Change 2007: Impacts, Adaptation and Vulnerability. Contrib. Work. Gr. II to Fourth As- sessment Rep. Intergov. Panel Clim. Chang.:653–685.

Boonstra R, Hubbs AH, Lacey EA, McColl CJ. 2001. Seasonal changes in glucocorticoid and testosterone concentrations in free-living arctic ground squirrels from the boreal forest of the Yukon. Can. J. Zool. 79:49–58.

Bosch J, Gabor CR, Davis DR, Fries JN. 2013. A non-invasive water-borne hormone assay for amphibians. Amphib. Reptil. 34:151–162.

Burek KA, Gulland FMD, O'Hara TM. 2008. Effects of climate change on arctic marine mammal health. Ecol. Appl. 18:S126–S134.

Burgess EA, Hunt KE, Kraus SD, Rolland RM. 2016. Get the most out of blow hormones:

validation of sampling materials, field storage and extraction techniques for whale respiratory vapour samples. Conserv. Physiol. 4:1–14.

Burgess EA, Hunt KE, Kraus SD, Rolland RM. 2018. Quantifying hormones in exhaled breath for physiological assessment of large whales at sea. Sci. Rep. 8:1–14.

Cooke SJ, O'Connor CM. 2010. Making conservation physiology relevant to policy makers and conservation practitioners. Conserv. Lett. 3:159–166.

COSEWIC. 2004. COSEWIC assessment and update status report on the beluga whale Delphinapterus leucas in Canada. Comm. Status Endanger. Wildl. Canada Ottawa:ix + 70 pp.

COSEWIC. 2016. Designatable Units for Beluga Whales (*Delphinapterus leucas*) in Canada. Committee on the Status of Endangered Wildlife in Canada. Ottawa. 73 pp.

Dwyer TM. 2004. Sampling Airway Surface Liquid: Non-Volatilesin the Exhaled Breath Condensate. Lung 182:241–250.

Effros RM, Murphy C, Ozker K, Hacker A. 1992. Kinetics of urea exchange in air-filled and fluid-filled rat lungs. Am. J. Physiol. Lung Cell. Mol. Physiol. 236:L619–L626.

Esther CR, Boysen G, Olsen BM, Collins LB, Ghio AJ, Swenberg JW, Boucher RC, Boucher RC. 2009. Mass spectrometric analysis of biomarkers and dilution markers in exhaled breath condensate reveals elevated purines in asthma and cystic fibrosis. Am. J. Physiol. Lung Cell. Mol. Physiol. 296:L987-93.

Frère CH, Krzyszczyk E, Patterson EM, Hunter S, Ginsburg A, Mann J. 2010. Thar She Blows! A Novel Method for DNA Collection from Cetacean Blow. Aramayo R, editor. PLoS One 5:e12299.

Gabor CR, Zabierek KC, Kim DS, da Barbiano LA, Mondelli MJ, Bendik NF, Davis DR. 2016. A Non-Invasive Water-Borne Assay of Stress Hormones in Aquatic Salamanders. Copeia 104:172–181.

Gagnon AS, Gough WA. 2005. Trends in the dates of ice freeze-up and breakup over Hudson Bay, Canada. Arctic 58:370–382.

Geoghegan JL, Pirotta V, Harvey E, Smith A, Buchmann JP, Ostrowski M, Eden J, Harcourt R, Id ECH. 2006. Virological Sampling of Inaccessible Wildlife with Drones. :1–7.

Hogg CJ, Rogers TL, Shorter A, Barton K, Miller PJO, Nowacek D. 2009. Determination of steroid hormones in whale blow: It is possible. Mar. Mammal Sci. 25:605–618.

Hunt KE, Moore MJ, Rolland RM, Kellar NM, Hall AJ, Kershaw J, Raverty SA, Davis CE, Yeates LC, Fauquier DA, et al. 2013. Overcoming the challenges of studying conservation physiology in large whales: a review of available methods. Conserv. Physiol. 1.

Hunt KE, Rolland RM, Kraus SD. 2014. Detection of steroid and thyroid hormones via immunoassay of North Atlantic right whale (*Eubalaena glacialis*) respiratory vapor. 30:796–809.

Kidd CE, Kidd MR, Hofmann HA. 2010. Measuring multiple hormones from a single water sample using enzyme immunoassays. Gen. Comp. Endocrinol. 165:277–285.

Kim K, Jahan SA, Kabir E. 2012. A review of breath analysis for diagnosis of human health. Trends Anal. Chem.

Knepper MA, Roch-Ramel F. 1987. Pathways of urea transport in the mammalian kidney. Kidney Int. 31:629–633.

Learmonth JA, MacLeod CD, Santos MB, Pierce GJ, Crick HQP, Robinson RA. 2006. Potential

effects of climate change on marine mammals. Oceanogr. Mar. Biol. - an Annu. Rev. Vol 44 44:431–464.

Mingramm FMJ, Dunlop RA, Blyde D, Whitworth DJ, Keeley T. 2019. Evaluation of respiratory vapour and blubber samples for use in endocrine assessments of bottlenose dolphins (*Tursiops* spp.). Gen. Comp. Endocrinol. 274:37–49.

Pitiranggon M, Perzanowski MS, Kinney PL, Xu D, Chillrud SN, Yan B. 2014. Determining urea levels in Exhaled breath condensate with minimal preparation steps and classic LC-MS. J. Chromatogr. Sci. 52:1026–1032.

Richard JT, Robeck TR, Osborn SD, Naples L, Mcdermott A, Laforge R, Romano TA, Sartini BL. 2017. General and Comparative Endocrinology Testosterone and progesterone concentrations in blow samples are biologically relevant in belugas (*Delphinapterus leucas)*. Gen. Comp. Endocrinol. 246:183–193.

Sheriff MJ, Dantzer B, Delehanty B, Palme R, Boonstra R. 2011. Measuring stress in wildlife: Techniques for quantifying glucocorticoids. Oecologia 166:869–887.

Shrivastava A, Gupta V. 2011. Methods for the determination of limit of detection and limit of quantitation of the analytical methods. Chronicles Young Sci. 2:21.

Thompson LA, Spoon TR, Goertz CEC, Hobbs RC, Romano TA, Bonier F, Martin P, Moore I, Wingfield J, Sheriff M, et al. 2014. Blow Collection as a Non-Invasive Method for Measuring Cortisol in the Beluga (*Delphinapterus leucas*). PLoS One 9:e114062.

Tynan CT, Demaster DP. 1997. Observations and Predictions of Arctic Climatic Change: Potential Effects on Marine. Source Arct. 50:308–322.

Wikelski M, Cooke SJ. 2006. Conservation physiology. Trends Ecol. Evol. 21:38–46.

Wingfield JC, Kitaysky AS. 2002. Endocrine Responses to Unpredictable Environmental Events: Stress or Anti-Stress Hormones? Integr. Comp. Biol. 42:600–609.

SUPPLEMENTARY INFORMATION

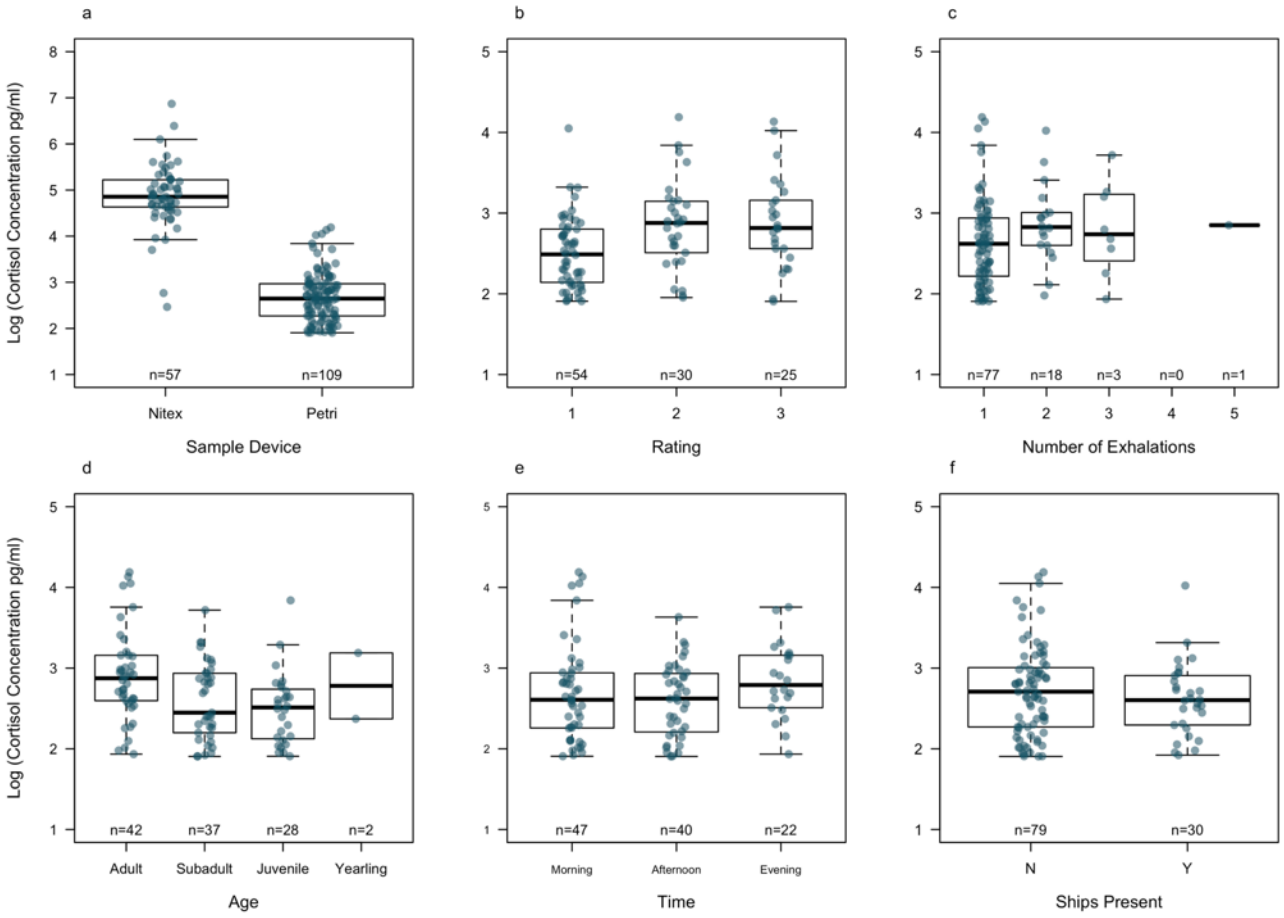

Figure S.6. Cortisol concentrations in relation to (a) sample device, (b) quantity rating, (c) number of exhalations, (d) age class, (e) time, and (f) ship presence. Cortisol values below the limit of detection (6.6 pg/ml) were removed, along with samples collected on nitex. (a) Samples collected on nitex had significantly higher cortisol measurements than those collected on petri dishes (X2= 102.33, df = 1, p-value < 2.2e-16). (b) Rating significantly influenced cortisol levels (X2= 12.423, df = 2, p-value = 0.002006), with 3 rated samples having higher cortisol than 2 and 1-rated samples and 2-rated samples having higher cortisol than 1-rated samples. (c) Number of exhalations had no effect of cortisol concentration (X2= 3.9445, df = 3, p-value = 0.2675). (d) Cortisol levels were influenced by age class (X2= 11.434, df = 3, p-value = 0.009598), with adults having significantly higher cortisol levels than subadults and juveniles. (e) Cortisol was not

influenced by time (X2=3.3129, df = 2, p-value = 0.1908). (f) Cortisol was not influenced by ship presence (X2= 0.63552, df = 1, p-value = 0.4253).

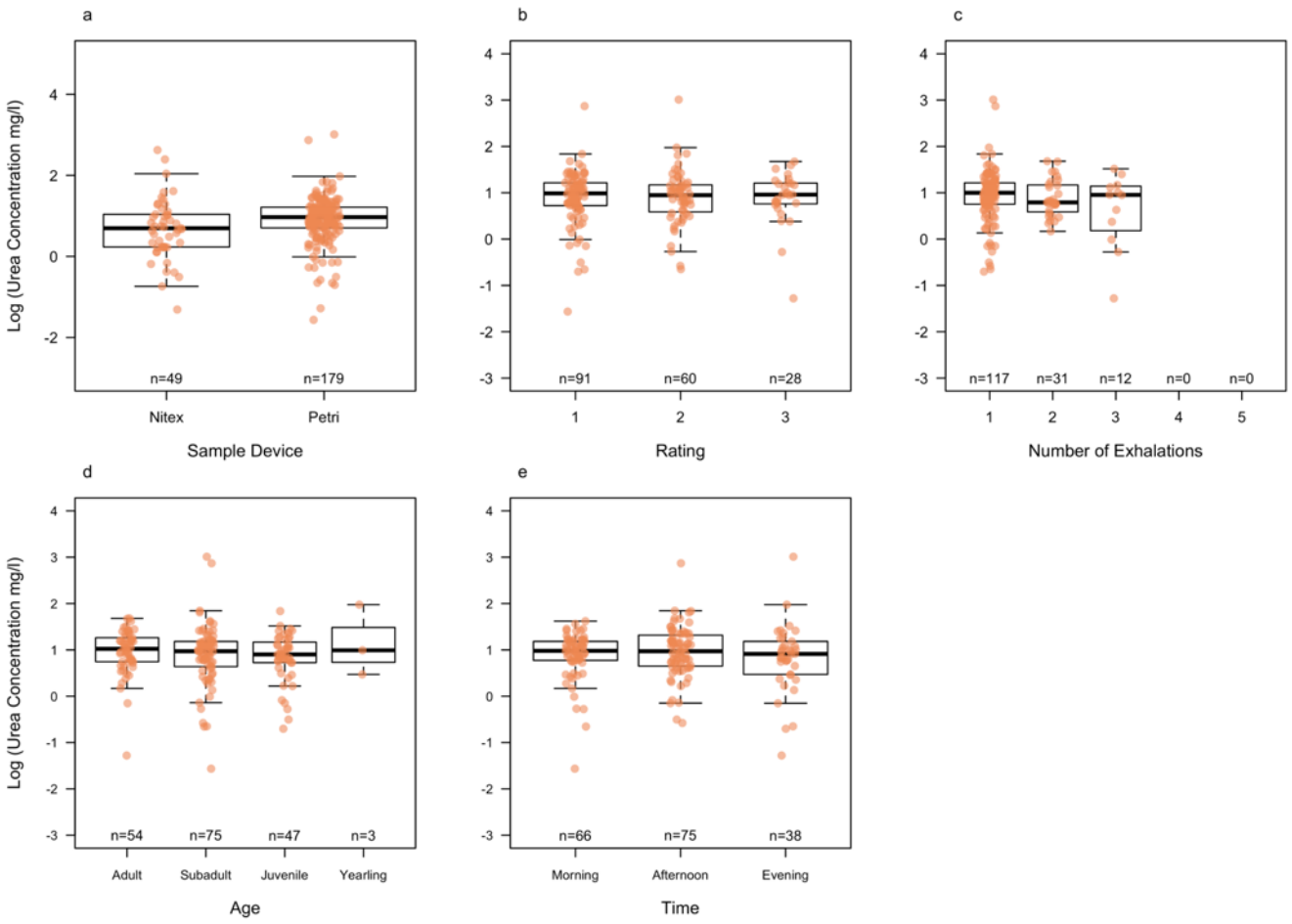

Figure S.7. Urea concentrations in relation to (a) sample device, (b) quantity rating, (c) number of exhalations, (d) age class, (e) time, and (f) ship presence. Cortisol values below the limit of detection (0.17 mg/l) were removed, along with samples collected on nitex. (a) Samples collected on nitex had significantly lower urea measurements than those collected on petri dishes (X2= 7.0983, df = 1, p-value = 0.007716). (b) Rating had no effect on urea concentrations (X2=0.094105, df = 2, p-value = 0.954). (c) Number of exhalations had no effect of urea concentration (X2= 2.2404, df = 2, p-value = 0.3262). (d) Urea levels were not influenced by age class (X2= 1.6421, df = 3, p-value = 0.6499). (e) Urea was not influenced by time (X2= 1.0845, df = 2, p-value = 0.5814).

CHAPTER 3: ASSESSING THE SEASONAL FORAGING ECOLOGY OF BOWHEAD WHALES (*BALAENA MYSTICETUS*) USING BALEEN THYROID HORMONES

Justine M. Hudson*[1,2], Cory J. D. Matthews[2], and Marianne Marcoux[2]

* Corresponding Author

[1]Univerisity of Manitoba, 66 Chancellors Circle, Winnipeg, Manitoba R3T 2N2

[2]Fisheries and Oceans Canada, Freshwater Institute, 501 University Crescent, Winnipeg, Manitoba R3T 2N6

ABSTRACT

With growing concerns over climate-change induced shifts in prey distribution, it is becoming increasingly important to understand the foraging ecology of Arctic marine mammals. Thyroid hormones play an important role in vertebrate metabolism and can be informative in foraging studies, as they decrease when some organisms experience a nutritional deficit. Previous studies have suggested that bowhead whales either forage year-round, reduce their winter foraging, or fast during the winter and in this study, we explored each of these foraging strategies using triiodothyronine (T3; a marker for nutritional stress), corticosterone (a stress-related hormone) and previously measured stable isotope values (a proxy for seasonal foraging). Baleen plates were collected between 1998-2011 from 8 subsistence-hunted bowhead whales across the Eastern Canadian Arctic. The plates were drilled at 2 cm increments from the base, down the entire length of the plate, representing ~30 days of growth. T3 concentrations ranged from 0.61-21.62 ng/g, while corticosterone concentrations ranged from 0.05-3.62 ng/g. T3 concentrations varied seasonally in two whales, while no seasonality was observed in the remaining 6 whales. The lack of regular cycles in T3 concentrations and the lack of correlation between T3 and stable isotope values in the majority of whales, suggest that bowhead whales forage year-round. Surprisingly, we observed a strong positive correlation between T3 and corticosterone in each whale, which could be the result of stressful foraging, moulting, or cross-talks between the hypothalamic-pituitary-thyroid axis and the hypothalamic-pituitary-adrenocortical axis. Based on our results, we conservatively conclude that the majority of bowhead whales forage year-round and recommend that the relationship between T3 and corticosterone be further explored.

3.1 INTRODUCTION

Accurate information on marine mammal diets, including how and where caloric requirements are met, is crucial for understanding the effects of environmental change (Bowen and Iverson 2012; Schaebs et al. 2016). Several studies have shown changes in zooplankton diversity, distribution, and phenology due to rapid changes in sea-ice and water temperatures (Edwards and Richardson 2003; Stempniewicz et al. 2007; Buchholz et al. 2010; Weydmann et al. 2014). These changes are likely to impact zooplankton-dependent predators like the bowhead whale (*Balaena mysticetus*). In the terrestrial system, for example, climate change has caused a mismatch in timing between breeding in migratory birds and the availability of their main prey, leading to a reduction in reproduction (Both et al. 2006). Reductions in caloric intake can also lead to declines in body condition and survival of individuals, which can ultimately have severe impacts on populations (Trites et al. 2003).

The Eastern Canada-West Greenland (EC-WG) bowhead whale is a migratory baleen whale that spends the entire year in Arctic and sub-Arctic waters. The population summers in Hudson Bay, Foxe Basin, and the Gulf of Boothia (Cosens and Innes 2000; Nunavut Wildlife Management Board 2000) and is thought to winter in northern Hudson Bay, Hudson Strait, and along the pack-ice lining the western coast of Greenland (Reeves and Heide-Jørgensen 1996; Koski et al. 2006; Ferguson et al. 2010; Figure 1). Bowhead whales are zooplankton specialists and feed on Arctic copepods, isopods, decapods, amphipods, mysids, and euphausiids (Laidre et al. 2007; Pomerleau, Ferguson, et al. 2011; Pomerleau et al. 2012).

Until recently, it was thought that bowhead whales foraged during the summer and fall, and fasted during the winter (Finley 2001). However, stable isotope analysis and tag data suggest that bowhead whales forage year round (Heide-Jørgensen et al. 2013; Matthews and Ferguson 2015). For stable isotope analysis, variation in carbon ($\delta^{13}C$) is associated with movement between geographic locations with isotopically distinct prey (Caraveo-Patiño and Soto 2005; Matthews and Ferguson 2015), while nitrogen ($\delta^{15}N$) is thought to reflect seasonal foraging and fasting cycles (Summers et al. 2006). Measuring $\delta^{15}N$ and $\delta^{13}C$, Matthews and Ferguson (2015) found that bowhead whales foraged year round and suggested that winter foraging could be important in their annual energy budget (Matthews and Ferguson 2015). However, stable isotope values in this study were not sufficiently discriminatory to confirm reductions in winter foraging.

Diet is often estimated using stomach content analysis, stable isotope analysis, quantitative fatty acid signature analysis, calorimetry, and genetic analysis of stomach contents and feces (Bowen and Iverson 2012), while satellite transmitters can provide information regarding foraging behaviour, including where, when, and how food is consumed (Laidre et al. 2007; Pomerleau, Patterson, et al. 2011). More recently, endocrine techniques have been employed to assess responses to food limitations and factors that might influence them (Wasser et al. 2011; Ayres et al. 2012; Schaebs et al. 2016; Wasser et al. 2017).

Thyroid hormones (THs; triiodothyronine, T3 and thyroxine, T4) play an important role in vertebrate metabolism, thermoregulation, growth, and reproduction (Wasser et al. 2010; Behringer et al. 2018). Many studies have focused on the role that THs play in metabolism, as THs decrease when some organisms experience a nutritional deficit, which slows metabolism

and allows the organism to conserve energy (Wasser et al. 2010). In humans, for example, acute and chronic fasting (i.e. low caloric intake) resulted in decreased TH levels and reduced metabolic rate (Fontana et al. 2006), whereas obesity (i.e. high caloric intake) resulted in increased TH levels (Reinehr and Andler 2002; Michalaki et al. 2006). The same pattern was observed in non-human vertebrates (Harris et al. 1987; Ayres et al. 2012; Gobush et al. 2014; Schaebs et al. 2016). Although both T3 and T4 have been used to study wildlife physiology, T3 is considered the more informative and biologically active TH, as it is produced in proportion to its use (Wasser et al. 2010).

Several studies have examined THs in whales. In the North Atlantic right whale (NARW; *Eubalaena glacialis*), entanglement (and reduced feeding) was associated with increased concentrations of THs, which was potentially caused by thyroid-promoted lipolysis, which would have allowed the animal to mobilize stored energy to meet energetic demands during periods of starvation (Lysiak et al. 2018). St. Aubin and Geraci (1988) found that beluga whales (*Delphinapterus leucas*) had significantly higher THs in the summer compared to the spring or fall and suggested that caloric intake was an unlikely factor based on a lack of observed feeding behaviour and stomach content analysis (St. Aubin and Geraci 1988). Instead they hypothesized that growth promotion could be a contributing factor (St. Aubin and Geraci 1988). St. Aubin and Geraci (1988) also found that THs decreased markedly during capture and handling of belugas and suggested that glucocorticoids (GCs) reduced circulating THs as a way to restore hormone balance; however, they also noted that THs could have been elevated during capture and the observed decrease was simply THs returning to their normal levels (St Aubin and Geraci 1988).

In addition to THs, several studies have measured GCs to assess the nutritional status of wildlife and potential mitigating factors (Ayres et al. 2012; Gobush et al. 2014). GCs increase in response to nutritional stress, and other threatening situations, including predation, entanglement in fishing gear, and capture (eg. Hayward et al. 2011; Ayres et al. 2012; Gobush et al. 2014; Thompson et al. 2014). For example, Rolland et al. (2017) found that entangled and stranded NARW had significantly higher fecal GCs than healthy whales or whales that were killed quickly after a ship strike (Rolland et al. 2017). NARW also had lower GCs in relation to reduced shipping noise (Rolland et al. 2012). Measuring THs and GCs in a single sample may provide insights into the physiological state (nutritional or psychological stress) of individuals (Wasser et al. 2010). However; it is exceedingly difficult to explore physiological changes in large cetaceans and a matrix is needed that would allow for long-term monitoring.

Baleen functions as the filter-feeding apparatus of mysticete (baleen) whales and consists of continuously growing keratin plates that line the left and right sides of the palate (Young et al. 2015). It is readily collected from stranded and necropsied whales (Hunt et al. 2017b), and in the Canadian Arctic, baleen is often collected from subsistence harvested whales. Recently, researchers have explored long-term patterns in hormones and have confirmed that fluctuations in hormones along baleen plates reflect known life histories (Hunt et al. 2014; Hunt et al. 2016; Hunt et al. 2017a; Hunt et al. 2017b; Lysiak et al. 2018). For example, progesterone levels measured along baleen plates collected from female NARW peaked during known pregnancies over a 10-year period (Hunt et al. 2016). Several studies have also examined long-term changes in diet by measuring stable isotopes along baleen plates (Aguilar et al. 2014; Matthews and Ferguson 2015).

Previous studies have suggested that bowhead whales either forage year-round, reduce their winter foraging, or fast during the winter (Finley 2001; Heide-Jørgensen et al. 2013; Matthews and Ferguson 2015). In this study, we aim to explore these foraging strategies using T3 (a marker for nutritional stress), corticosterone (a stress-related hormone), and previously measured stable isotope values (a proxy for seasonal foraging). If bowhead whales forage year-round, we predict to see a lack of seasonal variation in T3 and a lack of correlation between T3 and stable isotope values (which vary seasonally). Conversely, if bowhead whales reduce their winter feeding or fast during the winter, we predict to see a seasonal variation in T3, a negative correlation between T3 and corticosterone, and a positive correlation between T3 and stable isotope values.

3.2 METHODS

3.2.1 Baleen collection

Baleen plates were collected from subsistence harvested male bowhead whales (n=8) from the eastern Canadian Arctic between 1998-2011 (Figure 1). We focused on male bowhead whales to remove any variation caused by sex. Baleen plates were either removed from within the gumline, preserving the most recent growth (which would capture the most recent fluctuations in hormones) or cut at the gumline (which would exclude the most recent fluctuations in hormones; Table 1). Morphological data (Table 1) were collected and recorded within 24-48 hours of death and baleen plates were frozen at -25°C shortly after collection.

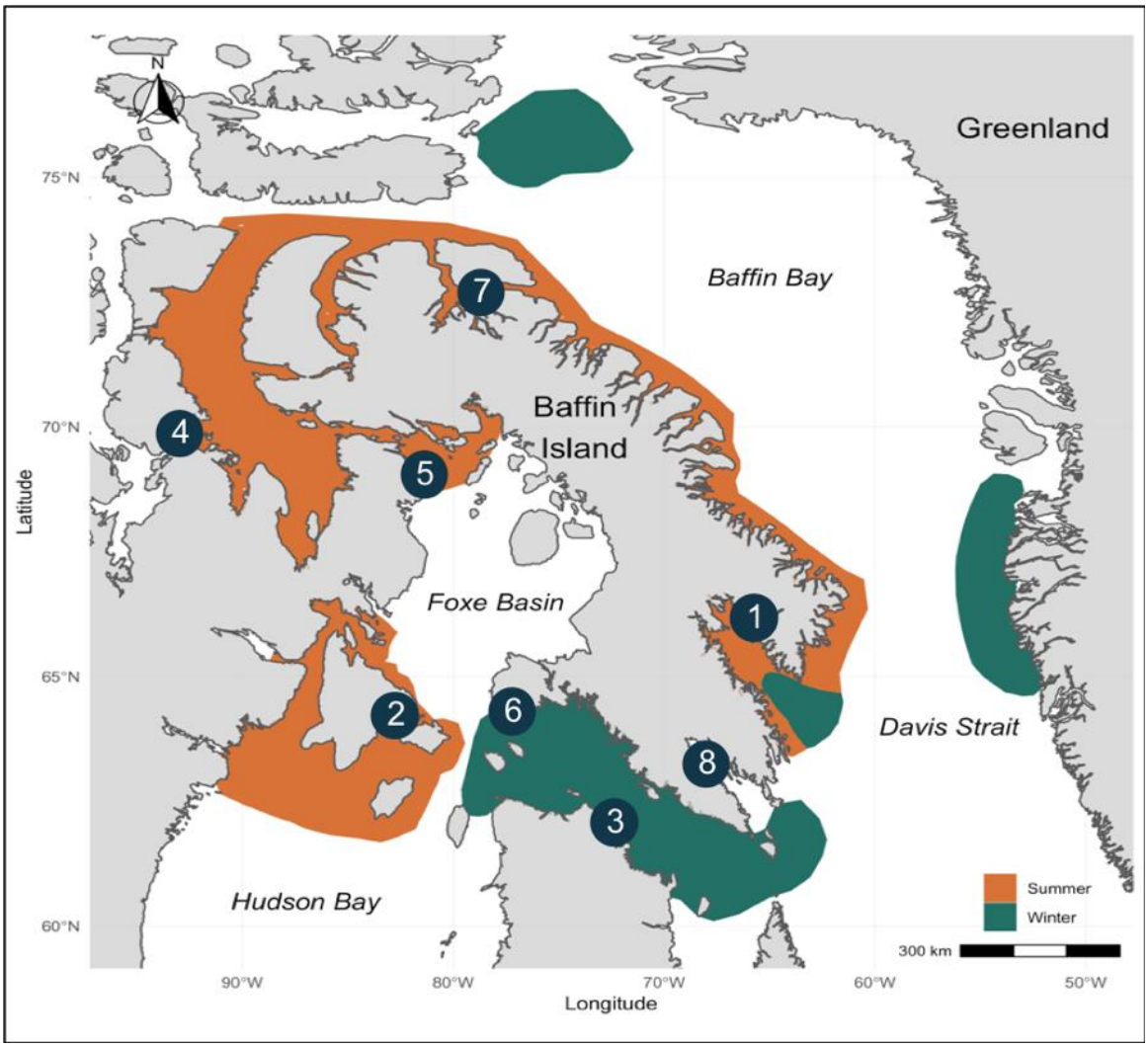

Figure 3.1. Locations where baleen was harvested from 8 bowhead whales from the Eastern Canada-West Greenland (EC-WG) population. Numbers on map correspond to whales listed in Table 1. Winter (teal) and summer (orange) distributions were reproduced from Matthews and Ferguson (2015).

Table 3.1 Morphological data for baleen plates collected from Eastern Canada-West Greenland Bowhead whales (Matthews and Ferguson 2015). Figure 1. shows the locations where each baleen plate was collected.

Whale Number	Whale ID	Date Collected	Total body length (m)	Baleen length (cm)
1	NSA-BM-98-01	July 1998	12.75	252[a]
2	BM-CH-2000-001	August 2000	11.65	256[b]
3	BM-01-2008	August 2008	14.88	296[a]
4	BM-NSA-2008-001	September 2008	10.51	174[a]
5	BM-NSA-2008-002	August 2008	13.43	228[a]
6	BM-NSA-2009-03	September 2009	15.77	314[a]
7	BM-NSA-2010-01	August 2010	12.80	206[a]
8	BM-NSA-2011-01	August 2011	14.33	286[a]

[a] Total length (removed from within the gumline)
[b] Erupted length (cut at the gumline)

3.2.2 Sample preparation and hormone extraction

Algae and other surface materials were removed from baleen plates using scrubbing pads and scalpels, and then plates were air dried (for details see Matthews and Ferguson 2015). Starting at the base (most recent growth), baleen plates were drilled at 2 cm increments down the entire length of the plate (2 cm intervals represent ~30 days of growth; Lysiak 2009) using a hand-held rotary tool with a 1/16-inch drill bit. Baleen powder from each 2 cm interval was collected in separate vials and frozen until the hormone extraction process.

For hormone extraction, we followed the protocol developed by Hunt et al. (2017). We placed 100 mg of baleen powder into a borosilicate glass tube, to which 6 ml of 100% methanol was added. The sample was put on a plate shaker for 2 hours, centrifuged for 15 minutes at 3000 rpm, and 4.5 ml of the supernatant was removed. The sample was then transferred to a new glass tube and dried under nitrogen at 45°C for 4-6 hours. Finally, samples were reconstituted in 0.50 ml of assay buffer (Triiodothyronine (T_3), Arbor Assays, Ann Arbor, MI,

USA), vortexed for 1 minute, and transferred to a cryovial. Extracted samples were frozen at -20°C until hormone analysis.

3.2.3 Hormone assays and validation

All samples were assayed using commercially available T3 and corticosterone enzyme immunoassay kits (EIA; catalogue #K056-H5 and K014-H5; Arbor Assays, Ann Arbor, MI). For T3, we modified the manufacturer's protocol by reducing the volume of standards and samples (50 µl of standards/samples; 75 ul of assay buffer in non-specific binding wells; 50 ul of assay buffer in zero wells) and we also included an additional standard to increase the range (39.06-5,000 pg/ml; Hunt et al. 2017b). We followed the manufacturer's protocol for corticosterone. For the T3 assay, the manufacturers reported the following specifications: sensitivity=37.4 pg/ml, limit of detection=46.6 pg/ml; cross-reactivities: thyroxine (T4) =0.88%, reverse T3 (3,3',5'-Triiodo-L-thyronine) =<0.1%. For the corticosterone assay, the manufacturers reported the following specifications: standard range=78.125-10,000 pg/ml, sensitivity=18.6 pg/ml, limit of detection=16.9; cross-reactivities: desoxycorticosterone=12.30%, tetrahydrocorticosterone=0.76%, aldosterone=0.62%, cortisol=0.38%, progesterone=0.24%, dexamethasone=0.12%, corticosterone-21-hemisuccinate=<0.1%, cortisone=<0.08%, estradiol=<0.08%.

Parallelism for T3 was tested by serially diluting a pool of baleen extract. The pooled sample was serially diluted in assay buffer and was compared to the T3 standard curve. Accuracy was tested by spiking an equal volume of a low concentration pooled extract (determined by parallelism test) with a standard of known concentration (2500, 1250, 625, 312.5, 156.25 pg/ml of T3). The spiked standards were assayed alongside a normal standard

curve and the unspiked pooled extract (to determine the amount of endogenous hormone). The results were graphed as observed (concentration from spiked solution minus unspiked solution) vs. expected (concentration of standard divided by 2). Corticosterone assays were validated by Hunt et al. 2017b.

We followed standard QA/QC protocols for hormone analysis, including running each sample or standard in duplicate and running each plate with a standard curve, non-specific binding wells, and zero wells. If duplicate samples resulted in a coefficient of variation (CV) greater than 10% they were re-assayed. Baleen extract was assayed neat (undiluted) and final results were converted to ng/g of baleen powder.

3.2.4 Stable Isotopes

Stable isotopes were previously measured, and the methods can be found in Matthews and Ferguson (2015).

3.2.5 Data analysis

T3 and corticosterone concentrations were calculated using GraphPad Prism (Version 8.1.2; San Diego, CA). Parallelism was tested by comparing the linear portions of a curve from a serially diluted pool of baleen extract to that of the standard curve and assessed using F statistics. Accuracy was tested using linear regression to determine the goodness-of-fit and the results were considered acceptable if the slope was between 0.7-1.3 and the R^2 was greater than 0.95 (Arbor Assay, personal communication, 2019). We investigated seasonal trends in T3, corticosterone, and $\delta^{15}N$ using autocorrelation and cross correlation functions in R Studio (R Studio Inc. Version 1.1.463; Boston, MA). Time series analysis, which includes autocorrelation

and cross correlation functions, is used to explore seasonality in data and since baleen grows linearly over time (Shao et al. 2004), it is an appropriate analysis for this study.

3.3 RESULTS

3.3.1 Validations

The T3 assay exhibited good parallelism and accuracy for bowhead baleen extract. The serially diluted pool was parallel to that of the standard curve (T3: $F_{1,9}$=1.908; P=0.20) and accuracy was considered acceptable (slope=0.94; R^2=0.98; Figure 2).

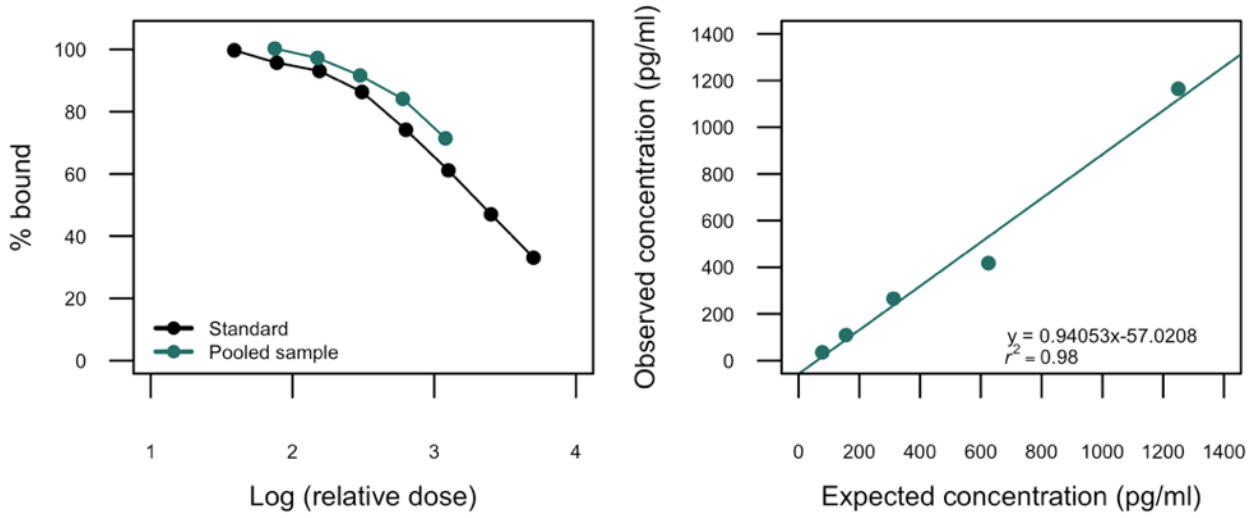

Figure 3.2. Validation plots for measuring T3 in bowhead whale baleen samples, using an enzyme immunoassay. Parallelism results for a serially diluted pooled baleen sample (1:1-1:16; teal circles; left) compared to the standard curve (39.06-5000 pg/ml; black circles; F1,9=1.908; P=0.20). Accuracy results showing the best-fit line and R2 of a T3 standard spiked with a low concentration pooled baleen extract (right).

3.3.2 Baleen hormones

T3 and corticosterone were detected in all baleen samples. T3 concentrations ranged from 0.61-21.62 ng/g, while corticosterone concentrations ranged from 0.05-3.62 ng/g (Figure 3). Autocorrelation analysis revealed seasonality in T3 concentrations in 2 baleen plates (BM-01-2008 and BM-NSA-2008-001); however, the remaining plates exhibited no seasonality (Figure 4). Plates that showed seasonality resulted in significant lags (sampling interval, i.e. 2 cm in this study) at lag 3 and lag 4 (for plates BM-NSA-2008-001 and BM-01-2008, respectively), indicating seasonality every 6-8 cm of growth.

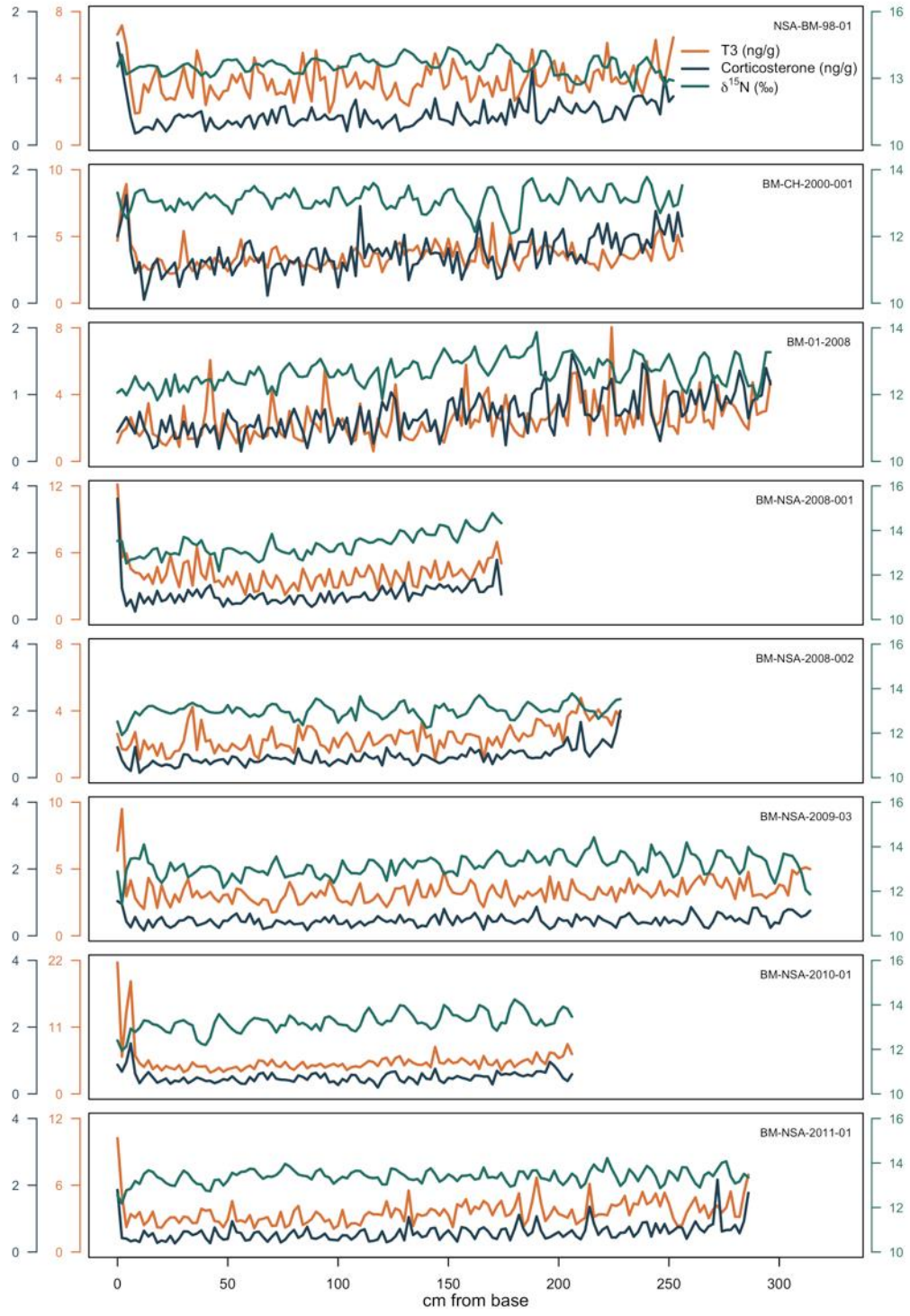

Figure 3.3. Profiles of T3 (orange; ng/g), corticosterone (navy blue; ng/g), and $\delta^{15}N$ (teal; $^{0}/_{00}$) along baleen plates. Note: y axes are not the same for each whale and 0 cm from base represents the most recent growth.

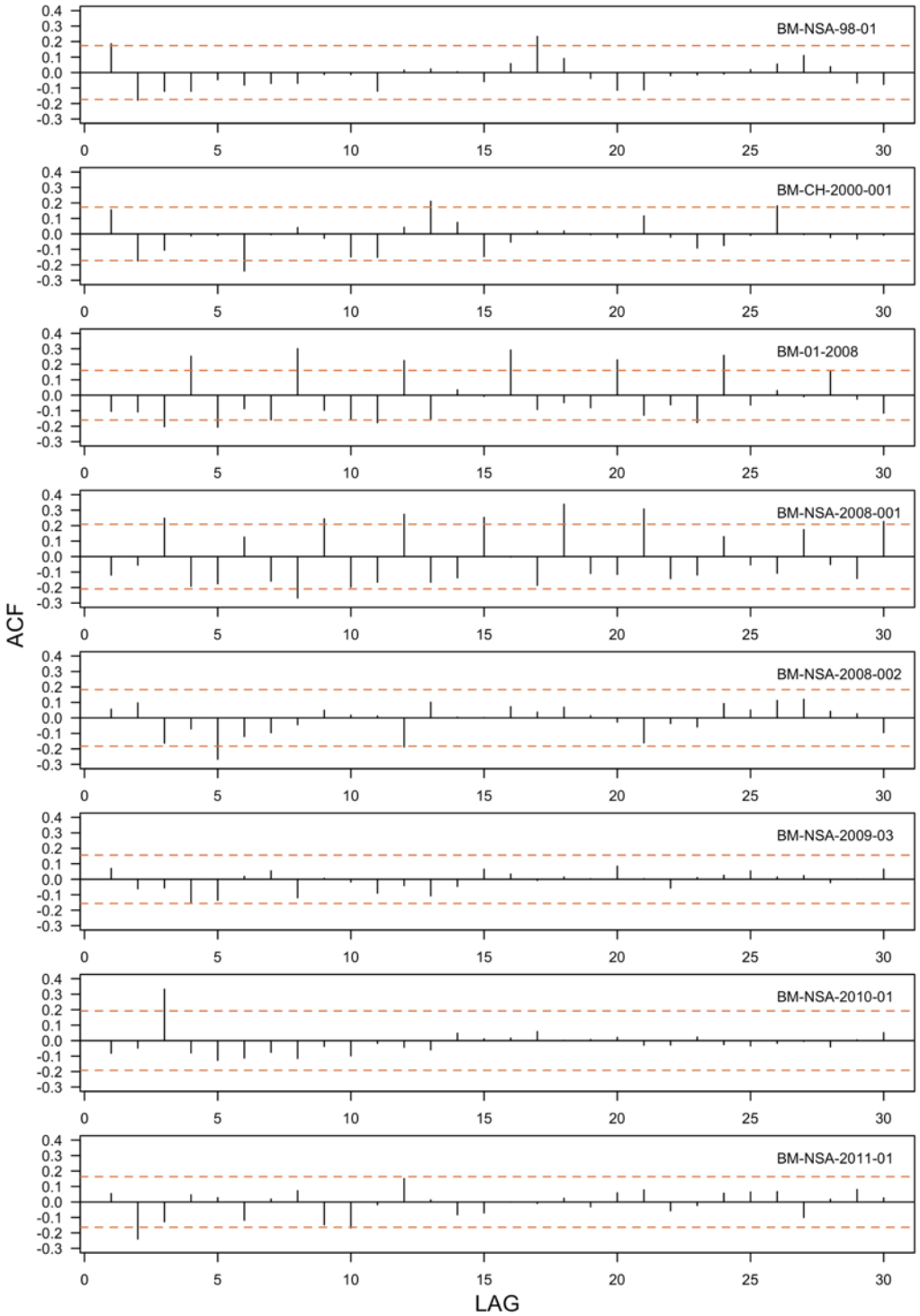
ACF
LAG
BM-NSA-98-01
BM-CH-2000-001
BM-01-2008
BM-NSA-2008-001
BM-NSA-2008-002
BM-NSA-2009-03
BM-NSA-2010-01
BM-NSA-2011-01
0.4
0.3
0.2
0.1
0.0
-0.1
-0.2
-0.3
0
5
10
15
20
25
30

Figure 3.4. Autocorrelation analysis of T3 along baleen plates collected from 8 Eastern Canada-West Greenland bowhead whales. Seasonality was observed in 2 of the 8 whales (BM-01-2008 and BM-NSA-2008-001), which was indicated by values that cross the dashed line (representing the 95% confidence interval) at repeated lags.

3.3.3 T3 and $\delta^{15}N$

Cross-correlation analysis revealed that T3 and $\delta^{15}N$ were positively correlated at lag 0 in plate BM-NSA-2008-001 and negatively correlated at lag 0 in plate BM-NSA-2009-03 (Figure 5). The remaining 6 plates showed no correlation between T3 and $\delta^{15}N$.

3.3.4 T3 and corticosterone

T3 concentrations cycled synchronously with corticosterone in each plate. Cross-correlation analysis indicated that T3 and corticosterone were positively correlated at lag 0 in all plates, with the exception of BM-01-2008 that showed the strongest positive correlation at lag 1 (Figure 5). A simple correlation graph (Figure 6) shows the relationship between T3 and corticosterone and the R^2, slopes, and P values are detailed in Table 2.

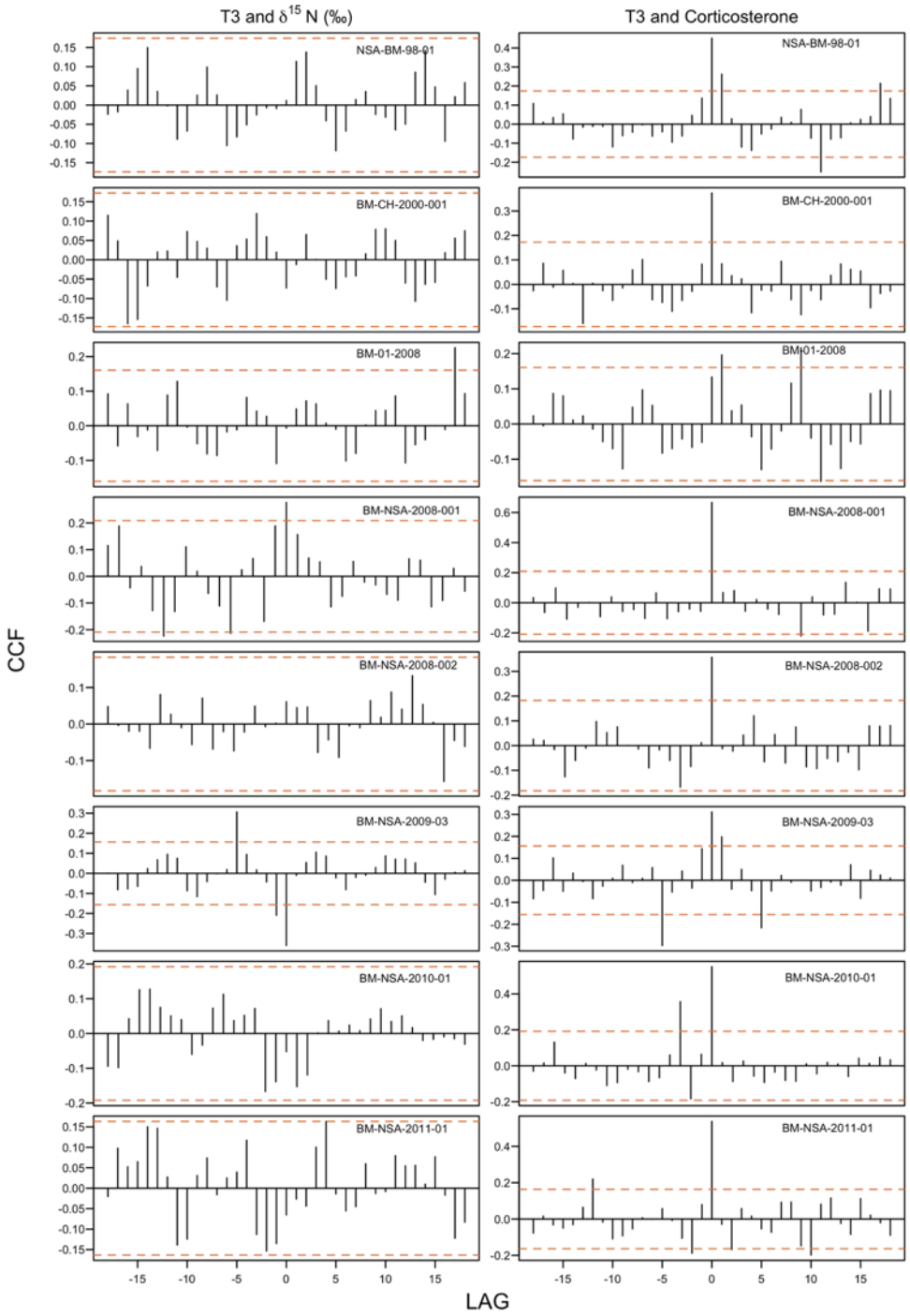

Figure 3.5. Cross-correlation analysis of T3 vs. $\delta^{15}N$ and T3 vs. corticosterone measured along baleen plates collected from 8 Eastern Canada-West Greenland bowhead whales. T3 and $\delta^{15}N$ were positively correlated in whales BM-NSA-2008-001 and negatively correlated in BM-NSA-2009-03, which is indicated by values that cross the dashed line representing the 95% confidence interval. T3 and corticosterone were positively correlated at lag in all whales, with the exception of BM-01-2008, which showed the strongest correlation at lag 1.

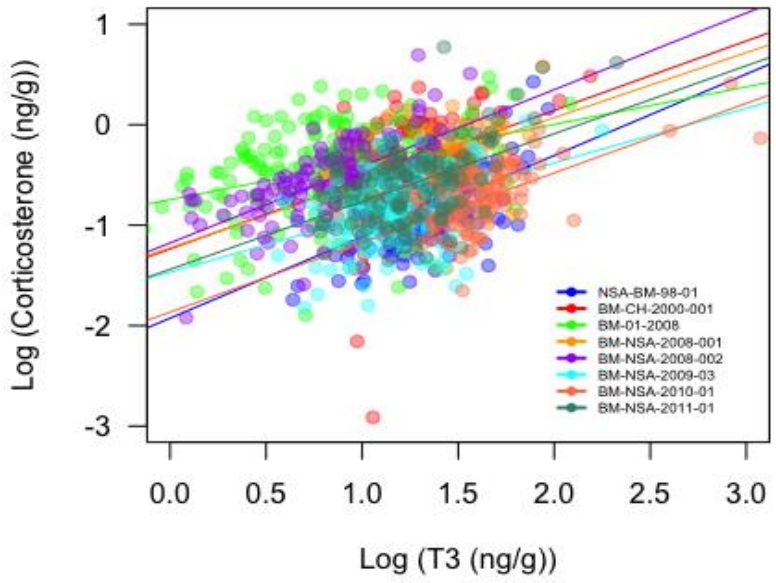

Figure 3.6. Correlation between T3 and corticosterone measured along baleen plates from 8 Eastern Canada-West Greenland bowhead whales. Slope, R^2, and P values are given in Table 2.

Table 3.2. Results of a linear regression model of the correlation between T3 and corticosterone.

Whale ID	Slope	R^2	P value
NSA-BM-98-01	0.8098	0.2869	5.22e-11
BM-CH-2000-001	0.6900	0.1283	1.827e-05
BM-01-2008	0.3747	0.1256	5.506e-06
BM-NSA-2008-001	0.6472	0.2422	6.664e-07
BM-NSA-2008-002	0.763	0.4142	5.229e-15
BM-NSA-2009-03	0.5438	0.1276	2.438e-06
BM-NSA-2010-01	0.6928	0.3666	5.961e-12
BM-NSA-2011-01	0.6754	0.2333	5.235e-10

3.4 DISCUSSION

The goal of this study was to explore 3 proposed foraging strategies of bowhead whales. They include foraging year-round, reduced winter foraging, and winter fasting. We predicted

that if bowhead whales foraged year-round, we would see no seasonal variation in T3 and corticosterone concentrations and no correlation between T3 and stable isotope values. Whereas, if bowhead whales were undergoing seasonal variation in foraging behaviour (i.e. reduced winter feeding or winter fasting), we predicted to see seasonal variation in T3 concentrations, a negative correlation between T3 and corticosterone, and a positive correlation between T3 and stable isotope values. In this study, we observed a lack of seasonal variation in T3 in the majority of bowhead whales and no correlation between T3 and stable isotope values, suggesting that bowheads do feed year-round. Unexpectedly, we also observed a significant positive correlation between T3 and corticosterone in each whale, suggesting that variation in T3 and corticosterone might be influenced by other factors.

Thyroid hormones play an important role in vertebrate metabolism but also in thermoregulation, and reproduction (Wasser et al. 2010; Behringer et al. 2018). During periods of fasting, the hypothalamus-pituitary-thyroid (HPT) axis becomes down-regulated, resulting in a reduction of THs (Boelen et al. 2008; Behringer et al. 2018). The reduction in THs due to fasting is thought to be an adaptive trait to conserve energy during times of nutritional deficits (Boelen et al. 2008). In this study, we observed a lack of seasonality in T3 in the majority of bowhead whales, confirming that bowhead whales feed year-round and do not undergo periods of seasonal foraging. Although we did not observe seasonal variation in T3, we did observe variation in T3 each whale. This could be the result of non-seasonal foraging behaviour or the result of other factors, such as thermoregulation, mating, or molting.

Two whales (BM-01-2008 and BM-NSA-2008-001) did experience seasonal changes in T3 that occurred 2 to 3 times per year, based on the assumption that 2 cm represents ~30 days of growth (Lysiak 2009). This could indicate individual differences in foraging behaviour or could be the result of other biological or environmental factors. Seasonal fluctuations in T3 could indicate seasonal changes in energy consumption; however, if this was the case, we would expect to see a positive relationship between T3 and $\delta^{15}N$, which was only observed in BM-NSA-2008-001. Thermoregulation, molting, and mating are other variables that influence T3 (Gobush et al. 2014; Cristóbal-Azkarate et al. 2016), and could potentially explain seasonal fluctuations observed in these whales, as we know that bowhead whales migrate between areas with different water temperatures, molt, and mate seasonally. In addition to BM-NSA-2008-001, BM-NSA-2009-03 also showed a correlation between T3 and $\delta^{15}N$; however, the relationship was negative and the reason behind the negative correlation is unclear.

We predicted to see a negative correlation between T3 and corticosterone in bowhead whales if they underwent periods of fasting or reduced winter feeding. Interestingly, our results showed a positive correlation between T3 and corticosterone in each whale, which could potentially be explained by 3 factors that are not mutually exclusive: 1) adequate yet stressful or intense foraging; 2) molting; and 3) cross-talk between the HPT axis and the hypothalamic-pituitary-adrenocortical (HPA) axis.

Bowhead whales are filter-feeders and use ram filtration to feed on Arctic zooplankton (Laidre et al. 2007; Simon et al. 2009; Pomerleau, Ferguson, et al. 2011; Pomerleau et al. 2012). Bowheads must hold their breath for long-periods of time in order to maximize the volume of prey consumed and limit their oxygen consumption (Simon et al. 2009). This could lead to a

number of physiological changes, including bradycardia, vascular redistribution, accumulation of lactic acid, and declines in body temperature (Kooyman et al. 1981; Atkinson et al. 2015). Although marine mammals are adapted to diving, the process could potentially be stressful for bowhead whales, which could explain the relationship between high GCs and THs. Gobush et al. (2014) also found a positive correlation between T3 and GCs in Hawaiian monk seals (*Monachus schauinslandi*) at 4 of their sampling sites and suggested that the relationship was due to increased physiological stress while foraging for prey; however, this study measured hormone metabolites from faeces, which would reflect shorter-term fluctuations in hormones than baleen.

T3 and corticosterone have also been observed to be positively correlated during times of molt. For example, in Hawaiian monk seals, molting was associated with high concentrations of T3 and GCs (Gobush et al. 2014). It was thought that the stress of the molt caused an increase in GCs, as monk seals were fasting, less rested, on high alert, and tended to have a high body temperatures, while T3 was thought to increase due to pelage growth (Gobush et al. 2014). Thyroid hormones also increased during the molting period of ringed seals (*Pusa hispida*) (Routti et al. 2010). Bowhead whales also molt to remove ectoparasites, diatoms, and to get rid of solar damaged skin and have been observed rubbing on rocks to aid in the process (Fortune et al. 2017). The process of molting and rubbing on rocks could be energetically costly; therefore, contributing to high concentrations of corticosterone, while new epidermal growth may be the cause of high T3 concentrations. If T3 and corticosterone were influenced by molting, we would expect to see seasonal fluctuations in both hormones, as bowhead whales have only been observed molting annually during the summer and fall (Fortune et al. 2017). We

did not observe seasonal variation in T3 and corticosterone; however, it is unknown whether bowhead whales molt solely in the summer and fall or if it is possible that they molt year-round.

The correlation between T3 and corticosterone also suggests cross-talk between the HPT and the HPA axes. The central nervous system produces THs when stimulated by environmental stressors (Castañeda Cortés et al. 2014). The hypothalamus produces thyrotropin-releasing hormone (TRH), which stimulates the anterior pituitary gland to release thyroid-stimulating hormone (TSH or thyrotropin) into the blood system (Castañeda Cortés et al. 2014; Behringer et al. 2018). TSH stimulates thyroid follicles to produce T3 and T4, while T3 and T4 regulate the production of TRH and TSH through a negative feedback mechanism (Behringer et al. 2018). In addition to TH production, it is thought that the HPT axis may also be influenced by the HPA axis (Castañeda Cortés et al. 2014; Behringer et al. 2018). Corticotropin-releasing hormone (CRH) stimulates the HPA axis, leading to the release of GCs from the adrenal cortex; however, CRH also stimulates the production of TSH in non-mammal vertebrates (Castañeda Cortés et al. 2014; Behringer et al. 2018).

The cross-talks between the two axes is thought to be an adaptive evolutionary trait for dealing with stressful environments (Behringer et al. 2018). For example, in tadpoles experiencing environmental stress, high CRH concentrations led to increased production of GCs and THs, which sped up metamorphosis (Bonett et al. 2010; Behringer et al. 2018). Additionally, injecting CRH into chicken embryos increased corticosterone production, along with T3 and T4 (Meeuwis et al. 1989). The interaction between the HPT and HPA axes is less understood in mammals; however, some studies have shown that stressful situations were associated with

high levels of GCs and low levels in T3 (Burr et al. 1976; Behringer et al. 2018; McCormley et al. 2018).

Although several studies have shown that T3 increases with energy intake (Ortiz et al. 2010; Wasser et al. 2010; Ayres et al. 2012; Gobush et al. 2014; Schaebs et al. 2016), the relationship between nutritional deficits and T3 in marine mammals is less clear, with some studies observing no change in T3 concentrations during times of fasting (Crocker et al. 2012; Kelso et al. 2012). This is thought to be a result of large thyroid glands possessed by cetaceans that produce higher concentrations of THs compared to terrestrial mammals (Behringer et al. 2018). Additionally, animals with greater fat stores may not need to down-regulate during periods of nutritional deficits (Behringer et al. 2018).

3.5 CONCLUSION

Baleen hormone analysis is a useful tool for assessing long-term physiological changes in bowhead whales. Based on the lack of seasonal fluctuations in T3 concentrations and no correlation between T3 and $\delta^{15}N$, we conservatively conclude that bowhead whales feed year-round and do not exhibit seasonal foraging patterns. A strong positive correlation between T3 and corticosterone in each whale suggest that there are other physiological factors at play and future studies should explore the effects of stressful foraging, molting, and cross-talk between the HPT and HPA axes on T3 and corticosterone. Furthermore, future studies should also examine the relationship between T3, GCs, and reproductive hormones to rule out reproductive influences and include female bowhead whales to explore sex-related differences. Lastly, these studies should focus on validating T3 concentrations in marine mammals, as

animals with large fat stores may respond differently to reduced energy intake. These results further our knowledge of bowhead whale foraging behaviour and can potentially be used to identify and protect important foraging habitat.

ACKNOWLEDGEMENTS

The authors thank the Hunters and Trappers Organizations and wildlife officers in Nunavut and Nunavik for collecting baleen samples. We also thank Dr. Kathleen Hunt and Dr. Loren Buck for their support in this project. Thank you also to Fisheries and Oceans Canada, the University of Manitoba, ArcticNet, and NSERC for providing funding and resources that made this project possible.

LITERATURE CITED

Aguilar A, Giménez J, Gómez-Campos E, Cardona L, Borrell A. 2014. δ15N value does not reflect fasting in mysticetes. PLoS One 9.

Atkinson S, Crocker D, Houser D, Mashburn K. 2015. Stress physiology in marine mammals: how well do they fit the terrestrial model? J. Comp. Physiol. B 185:463–486.

Ayres KL, Booth RK, Hempelmann JA, Koski KL, Emmons CK, Baird RW, Balcomb-Bartok K, Hanson MB, Ford MJ, Wasser SK. 2012. Distinguishing the Impacts of Inadequate Prey and Vessel Traffic on an Endangered Killer Whale (*Orcinus orca*) Population. PLoS One 7:e36842.

Behringer V, Deimel C, Hohmann G, Negrey J, Schaebs FS, Deschner T. 2018. Applications for non-invasive thyroid hormone measurements in mammalian ecology, growth, and maintenance. Horm. Behav. 105:66–85.

Boelen A, Wiersinga WM, Fliers E. 2008. Fasting-induced changes in the hypothalamus-pituitary-thyroid axis. Thyroid 18:123–129.

Bonett RM, Hoopfer ED, Denver RJ. 2010. Molecular mechanisms of corticosteroid synergy with thyroid hormone during tadpole metamorphosis. Gen. Comp. Endocrinol. 168:209–19.

Both C, Bouwhuis S, Lessells CM, Visser ME. 2006. Climate change and population declines in a long-distance migratory bird. Nature 441:81–83.

Bowen WD, Iverson SJ. 2012. Methods of estimating marine mammal diets: A review of validation experiments and sources of bias and uncertainty. Mar. Mammal Sci. 29:n/a-n/a.

Buchholz F, Buchholz C, Weslawski JM. 2010. Ten years after: Krill as indicator of changes in the

macro-zooplankton communities of two Arctic fjords. Polar Biol. 33:101–113.

Burr WA, Ramsden DB, Griffiths RS, Black EG, Hoffenberg R. 1976. Effect of a single dose of dexamethasone on serum concentrations of thyroid hormones. Lancet:58–61.

Caraveo-Patiño J, Soto LA. 2005. Stable carbon isotope ratios for the gray whale (*Eschrichtius robustus*) in the breeding grounds of Baja California Sur, Mexico. Hydrobiologia 539:99–107.

Castañeda Cortés DC, Langlois VS, Fernandino JI. 2014. Crossover of the hypothalamic pituitary-adrenal/interrenal, -thyroid, and -gonadal axes in testicular development. Front. Endocrinol. (Lausanne). 5:139.

Cosens SE, Innes S. 2000. Distribution and numbers of bowhead whales (*Balaena mysticetus*) in northwestern Hudson Bay in August 1995. Arctic 53:36–41.

Cristóbal-Azkarate J, Maréchal L, Semple S, Majolo B, MacLarnon A. 2016. Metabolic strategies in wild male Barbary macaques: evidence from faecal measurement of thyroid hormone. Biol. Lett. 12.

Crocker DE, Ortiz RM, Houser DS, Webb PM, Costa DP. 2012. Hormone and metabolite changes associated with extended breeding fasts in male northern elephant seals (*Mirounga angustirostris*). Comp. Biochem. Physiol. - A Mol. Integr. Physiol. 161:388–394.

Edwards M, Richardson AJ. 2003. Impact of climate change on marine pelagic phenology and trophic mismatch. Nature 4030:881.

Ferguson SH, Dueck L, Loseto LL, Luque SÁP. 2010. Bowhead whale *Balaena mysticetus* seasonal selection of sea ice. Mar. Ecol. Prog. Ser. 411:285–297.

Finley KJ. 2001. Natural History and Conservation of the Greenland Whale, or Bowhead, in the

Northwest Atlantic. Arctic 54:55–76.

Flower JE, Allender MC, Giovanelli RP, Summers SD, Spoon TR, Leger JA St., Goertz CEC, Dunn JL, Romano TA, Hobbs RC, et al. 2015. Circulating concentrations of thyroid hormone in beluga whales (*Delphinapterus leucas*): influence of age, sex, and season. J. Zoo Wildl. Med. 46:456–467.

Fontana L, Klein S, Holloszy JO, Premachandra BN. 2006. Effect of Long-Term Calorie Restriction with Adequate Protein and Micronutrients on Thyroid Hormones. J. Clin. Endocrinol. Metab. 91:3232–3235.

Fortune SME, Koski WR, Higdon JW, Trites AW, Baumgartner MF, Ferguson SH. 2017. Evidence of molting and the function of “rock-nosing” behavior in bowhead whales in the eastern Canadian Arctic. PLoS One 12:e0186156.

Gobush KS, Booth RK, Wasser SK. 2014. Validation and application of non-invasive glucocorticoid and thyroid hormone measures in free-ranging Hawaiian monk seals. Gen. Comp. Endocrinol. 195:174–182.

Harris ARC, Fang S-L, Azizi F, Lipworth L, Vagenakis AG, Braverman LE. 1987. Effect of starvation on biochemical indices of renal function in the rat. Br. J. Exp. Pathol. 68:767–775.

Hayward LS, Bowles AE, Ha JC, Wasser SK. 2011. Impacts of acute and long-term vehicle exposure on physiology and reproductive success of the northern spotted owl. Ecosphere 2:art65.

Heide-Jørgensen MP, Laidre KL, Nielsen NH, Hansen RG, Røstad A. 2013. Winter and spring diving behavior of bowhead whales relative to prey. Anim. Biotelemetry 1:15.

Hunt KE, Lysiak NS, Moore M, Rolland RM. 2017a. Multi-year longitudinal profiles of cortisol

and corticosterone recovered from baleen of North Atlantic right whales (*Eubalaena glacialis*). Gen. Comp. Endocrinol. 254:50–59.

Hunt KE, Lysiak NS, Moore MJ, Rolland RM. 2016. Longitudinal progesterone profiles in baleen from female North Atlantic right whales (*Eubalaena glacialis*) match known calving history. Conserv. Physiol. 4:cow014.

Hunt KE, Lysiak NS, Robbins J, Moore MJ, Seton RE, Torres L, Buck CL. 2017b. Multiple steroid and thyroid hormones detected in baleen from eight whale species. Conserv. Physiol. 5.

Hunt KE, Stimmelmayr R, George C, Hanns C, Suydam R, Brower H, Rolland RM. 2014. Baleen hormones: A novel tool for retrospective assessment of stress and reproduction in bowhead whales (*Balaena mysticetus*). Conserv. Physiol. 2:1–12.

Kelso EJ, Champagne CD, Tift MS, Houser DS, Crocker DE. 2012. Sex differences in fuel use and metabolism during development in fasting juvenile northern elephant seals. J. Exp. Biol. 215:2637–2645.

Kooyman GL, Castellini MA, Davis RW. 1981. Physiology of diving in marine mammals. Annu. Rev. Physiol. 43:343–356.

Koski WR, Heide-Jørgensen MP, Laidre KL. 2006. Winter abundance of bowhead whales, *Balaena mysticetus*, in the Hudson Strait, March 1981. J. Cetacean Res. Manag. 8:139–144.

Laidre KL, Heide-Jørgensen MP, Nielsen TG. 2007. Role of the bowhead whale as a predator in West Greenland. Mar. Ecol. Prog. Ser. 346:285–297.

Lysiak N. 2009. Investigating the migration and foraging ecology of North Atlantic right whales with stable isotope geochemistry of baleen and zooplankton. Boston University.

Lysiak NSJ, Trumble SJ, Knowlton AR, Moore MJ. 2018. Characterizing the Duration and Severity

of Fishing Gear Entanglement on a North Atlantic Right Whale (*Eubalaena glacialis*) Using Stable Isotopes, Steroid and Thyroid Hormones in Baleen. Front. Mar. Sci. 5:168.

Matthews CJD, Ferguson SH. 2015. Seasonal foraging behaviour of eastern Canada-West Greenland bowhead whales: An assessment of isotopic cycles along baleen. Mar. Ecol. Prog. Ser. 522:269–286.

McCormley MC, Champagne CD, Deyarmin JS, Stephan AP, Crocker DE, Houser DS, Khudyakov JI. 2018. Repeated adrenocorticotropic hormone administration alters adrenal and thyroid hormones in free-ranging elephant seals. Conserv. Physiol. 6.

Meeuwis R, Michielsen R, Decuypere E, Kuhn ER. 1989. Thyrotropic activity of the ovine corticotropin-releasing factor in the chick embryo. Gen. Comp. Endocrinol. 76:357–363.

Michalaki MA, Vagenakis AG, Leonardou AS, Argentou MN, Habeos IG, Makri MG, Psyrogiannis AI, Kalfarentzos FE, Kyriazopoulou VE. 2006. Thyroid Function in Humans with Morbid Obesity. Thyroid 16:73–78.

Nunavut Wildlife Management Board. 2000. Final report of the Inuit bowhead knowledge study.

Ortiz RM, Long B, Casper D, Ortiz LL, Williams TM. 2010. Biochemical and hormonal changes during acute fasting and re-feeding in bottlenose dolphins (*Tursiops truncatus*). Mar. Mammal Sci. 26:409–419.

Pomerleau C, Ferguson SH, Walkusz W. 2011. Stomach contents of bowhead whales (*Balaena mysticetus*) from four locations in the Canadian Arctic. Polar Biol. 34:615–620.

Pomerleau C, Lesage V, Ferguson SH, Winkler G, Petersen SD, Higdon JW. 2012. Prey assemblage isotopic variability as a tool for assessing diet and the spatial distribution of

bowhead whale *Balaena mysticetus* foraging in the Canadian eastern Arctic. Mar. Ecol. Prog. Ser. 469:161–174.

Pomerleau C, Patterson T, Luque S, Lesage V, Heide-Jørgensen M, Dueck L, Ferguson S. 2011. Bowhead whale *Balaena mysticetus* diving and movement patterns in the eastern Canadian Arctic: implications for foraging ecology. Endanger. Species Res. 15:167–177.

Reeves RR, Heide-Jørgensen MP. 1996. Recent status of bowhead whales, *Balaena mysticetus*, in the wintering grounds off West Greenland. Polar Res. 15:115–125.

Reinehr T, Andler W. 2002. Thyroid hormones before and after weight loss in obesity. Arch. Dis. Child. 87:320–323.

Rolland RM, McLellan WA, Moore MJ, Harms CA, Burgess EA, Hunt KE. 2017. Fecal glucocorticoids and anthropogenic injury and mortality in North Atlantic right whales *Eubalaena glacialis*. Endanger. Species Res. 34:417–429.

Rolland RM, Parks SE, Hunt KE, Castellote M, Corkeron PJ, Nowacek DP, Wasser SK, Kraus SD. 2012. Evidence that ship noise increases stress in right whales. Proceedings. Biol. Sci. 279:2363–8.

Routti H, Jenssen BM, Lydersen C, Bäckman C, Arukwe A, Nyman M, Kovacs KM, Gabielsen GW. 2010. Hormone, vitamin and contaminant status during the moutling/fasting period in ringed seals (*Pusa* [*Phoca*] *hispida*) from Svalbard. Comp. Biochem. and Physiol, Part A 155: 70-76

Schaebs FS, Wolf TE, Behringer V, Deschner T. 2016. Fecal thyroid hormones allow for the non-invasive monitoring of energy intake in capuchin monkeys.

Shao Q, Wilson MD, Romanek CS, Hobson KA. 2004. Time series analysis of elemental and

isotopic data from biomineralized whale tissue. Enviro. and Ecol. Stats 11:323-337

Simon M, Johnson M, Tyack P, Madsen PT. 2009. Behaviour and kinematics of continuous ram filtration in bowhead whales (*Balaena mysticetus*). Proc. R. Soc. B Biol. Sci. 276:3819–3828.

St Aubin D. J., Geraci JR. 1988. Seasonal variation in thyroid morphology and secretion in the white whale, *Delphinapterus leucas*. Can. J. Zool. 67:263–267.

St Aubin David J., Geraci JR. 1988. Capture and Handling Stress Suppresses Circulating Levels of Thyroxine (T4) and Triiodothyronine (T3) in Beluga Whales *Delphinapterus leucas*. Physio. Zool. 61:170-175

Stempniewicz L, Błachowiak-Samołyk K, Wesławski JM. 2007. Impact of climate change on zooplankton communities, seabird populations and arctic terrestrial ecosystem-A scenario. Deep. Res. Part II Top. Stud. Oceanogr. 54:2934–2945.

Summers EL, Estrada JA, Zeeman SI. 2006. A note on geographic and seasonal fluctuations in the isotopic composition of baleen in four North Atlantic right whales (*Eubalaena glacialis*). J. Cetacean Res. Manag. 8:241–245.

Trites W, Donnelly CP, Trites AW. 2003. The decline of Steller sea lions *Eumetopias jubatus* in Alaska: a review of the nutritional stress hypothesis.

Wasser SK, Azkarate JC, Booth RK, Hayward L, Hunt K, Ayres K, Vynne C, Gobush K, Canales-Espinosa D, Rodríguez-Luna E. 2010. Non-invasive measurement of thyroid hormone in feces of a diverse array of avian and mammalian species. Gen. Comp. Endocrinol. 168:1–7.

Wasser SK, Keim JL, Taper ML, Lele SR. 2011. The influences of wolf predation, habitat loss, and human activity on caribou and moose in the Alberta oil sands. Front. Ecol. Environ. 9:546–551.

Wasser SK, Lundin JI, Ayres K, Seely E, Giles D, Balcomb K, Hempelmann J, Parsons K, Booth R. 2017. Population growth is limited by nutritional impacts on pregnancy success in endangered Southern Resident killer whales (*Orcinus orca*). PLoS One 12.

Weydmann A, Carstensen J, Goszczko I, Dmoch K, Olszewska A, Kwasniewski S. 2014. Shift towards the dominance of boreal species in the Arctic: Inter-annual and spatial zooplankton variability in the West Spitsbergen Current. Mar. Ecol. Prog. Ser. 501:41–52.

Young S, Deméré TA, Ekdale EG, Berta A, Zellmer N. 2015. Morphometrics and Structure of Complete Baleen Racks in Gray Whales (*Eschrichtius robustus*) From the Eastern North Pacific Ocean. Anat. Rec. 298:703–719.

CHAPTER 4: SUMMARY AND CONCLUSION

SUMMARY

To improve our understanding of how Arctic marine mammals will respond to climate change and associated threats, I conducted two studies examining the physiology of beluga and bowhead whales. The first objective of Chapter 2 was to determine whether it was possible to collect blow from free-swimming beluga whales. Although blow has been collected from wild-captured beluga whales (Thompson et al. 2014), to date, no studies have attempted to collect blow from free-swimming beluga whales. The second objective of Chapter 2 was to determine what factors influenced cortisol and urea concentrations, including collection device, quantity rating, number of exhalations, age class, time, and ship presence. One of the biggest challenges with blow collection is the inability to quantify blow sample volumes due to seawater contamination and evaporation (Burgess et al. 2018). In an attempt to solve this issue, the third and final objective of Chapter 2 was to assess whether urea could be used as a dilution marker to normalize blow samples.

The overall objective of Chapter 3 was to assess the foraging ecology of Eastern Canada-West Greenland (EC-WG) bowhead whales by measuring triiodothyronine (T3) and corticosterone in baleen plates and comparing them with previously measured stable isotope values. T3 was used as a proxy for nutritional stress, corticosterone was used as a proxy for psychological stress, and stable isotopes were used as a proxy for foraging behaviour. I explored 3 foraging strategies brought forward by previous studies: 1) bowhead whales forage year-round; 2) bowhead whales reduce their winter foraging; and 3) bowhead whales fast during the winter.

FINDINGS

Blow collection has been used to study both reproductive (Hogg et al. 2009; Hunt, Rolland, et al. 2014; Thompson et al. 2014; Richard et al. 2017; Mingramm et al. 2019) and stress hormones (Hunt, Rolland, et al. 2014; Thompson et al. 2014; Burgess et al. 2018; Mingramm et al. 2019), genetics (Frère et al. 2010), and disease (Geoghegan et al. 2006; Acevedo-Whitehouse et al. 2010) in a variety of whale species and in Chapter 2, I demonstrated, for the first time, that blow could be collected from free-swimming beluga whales. Knowing that blow can be successfully collected from the WHB beluga population allows future researchers to use blow collection as a way to explore beluga physiology and other aspects of beluga health. The results from this study also found that urea was an unsuitable dilution marker to normalize blow samples. This finding corroborates results from Mingramm et al. (2019) that also found that urea was an unsuitable dilution marker.

Although I was unable to normalize blow samples, I found that sample device, quantity rating, and age class significantly influenced absolute cortisol concentrations. Specifically, samples collected on nitex, 3-rated samples (highest quantity rating), and adults had the highest cortisol concentrations, which is most likely due to increased sample volume. Nitex and veil-like materials have been used for a number of blow studies (Hogg et al. 2009; Hunt, Rolland, et al. 2014; Thompson et al. 2014), as they are thought to retain greater volumes of sample. However, I also found that nitex resulted in high amounts of assay interference, which could also explain why cortisol levels were higher for samples collected on nitex. This information can be useful for future studies that aim to collect blow from free-swimming beluga whales or other small cetaceans and also for studies hoping to collect blow to answer

other physiological questions. These results also highlight the importance of evaluating collection materials and factors that may influence hormone concentrations.

Chapter 3 assessed the foraging ecology of EC-WG bowhead whales by measuring T3 and corticosterone along baleen plates and comparing them with previously measured stable isotope values. Based on a lack of seasonal variation in T3 concentrations and a lack of correlation between T3 and stable isotope values, I conservatively concluded that the majority of bowhead whales foraged year-round. Although most whales did not exhibit seasonality in T3 concentrations, 2 whales did. This could potentially indicate individual differences in foraging behaviour or could be the result of other environmental or biological factors that influence T3, such as thermoregulation, molting, or mating.

Interestingly, I also found a strong positive correlation between T3 and corticosterone in each whale. One potential explanation for this relationship is adequate yet stressful foraging. Gobush et al. (2014) found a similar correlation between T3 and glucocorticoids (GCs) in Hawaiian monk seals and suggested that although the seals were consuming adequate amounts of prey (high T3), the foraging process could have been stressful (high GCs). Since bowhead whales must dive to consume prey, which can be physiologically taxing (Kooyman et al. 1981; Atkinson et al. 2015), I hypothesized that this could also explain our results. Molting is another potential cause, as Gobush et al (2014) also found that T3 and GCs increased during periods of molt in Hawaiian monk seals. Bowhead whales have also been observed molting, which may explain the patterns I saw. A third potential cause for a positive correlation between T3 and corticosterone is cross-talk between the hypothalamic-pituitary-thyroid (HPT) axis and the hypothalamic-pituitary-adrenocortical (HPA) axis, which has been documented in a several

studies (Meeuwis et al. 1989; Bonett et al. 2010; Behringer et al. 2018). Further analyses are required to explain the relationship between T3 and corticosterone.

FUTURE WORK & CONCLUSIONS

Climate change has caused significant warming in the Arctic, which has led to a decrease in sea-ice extent and duration, an increase in ocean temperatures, alterations in prey distribution, the emergence of infectious disease, and an increase in predators and anthropogenic activity (Tynan and Demaster 1997; Learmonth et al. 2006; Anisimov et al. 2007; Burek et al. 2008). The WHB beluga population and the EC-WG bowhead whale population are likely to feel the effects of climate change and understanding how they will respond is important for their conservation and management.

In Chapter 2, I determined that urea was an ineffective dilution marker to normalize cortisol in beluga blow samples. Before blow sampling can become a reliable method for monitoring cetacean physiology, there needs to be a way to account for unknown amounts of seawater contamination and evaporation. I suggest that future studies identify other potential dilution markers or develop a method to quantify blow sample volumes. Future studies should explore dilution markers that have been identified in human breath studies and examine their feasibility in cetacean blow research. Other dilution markers include total lipid, total protein, and specific gravity (Chan et al. 2009; Kubáň and Foret 2013). It might also be possible to look at abiotic markers in water samples, such as chemical pollutants or microplastics. Future studies should also focus on collecting blow samples from known individuals, with known life histories, as this may help determine appropriate dilution markers. With additional research, blow

sampling could become the new gold standard for monitoring the physiology of free-swimming cetaceans.

Until recently, it was thought that bowhead whales foraged only during the summer and fall, and fasted throughout the winter (Finley 2001). However, the results from my study, along with stable isotope (Matthews and Ferguson 2015) and tag data (Heide-Jørgensen et al. 2013), suggest that bowhead whales forage year-round. My results are based on a lack of observed seasonal variation in T3 levels and a lack of correlation between T3 and stable isotope values. Although I concluded that bowhead whales do forage year-round, future studies should examine the relationship between energy consumption and thyroid hormones in marine mammals, as animals with large fat stores may respond differently to changes in diet (Behringer et al. 2018).

One of the most perplexing results from Chapter 3 was the strong positive correlation between T3 and corticosterone. I hypothesized 3 potential explanations for this relationship: 1) adequate yet stressful foraging; 2) molting; and 3) cross-talks between the HPT and HPA axes. Future studies should explore this relationship further, potentially by looking at T3 and corticosterone in captive species like beluga whales. To explore the relationship between THs and energy consumption in wild populations, researchers could use photogrammetry and blubber biopsies to explore the relationship between body condition and THs bowhead whales. Future studies should also look at the relationship between T3 and reproductive hormones and include female whales to determine any sex-related effects.

Together, Chapter 2 and Chapter 3, highlight the importance of using physiological tools to monitor the health of Arctic cetaceans in a warming climate. This research has furthered our

knowledge of the best practices for collecting blow samples to assess the physiology of free-swimming beluga whales. Additionally, it has contributed to filling a knowledge gap in understanding the foraging ecology of bowhead whales. I have also established important considerations for carrying out physiological studies and recommend that further research be done to validate my findings and to improve my methods.

LITERATURE CITED

Acevedo-Whitehouse K, Rocha-Gosselin A, Gendron D. 2010. A novel non-invasive tool for disease surveillance of free-ranging whales and its relevance to conservation programs. Anim. Conserv. 13:217–225.

Anisimov OA, Vaughan DG, Callaghan T, Furgal C, Marchant H, Prowse TD. 2007. Chapter 15. Polar regions (Arctic and Antarctic) : Climate Change 2007: Impacts, Adaptation and Vulnerability. Contrib. Work. Gr. II to Fourth As- sessment Rep. Intergov. Panel Clim. Chang.:653–685.

Atkinson S, Crocker D, Houser D, Mashburn K. 2015. Stress physiology in marine mammals: how well do they fit the terrestrial model? J. Comp. Physiol. B 185:463–486.

Behringer V, Deimel C, Hohmann G, Negrey J, Schaebs FS, Deschner T. 2018. Applications for non-invasive thyroid hormone measurements in mammalian ecology, growth, and maintenance. Horm. Behav. 105:66–85.

Bonett RM, Hoopfer ED, Denver RJ. 2010. Molecular mechanisms of corticosteroid synergy with thyroid hormone during tadpole metamorphosis. Gen. Comp. Endocrinol. 168:209–19.

Burek KA, Gulland FMD, O'Hara TM. 2008. Effects of climate change on arctic marine mammal health. Ecol. Appl. 18:S126–S134.

Burgess EA, Hunt KE, Kraus SD, Rolland RM. 2018. Quantifying hormones in exhaled breath for physiological assessment of large whales at sea. Sci. Rep. 8:1–14.

Chan HP, Lewis C, Thomas PS. 2009. Exhaled breath analysis: Novel approach for early detection of lung cancer. Lung Cancer 63:164–168.

Finley KJ. 2001. Natural History and Conservation of the Greenland Whale, or Bowhead, in the

Northwest Atlantic. Arctic 54:55–76.

Frère CH, Krzyszczyk E, Patterson EM, Hunter S, Ginsburg A, Mann J. 2010. Thar She Blows! A Novel Method for DNA Collection from Cetacean Blow. Aramayo R, editor. PLoS One 5:e12299.

Geoghegan JL, Pirotta V, Harvey E, Smith A, Buchmann JP, Ostrowski M, Eden J, Harcourt R, Id ECH. 2006. Virological Sampling of Inaccessible Wildlife with Drones. :1–7.

Gobush KS, Booth RK, Wasser SK. 2014. Validation and application of noninvasive glucocorticoid and thyroid hormone measures in free-ranging Hawaiian monk seals. Gen. Comp. Endocrinol. 195:174–182.

Heide-Jørgensen MP, Laidre KL, Nielsen NH, Hansen RG, Røstad A. 2013. Winter and spring diving behavior of bowhead whales relative to prey. Anim. Biotelemetry 1:15.

Hogg CJ, Rogers TL, Shorter A, Barton K, Miller PJO, Nowacek D. 2009. Determination of steroid hormones in whale blow: It is possible. Mar. Mammal Sci. 25:605–618.

Hunt KE, Rolland RM, Kraus SD. 2014. Detection of steroid and thyroid hormones via immunoassay of North Atlantic right whale (*Eubalaena glacialis*) respiratory vapor. 30:796–809.

Kooyman GL, Castellini MA, Davis RW. 1981. Physiology of diving in marine mammals. Annu. Rev. Physiol. 43:343–356.

Kubáň P, Foret F. 2013. Exhaled breath condensate: Determination of non-volatile compounds and their potential for clinical diagnosis and monitoring. A review. Anal. Chim. Acta 805:1–18.

Learmonth JA, MacLeod CD, Santos MB, Pierce GJ, Crick HQP, Robinson RA. 2006. Potential

effects of climate change on marine mammals. Oceanogr. Mar. Biol. - an Annu. Rev. Vol 44 44:431–464.

Matthews CJD, Ferguson SH. 2015. Seasonal foraging behaviour of eastern Canada-West Greenland bowhead whales: An assessment of isotopic cycles along baleen. Mar. Ecol. Prog. Ser. 522:269–286.

Meeuwis R, Michielsen R, Decuypere ~ E, Kuhn ER. 1989. Thyrotropic activity of the ovine corticotropin-releasing factor in the chick embryo. Gen. Comp. Endocrinol. 76:357–363.

Mingramm FMJ, Dunlop RA, Blyde D, Whitworth DJ, Keeley T. 2019. Evaluation of respiratory vapour and blubber samples for use in endocrine assessments of bottlenose dolphins (*Tursiops spp*.). Gen. Comp. Endocrinol. 274:37–49.

Richard JT, Robeck TR, Osborn SD, Naples L, Mcdermott A, Laforge R, Romano TA, Sartini BL. 2017. General and Comparative Endocrinology Testosterone and progesterone concentrations in blow samples are biologically relevant in belugas (*Delphinapterus leucas*). Gen. Comp. Endocrinol. 246:183–193.

Thompson LA, Spoon TR, Goertz CEC, Hobbs RC, Romano TA, Bonier F, Martin P, Moore I, Wingfield J, Sheriff M, et al. 2014. Blow Collection as a Non-Invasive Method for Measuring Cortisol in the Beluga (*Delphinapterus leucas*). PLoS One 9:e114062.

Tynan CT, Demaster DP. 1997. Observations and Predictions of Arctic Climatic Change: Potential Effects on Marine. Source Arct. 50:308–322