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Thiamine deficiency and seabirds in Norway

A pilot study

Børge Moe, Sveinn Are Hanssen, Bjørnar Ytrehus, Lennart Balk, Olivier Chastel, Signe Christensen-Dalsgaard, Hanna Gustavsson, Magdalene Langset

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Common eiders in Kongsfjorden, Svalbard © Photo: Børge Moe

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Abstract

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Thiamine (vitamin B1) is vital for life-sustaining enzymes in cells. Previous studies have reported episodes of thiamine deficiency in marine ecosystems, and suggested that this have contributed to population declines of seabirds breeding in the Baltic Sea and elsewhere. Many Norwegian seabird populations have shown a strong decline in population size, but thiamine status has never been assessed. The objective of this pilot study was, thus, to document thiamin levels in selected species and their associated food webs, and explore methodological issues relevant for future studies or monitoring.

The methodological tests showed that storage freezing temperature did not affect thiamine levels in egg yolk samples, and that thiamine levels in eggs could not be corrected for incubation time. Furthermore, the quantified thiamine levels differed between two laboratories, and we developed a predictive equation to convert thiamine levels in egg yolk samples between the laboratories.

This pilot study has, for the first time, investigated thiamine levels in seabird eggs from selected species and populations in Norway mainland and Svalbard, and in their food webs. We revealed variation among species, populations and prey types. The lowest levels were found in eggs from common eiders and in blue mussel which is their prey. Eggs from herring gulls had also relatively low levels. The levels for common eiders and herring gulls were higher than previously reported from the Baltic Sea. Nevertheless, the levels from common eiders, herring gulls and blue mussels should be classified as thiamine deficient according to effect-ranges reported in these previous studies. The highest levels were found in eggs from kittiwakes and Atlantic puffins. The diet samples from kittiwakes and Atlantic puffins had higher thiamine levels compared to blue mussels. This is the first time thiamine levels are reported for kittiwakes and Atlantic puffins.

This pilot-study cannot answer whether Norwegian seabird population sizes are affected by thiamine levels, but we cannot rule out that thiamine can be a limiting factor for some Norwegian seabird populations. This report identifies knowledge gaps and provides recommendations for future studies and monitoring. We suggest more sampling of levels to better understand variation among years, areas, species and populations, and also clinical examinations and surveys. Studies investigating potential effects on reproduction and survival is ultimately need to better understand potential effects on population dynamics.

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Sammendrag

Moe, B., Hanssen, S. A., Ytrehus, B., Balk, L., Chastel, O., Christensen-Dalsgaard, S., Gustavsson, H. & Langset, M. 2020. Tiaminmangel og sjøfugl i Norge. En pilotstudie. NINA Rapport 1720. Norsk institutt for naturforskning.

Timanin (vitamin B1) er vitalt for livsopprettholdende enzymer i celler. Tidligere studier har rapportert at det kan forekomme episoder med tiaminmangel i marine økosystemer, og foreslått at det har bidratt til bestandsnedgang hos sjøfugler som hekker i Østersjøen og andre steder. Mange norske sjøfuglbestander har vist kraftig tilbakegang, men tiaminstatus har ikke blitt undersøkt. Målsettingen med denne pilotstudien var derfor å dokumentere tiaminnivåer i utvalgte arter og deres næringskjede, og teste metodiske forhold relevant for framtidige studier og overvåkning.

Metodetestene viste at frysetemperatur ikke påvirket tiaminnivåene i eggeplomme, og at tiaminnivåene i eggeplomme ikke kunne korrigeres for rugetid. Vi fant også at tiaminnivå ble kvantifisert ulikt av to laboratorier, og vi utviklet en ligning for å omregne tiaminnivå i eggeplomme mellom laboratoriene.

Denne pilotstudien har for første gang undersøkt tiaminnivå i sjøfuglegg fra utvalgte arter og bestander på fastlandet i Norge og Svalbard, samt i deres næringskjeder. Vi fant variasjon mellom arter, bestander og næringsemner. De laveste nivåene var i ærfuglegg og blåskjell som er føde til ærfugl. Det var også relativt lave nivåer i egg fra gråmåke. Nivåene som ble funnet i ærfugl og gråmåke var høyere enn det som tidligere er rapportert fra Østersjøen. Likevel, både nivåene i egg fra ærfugl og gråmåke, samt nivåene i blåskjell, skal karakteriseres som tiaminmangel i henhold til effektnivåer funnet i tidligere studier fra Østersjøen. Egg fra krykkje og lunde hadde de høyeste tiaminnivåene i denne studien. Næringsemnene til disse hadde også høyere tiaminnivåer enn blåskjell. Dette er første gang tiaminnivåer er målt i krykkje og lunde.

Denne pilotstudien kan ikke svare på om størrelsen til norske sjøfuglbestander er påvirket av tiaminnivåer, men vi kan ikke utelukke at tiamin kan være en begrensende faktor for noen norske sjøfuglbestander. Denne rapporten identifiserer kunnskapshull og gir anbefalinger for nye studier og overvåkning. Vi foreslår mer innsamling og måling av tiaminnivåer for å bedre forstå variasjon mellom år, områder, arter og bestander, og også kliniske undersøkelser og kartlegging. Studier som undersøker effekter på reproduksjon og overlevelse behøves for å bedre forstå den potensielle effekten på bestandsutvikling.

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Contents

Foreword

This project was initiated by the Norwegian Environment Agency. With the episodes of thiamine deficiency documented for seabirds in the Baltic Sea and the strong declines in many Norwegian seabird populations, the aim was to investigate whether seabirds in Norway experiece thiamine deficiency. The objective of the pilot study was thus to document thiamin levels in selected species and their associated food web, and explore methodological issues relevant for future studies or monitoring.

We are grateful to Halvar Ludvigsen, Céline Albert, Don-Jean Léandri-Breton, William Jouanneau and Kjersti Selstad Utaker for efficient field work and collection of samples. A special thanks to Tom Zahl Pedersen and Nesodden kommune for logistical support at Nesodden and Haakon Braathu Haaverstad and SNO for logistic support at Hvaler.

All samples were analyzed at ACES at Stockholm University. We thank Annbjørg Bøkevoll and the Institute for Marine Research (IMR) in Bergen for analysing a set of duplicate samples for inter-lab calibration.

Contact persons at Norwegian Environment Agency have been Marte Rusten, Bård Nordbø, Gunn Lise Haugestøl and Øystein Tennfjord. We thank all for their enthusiastic cooperation and support.

10th December 2020, Børge Moe, project leader

1 Introduction

Population numbers of many species of seabirds are declining in Norway. Declines are pronounced both in the southern part (North Sea and Skagerrak) and in the northern part of Norway (Norwegian sea and Barents sea) (Fauchald et al. 2015a). Several explanations have been proposed for this ongoing decline, such as overfishing, by-catch, pollution, but no single factor seem to be able to explain the general decline for many species (Fauchald et al. 2015b). Complicated food web effects including indirect effects, sea warming which leads to fish moving further north and spawning at different times, an increase in avian predators in seabird colonies are probably important explanatory factors. Common for population limiting factors like food shortage and predation is that they are stress inducing. Increased stress may lead to increased sensitivity to other disturbing factors like infectious organisms, pollution and intraspecific competition, ultimately reducing reproductive investment and survival.

Disease is not only caused by infectious organisms, but may also be caused by e.g. lack of essential nutrients. Vitamin B_1 (thiamine) is an essential nutrient that seabirds cannot synthesise themselves but have to aquire via food. Inside animal cells, non-phosphorylated thiamine (T) is phosphorylated to thiamine diphosphate (TDP), which functions as a cofactor for life-sustaining enzymes required for basic cellular metabolism, and thiamine monophosphate (TMP) is a degradation product, which is recycled or excreted.

Low levels of thiamine may lead to for instance immunosuppression and neurological disorders. Lately, thiamine deficiency has been documented in several species of fish, birds and shellfish (Balk et al. 2016). In seabirds, thiamine deficiency has been linked to reduced reproduction and population declines in common eiders (*Somateria mollissima*) and herring gulls (*Larus argentatus*) in the Baltic Sea (Balk et al. 2009, Mörner at al. 2017).

However, levels of thiamine have not been investigated in Norwegian seabirds, and it has remained an open question if thiamine deficiency may play a role in the ongoing decline of Norwegian seabird populations. The Norwegian Environment Agency have therefore funded a pilot study/screening of relevant seabirds species in several locations along the coast of Norway and Svalbard. The results from this screening in 2019 are presented in this report.

The main aim of the present pilot study is to screen levels of thiamine in seabirds and their associated food webs and assess potential occurrence of thiamine deficiency. We focused on four seabird species to collect eggs and diet samples from: common eider, herring gull, black-legged kittiwake (*Rissa tridactyla*, hereafter kittiwake), and Atlantic puffin (*Fratercula arctica*). Furthermore, we focused on three regions: Oslofjord, North-Norway and Svalbard. As such, we could compare results with those from Balk et al. (2009, 2016) and Mörner et al. (2017) who reported thiamine deficiency in common eiders and herring gulls in the Baltic Sea. Also, we aimed to cover a large geographic scale by including the areas where we have the largest seabird populations in Norway, such as Atlantic puffins and kittiwakes in Northern Norway and Svalbard.

It can be challenging to sample eggs and biological material in remote locations, usually with poor infra-structure, and sampling requirements can constrain the ability to perform efficient monitoring or screening of thiamine levels. As such, our sub-goals were to test methodological aspects that would aid designing future monitoring and screening of thiamine levels in seabird populations. We aimed to 1) test whether storage freezing temperature affected thiamine levels in egg yolk samples, 2) test whether incubation time and embryo development affected thiamine levels in egg yolk samples and 3) compare thiamine levels quantified by a Norwegian laboratory with those quantified by the Swedish laboratory used by Balk et al. (2009, 2016). We also aimed to establish methods for clinical observations and necropsy in order to provide a foundation for standardized and reproducible registration of clinical signs and determination of cause of disease and death. Finally, we identify knowledge gaps and provide suggestion for future sampling and monitoring.

2 Methods

2.1 Study area and species

Field work was conducted at four locations in Norway and Svalbard (**Figure. 2.1, Table 2.1)**. In Oslofjord we collected eggs from common eiders and herring gulls. Oslofjord was chosen for comparative reasons, because it is located close to Sweden and the Baltic Sea, where episodes of thiamine deficiency have been reported for common eiders and herring gulls (Balk et al. 2009, 2016, Mørner et al. 2017). Extending on this, we also collected eggs from common eiders in Troms and in Svalbard, herring gull eggs in Nordland, kittiwake eggs in Nordland and Svalbard and Atlantic puffin eggs in Nordland. As such, we aimed to cover a large geographic scale including the areas where we have the largest seabird populations in Norway. The different seabird species also represent different ecological groups, with common eiders and herring gulls being coastal benthic and surface feeders, respectively. Kittiwakes and Atlantic puffins are pelagic surface feeders and pelagic divers, respectively. Covering different parts of the marine ecosystem we also aimed at investigating thiamine levels in their food webs. Therefore, we collected blue mussels (*Mytulis edulis*) which are key prey for common eiders, in Oslofjord and Troms. Finally, we also collected diet samples from kittiwakes and Atlantic puffins. These seabirds species catch their prey in the ocean and bring it back to the colony to feed their chicks. When being caught and handled by the field workers, they will drop prey carried in the beak (Atlantic puffins) or regurgitate any prey stored in the crop (kittiwake), and this is collected as diet samples.

All field work in this pilot study was done in 2019 (**Figure 2.1, Table 2.1)**. The main task was to collect freshly laid eggs. We started in Oslofjord where breeding starts earlier. The permission to do the field work and collect eggs, however, was granted 1-2 weeks later than we aimed for. Hence, incubation had already started and most of the eggs collected were already incubated for some days. At the other field locations we could do the field work in time to collect freshly laid eggs. In Svalbard we also collected eggs from common eider nests with known incubation time for a methodological test (see later section of this chapter).

Permissions and dispensations were granted by the County Governor of Oslo and Viken (2019/28136, 2020/14944, 2020/14944 3 - 432.3), County Governor of Nordland (2019/3197), Governor of Svalbard (19/01105-2) and Norwegian Environment Agency (2019/5862, 2019/6266).

***Table 2.1**. Overview of where eggs and diet/prey were sampled in 2019. Diet/regurgitate and diet/dropped refer to prey caught by the seabirds (e.g. fish) and dropped or regurgitated when being handled by field workers.*

	Region	Location	Egg collected	Diet/prey samples
1.	Oslofjord	Nesodden	Common eider	Blue mussels
			Herring gull	
2.	Nordland	Anda	Kittiwake	Diet/regurgitate
			Atlantic puffin	Diet/dropped
3.	Troms	Brensholmen	Common eider	Blue mussels
		Tromsø		
4.	Svalbard	Kongsfjorden	Common eider	
			Kittiwake	Diet/regurgitate

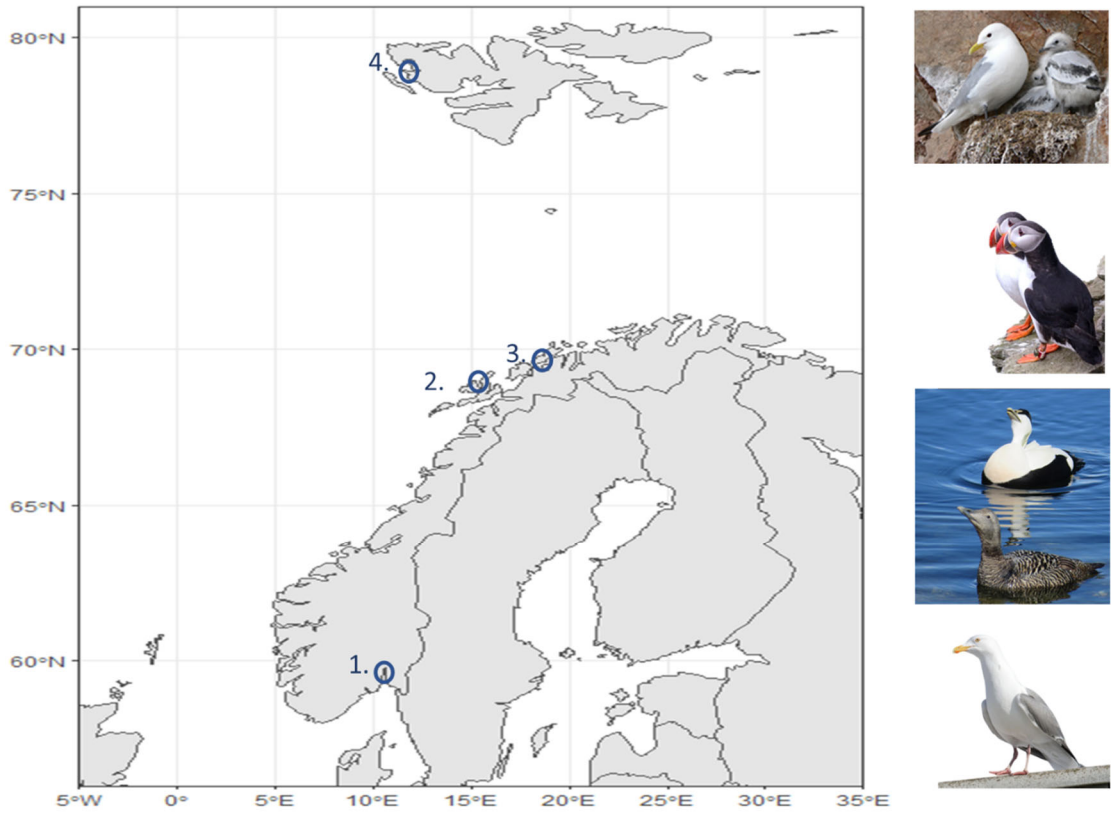

***Figure 2.1**. Overview of the study species, kittiwake, Atlantic puffin, common eider and herring gull and field sites; 1. Oslofjord, 2. Anda in Nordland, 3. Brensholmen and Tromsø in Troms and 4. Kongsfjorden in Svalbard. Seabird eggs were collected in the breeding colonies. In addition we collected blue mussels close to the colonies and diet samples from kittiwakes and Atlantic puffins when handling of the birds in the colonies.*

Thiamine concentrations were also analyzed in eggs from common eiders collected from Nesodden and Hvaler in 2020 in another study (Hanssen et al. 2020a, b). The results from those analyzes are included in this report, to obtain a large sample size from Oslofjord. Nesodden is located in the inner part of Oslofjord, and Hvaler in the outer part. Nesodden is referred to as Oslofjord and Hvaler as Oslofjord 2 in this report.

2.2 Sample collection and freezing

After being collected, eggs were stored in a protective case when working in the field. At the end of each daily field session the collected eggs were processed. The length and width were measured with a caliper (precision xx mm) and the mass was measured with a digital scale (precision 0.01 g) before the egg was cracked into a petri dish. Care was taken to preserve the egg yolk membrane. We took a picture of each egg in the petri dish, to document that it was freshly laid or to aid assessment of embryo age (incubation time), i.e. if the egg was incubated and development had started.

We used a syringe without a needle to collect a homogenous yolk sample. The yolk sample was allocated into 2 ml cryotubes which were subsequently frozen and stored in a dry-shipper (-150°C, see further description of dry-shipper in section 2.4.1). Egg yolk and mussel samples were kept in the dry-shipper until returning from the field site back to NINA. Depending on field location this was a period of 2- 14 days. At NINA the samples were stored in a low temperature freezer (-80°C).

The diet samples were heterogenous in terms of prey composition, not just among samples and individual birds but also sometimes within the sample. While Atlantic puffins only carry fish in their beaks, kittiwakes may collect a mix of fish and zooplankton in their crop. The prey items were identified to prey group (fish, fish larvae, krill, sea butterfly), and when possible to species (Polar cod *Boregadus saida*, Herring *Clupea harengus*, Sandeel *Ammodytes sp*, krill Thyssanoessa inermis, sea butterfly Thecosomata sp.).

Diet samples were collected over several weeks during the breeding-rearing period, while the eggs were collected during the first week of incubation period (on the population level). Since temperature inside the dry-shipper will increase over time, it was not feasible to keep a dry-shipper at the designated locations for the entire breeding season (~2 months) without recharging/refilling with liquid nitrogen. We had access to liquid nitrogen in Kongsfjorden, Svalbard, but not at Anda in Nordland. For practical reasons and for standardizing the methods, we stored diet samples from both Kongsfjorden and Anda in a -20°C freezer.

The blue mussel samples were not homogenized, but we cut a small cube of the soft tissue and used this for further analysis. The yolk samples were already very homogenous at collection, and were not subjected to any further homogenization. The diet samples, however, needed homogenization. To avoid high temperature and potential degradation or phosphorylation of the samples, the homogenization was performed in a freezer-room with temperature < -10°C using a glass mortar.

2.3 Quantification and calculations

All samples were analyzed at Department of Environmental Science and Analytical Chemistry (ACES) at Stockholm University. ACES quantified the three forms of thiamine, non-phosphorylated thiamine (T), thiamine monophosphate (TMP), and thiamine diphosphate (TDP) in the biological material and the method consisted homogenization and extraction, centrifugation, clean-up, derivatisation followed by separation and analysis on high performance liquid chromatography (HPLC, NH2 column) with fluorescence detection. Concentrations of T, TMP and TDP were summed to estimate total thiamine concentration ('sum T' hereafter). ACES also performed the thiamine analyses in the studies by Balk et al. (2009, 2016) and Mörner et al. (2017), and we refer to Balk et al. (2016) for further details of this method.

Concentrations were quantified in nmol/g on a wet-weight basis (ww). For the egg yolk samples limit of detection (LOD) were 0.2, 0.05 and 0.2 nmol/g for T, TMP and TDP, respectively. Limits of quantification (LOQ) were 0.2, 0.4 and 0.1 nmol/g for T, TMP and TDP, respectively. For the blue mussel and the diet samples, LOD and LOQ depended on sample mass. The average sample mass used in quantification was around 0.5 g (max 0.85, min 0.2 g). In average, blue mussel LOD was 0.01, 0.02 and 0.02 nmol/g for T, TMP and TDP, respectively, and diet LOD was 0.02, 0.03 and 0.05 nmol/g for T, TMP and TDP, respectively. In average, blue mussel LOQ was 0.03, 0.03 and 0.09 nmol/g for T, TMP and TDP, respectively, and diet LOQ was 0.06, 0.05 and 0.10 nmol/g for T, TMP and TDP, respectively. Concentrations <LOD or <LOQ were assigned with 0.5 x LOD or 0.5 x LOQ.

In addition to the analyses at ACES, a set of egg yolk samples were ananlyzed at the Institute for Marine Research (IMR) in Bergen for inter-lab calibration and comparison. We refer to this as duplicate samples. From the same egg, one egg yolk sample was sent to and analysed by ACES while an other egg yolk sample was sent to and analysed by IMR (see section 2.4).

IMR is a laboratory certified by Norwegian Accreditation with registration number TEST 050. IMR quantify the total concentration of thiamine (sum T) and does not quantify any of the three forms separately. The IMR method (239- Thiamine-HCl) cosists of extraction, hydrolysis, enzyme treatment, derivation of thiamine to thiochrome and quantification with reverse phase HPLC and

fluorescence detection. Concentrations were quantified in mg/kg on a wet-weight basis (ww). LOQ and LOD were 0.1 and 0.03 mg/kg ww, respectively. The IMR method has been approved in interlaboratory tests using reference material in 2019, but with other biological material than egg yolk. Uncertainty of measurement is 15 and 25% for concentrations ranging 0.1- 3 and 3-75 mg/kg, respectively. The chromatograms for the egg yolk samples were assessed to be very good, with clear peaks and no interference.

In this report we also include results from Hanssen et al. (2020b) to present more data from common eiders from Oslofjord. IMR quantified the thiamine levels in these samples, and we consequently converted to ACES levels using the predictive equation obtained from the interlaboratory calibration (see below, section 2.4.2), and finally converted from mg/kg to nmol/g using the molecular weight of T, since virtually all thiamine is in the form of T in eggs.

2.4 Methodological tests

2.4.1 Storage freezing temperature

This pilot study included three methodological tests relevant for how to design monitoring of thiamine levels in seabirds. The first test was motivated by the potential challenging sampling conditions in seabird colonies, which are often situated at remote locations with poor infra structure and limited facilities. Under such conditions it can be impossible to store and transport samples in liquid nitrogen. The alternative is using a dry-shipper (-150°C) in the field and during transportation from the field site, and a low temperature freezer (-80°C) when returning to the research institute. The dry-shipper is a portable container with absorbed liquid nitrogen, providing cold temperature (-150°C) for a short time (days) and safe conditions for field workers (no liquid nitrogen that can be spilled). It can also be brought on flights according to IATA regulations. Under some field conditions, however, the best available alternative is standard freezers at -20°C. The concern is that thiamine is not equally preserved at -20°C as compared to lower temperatures. We therefore tested whether temperature affected the thiamine levels quantified. **Figure 2.2** illustrates the design of this test. From 14 eggs from common eiders breeding in Svalbard we allocated yolk samples in duplicate tubes. One of the tubes were stored at -20°C in the field (2 weeks) and at -20°C at the Norwegian Institute for Nature Research (NINA, 2 months) after returning from the field. The other tube was stored in a dry-shipper in the field (-150°C, 2 weeks) and subsequently at -80°C at NINA. All samples were finally analyzed at the ACES laboratory. In the result section, we refer to -20°C and -150/-80°C as high and low temperature, respectively.

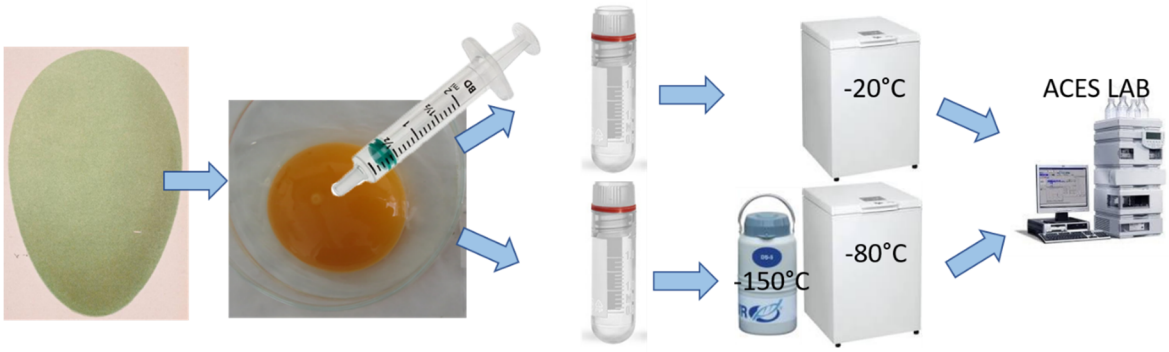

***Figure 2.2**. Illustration of study design for testing whether storage temperature affected thiamine levels quantified in egg yolk samples. We collected eggs from common eiders breeding in Svalbard. Duplicate yolk samples from the same eggs were collected in duplicate tubes. One of the tubes were stored at -20°C (in the field, and at NINA) while the other was stored at -150 (in the field) and -80°C (at NINA), and finally analyzed at the ACES lab.*

2.4.2 Interlaboratory calibration

The two laboratories used different methods to quantify thiamine. While ACES quantified each of the three forms (T, TMP, TDP), IMR quantified the sum T. The second methodological test was therefore to test whether the two laboratories, IMR and ACES, provided comparable thiamine levels. For that test we used duplicate samples of egg yolk from Svalbard breeding common eiders. **Figure 2.3** illustrates the design of this test. From 15 eggs from common eiders breeding in Svalbard we allocated yolk samples in duplicate tubes. Both tubes were stored at -20°C in the field (2 weeks) and at NINA (2 months) after returning from the field. One of the samples was analyzed at the ACES lab, while the other sample was analyzed at IMR.

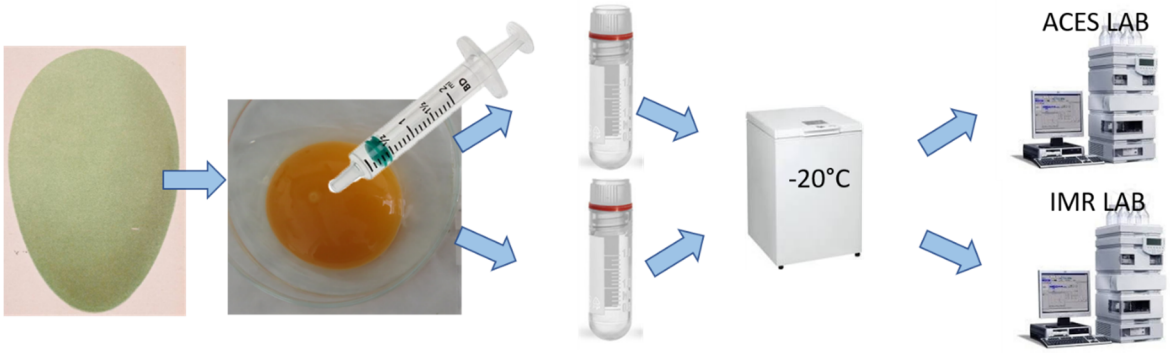

***Figure 2.3**. Illustration of study design for inter-lab comparison among ACES and IMR. We collected eggs from common eiders breeding in Svalbard. Duplicate yolk samples from the same eggs were collected in duplicate tubes, frozen and stored at -20°C, and analyzed at the two labs.*

ACES measured concentrations in nmol/g while IMR analyzed thiamine in mg/kg. Concentrations in nmol/g can be converted to mg/kg, or vice a versa, by using the molecular weight of the three forms. Hence, conversion can be done accurately if the composition and relative contribution of T, TMP and TDP is known. In this report, we converted sum T from ACES (T+TMP+TDP; nmol/g) to mg/kg for comparison with IMR.

2.4.3 Incubation time

It is challenging to collect freshly laid eggs. In order to achieve this, the field work has to be finely tuned to the breeding phenology of the birds and timing of egg-laying. The concern with eggs that have been incubated by the parent birds, is that thiamine may be metabolized by the embryo. If this is the case, the thiamine concentration may decrease over the course of incubation. Our motivation for testing the potential effect of incubation time on thiamine levels, was to establish a statistical relationship between incubation time and thiamin concentration. This could be used to statistically correct for incubation time if incubated eggs were collected unintentionally or if egg collection was impossible, for logistical or other reasons, around egg-laying.

To carry out this test, we first collected one freshly laid egg from eight different nests of common eiders in Svalbard. We collected another egg after 5 days of incubation in the same nests and in some cases a third egg >10 days after the first. As such, we obtained a design with nest as the grouping variable. Under the assumption that thiamine levels do not vary among eggs laid by the same female, this repeated sampling within the same nests, with known egg-laying date and known incubation time, would provide a strong test of the effect of incubation time. Low variation in thiamine levels among eggs from the same female has been observed and support the assumption (L Balk, unpublished data). However, in the result section we show that there is

another factor that needs to be considered more closely, which violated the experimental design for testing the effect of incubation time.

2.5 Clinical and pathological investigations

We aimed to perform detailed clinical, pathological investigations and necropsy of dying birds with strong symptoms associated with thiamine deficiency or dead birds with potential/suspected thiamine deficiency. During the field season in 2019, no such cases were observed in any of the focal seabird colonies (**Figure 2.1**). Hence, no birds or carcasses were subjected to such investigations.

A carcass should be brought to a veterinary pathologists with experience in central nervous system histopathology to undertake a necropsy while the carcass is still fresh. The majority of Norwegian seabird populations breed in remote locations, and it is not always possible to transport a dead bird to a veterinary pathologist sufficiently fast. In such cases, it would be useful to undertake a field necropsy. To facilitate this, we have developed a standard procedure for field necropsy as part of this pilot study. This procedure is presented in Ytrehus & Work (2019).

2.6 Statistical analyses

All statistical analyses and graphs were conducted using R version 3.6.3 (R Core Team 2020), and estimates are provided with ± 1 standard error (SE) unless otherwise stated.

Linear models were performed using the lm function in the `stats` package (R Core Team 2020) to test for differences among means (`anova` option). The `summary` option was used to provide parameter estimates.

Graphs, including predictive lines and confidence intervals, were created using the `ggplot2` package (Wickham 2016). When all variables are between-subjects, it is straightforward to plot standard error or confidence intervals. However, when there are within-subjects variables (repeated measures), plotting the standard error or regular confidence intervals may be misleading for making inferences about differences between conditions. We used the `summarySEwithin` function in the `Rmisc` package (Morey 2008, Hope 2013) to calculate standard error and confidence intervals for the within subjects variables when comparing the duplicated samples among IMR and ACES (paired test).

In box plots the upper and lower boundaries of the box represent the 25th and 75th percentile, the horizontal line is the median. The whiskers extends from boundaries to the smallest or highest value at most 1.5 x interquartile range (IQR) of the boundaries. Data beyond the whiskers are plotted as points (outliers).

3 Results

3.1 Methodological tests

3.1.1 Storage freezing temperature

Duplicate egg yolk samples of common eiders from Svalbard were stored at high (-20°C) or low (-150 and -80°C) temperature, respectively, and subsequently thiamine levels were quantified in all samples by ACES. Average sum T was 21.0 ± 1.59 and 20.9 ± 1.59 nmol/g in the two groups and were not significantly different (df=13, t=-0.04, p=0.97, **Figure 3.1, Table 3.1**).

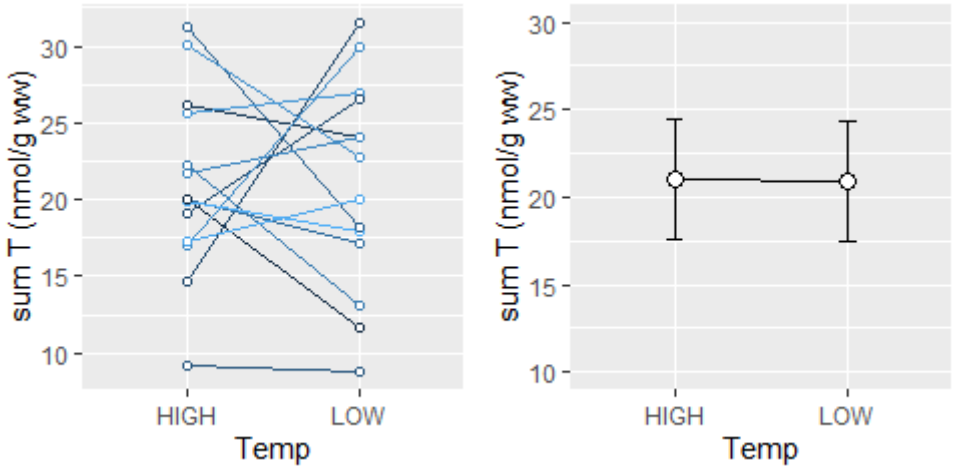

Figure 3.1 *Left panel shows thiamine concentration of duplicate egg yolk samples stored at high (-20°C) or low (-150 and -80°C) temperature. The eggs (N=14) are from common eiders breeding in Svalbard, and all samples were analyzed by ACES. The pairs of duplicate samples are connected with lines. Right panel shows the means and 95% confidence intervals calculated with the* `summarySEwithin` *function in the* `Rmisc` *package in R (Morey 2008, Hope 2013).*

Table 3.1 *Descriptive statistics for duplicate samples of common eider egg yolk stored at high (-20°C) or low (-150 and -80°C) temperature. Thiamine concentration is given in nmol/g ww. Standard deviation (sd), standard error (se) and 95% confidence interval (95% ci) are calculated with the* `summarySEwithin` *function in the* `Rmisc` *package in R (Morey 2008, Hope 2013).*

Temp	N	mean	sd	se	95% ci
High	14	21.0	5.94	1.59	3.43
Low	14	20.9	5.94	1.59	3.43

3.1.2 Incubation time

Sum T in egg yolk as a function of incubation time of eggs is shown in **Figure 3.2**. We do not provide any statistics for this, because the main results from this part of the study, is that the yolk membrane broke when opening the eggs that had been incubated (**Figure 3.3**). Despite careful effort to avoid it, this happened in all cases for incubated eggs, while the yolk membrane was preserved for freshly laid eggs. When the yolk membrane is broken, the albumen and yolk easily mix. Virtually all the thiamine is located within the yolk while the albumen has more or less no thiamine. Hence, mixing of albumen and yolk will result in dilution of the sample. Although we tried to target the yolk only and avoid albumen, it was virtually impossible to see whether or how much albumen was sucked into the syringe. Some of the sharp declines in sum thiamine from day 0 to day 5 of incubation could obviously be a result of mixing of albumen and yolk during the sampling (**Figure 3.2, 3.3**). Hence, in this case the results may reflect dilution from mixing yolk and albumen, and should not be used to make inference from incubation length.

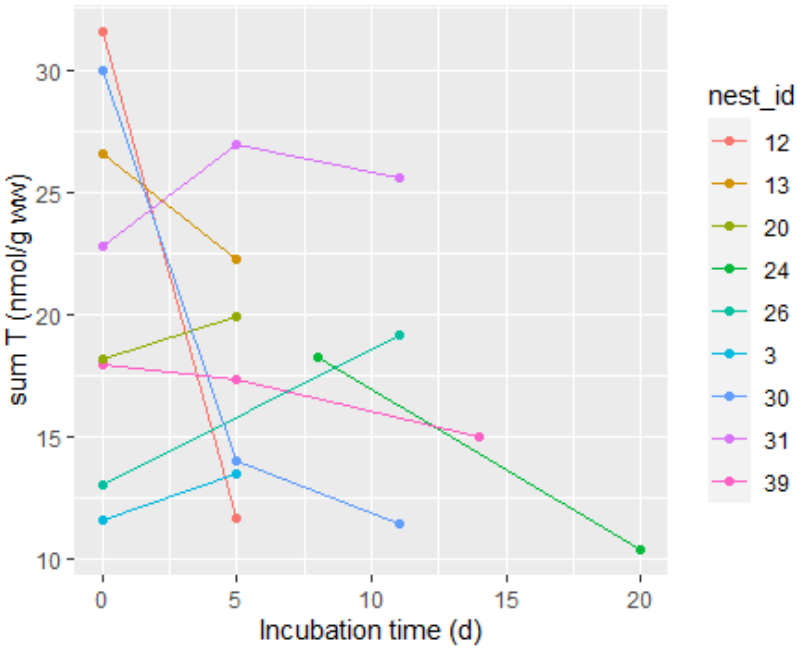

Figure 3.2. *Sum T as a function of incubation time (days). The grouping variable is nest (nest_id) and the lines connect concentrations of eggs from the same nest but with different incubation time. Eggs were collected from common eider nests in Svalbard where timing of egg laying and incubation was known.*

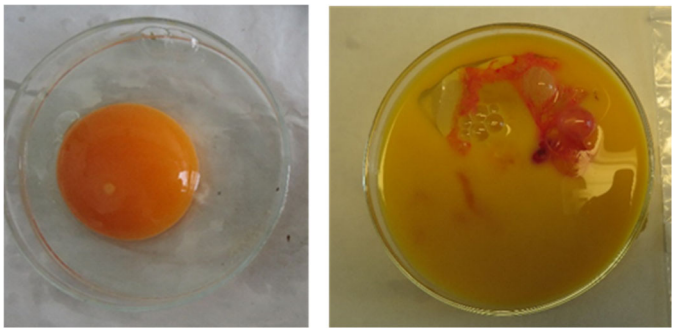

Figure 3.3*. Example of freshly laid egg (left) and egg incubated for 5 days (right) from the same nest. Note that the yolk membrane is broken and the yolk and albumen has mixed in the incubated egg in the petri dish. Eggs were collected from the same common eider nest in Svalbard at different stages of incubation.*

3.1.3 Interlaboratory calibration

We tested whether the two laboratories, IMR in Norway and ACES in Sweden, provided comparable thiamine levels in duplicate samples of egg yolk from Svalbard breeding common eiders. Sum T from IMR correlated significantly to sum T from ACES (r=0.52, df=13, t=2.2, p=0.04, **Figure 3.4**). The relationship was predicted by the equation for the regression line (**Figure 3.4**):

(1) $Y = 0.90\ (\pm 0.41)\ x + 2.36\ (\pm 1.48)$

In this equation, Y is sum T ACES and x is sum T IMR. The slope estimate (0.90 ±0.41) indicates an increase by 0.90 for each unit increase at the x axis (**Figure 3.4**).

Average sum T from ACES was 2.0 mg/kg higher than IMR for the duplicate samples (t = -6.0, df = 14, p <0.001, **Fig 3.5, Table 3.2**).

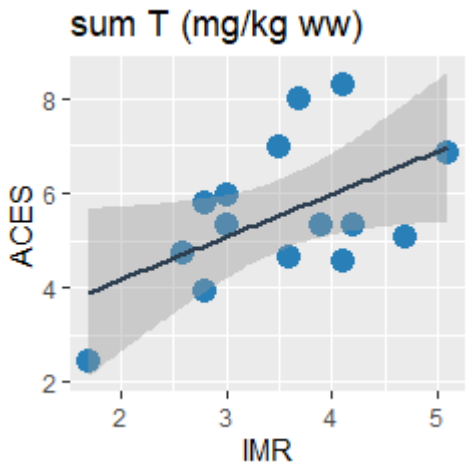

***Figure 3.4**. Regression of sum T (mg/kg) of duplicate egg yolk sample quantified at IMR and ACES. Sum T from ACES was converted from nmol/g to mg/kg using the molecular weight of T, TMP and TDP.*

***Table 3.2**. Descriptive statistics for duplicate samples of common eider egg yolk analysed at ACES and IMR. Thiamine concentration is given in mg/kg ww. Standard deviation (sd), standard error (se) and 95% confidence interval (95% ci) are calculated with the `summarySEwithin` function in the `Rmisc` package in R (Morey 2008, Hope 2013).*

Lab	N	mean	sd	se	95% ci
ACES	15	5.54	0.92	0.24	0.51
IMR	15	3.52	0.92	0.24	0.51

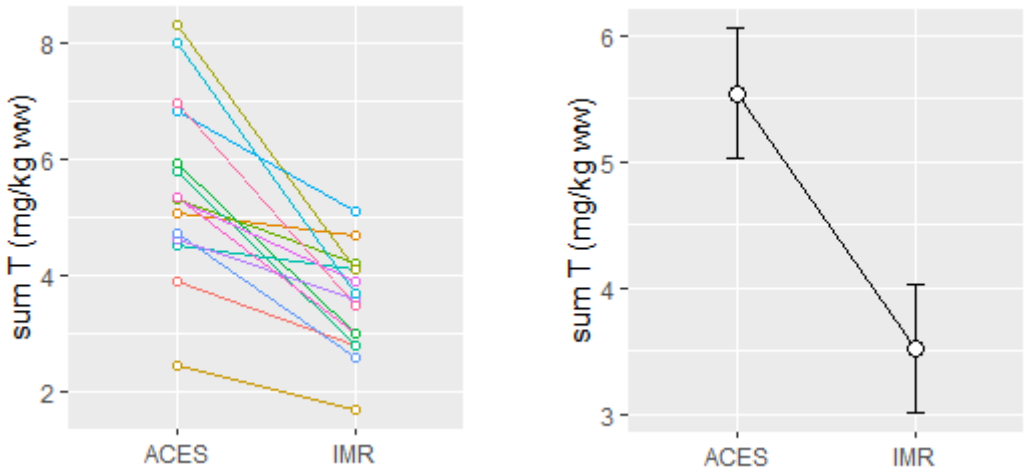

Figure 3.5 *Left panel shows thiamine concentration of individual duplicate samples analyzed at ACES and IMR from yolk samples of 15 eggs of common eiders breeding in Svalbard. The pairs of duplicate samples are connected with lines. Right panel shows the means and 95% confidence intervals calculated with the* `summarySEwithin` *function in the* `Rmisc` *package in R (Morey 2008, Hope 2013).*

3.2 Thiamine levels and composition in seabird eggs

3.2.1 Common eider

Sum T in egg yolk samples from common eiders differed significantly among populations ($F_{2, 28}$=9.9, p<0.001). Sum T was highest in the Svalbard breeding common eiders (**Figure 3.6A, Table 3.3**). The concentrations in eggs from Oslofjord showed an intermediate level. However, only two eggs of those collected were freshly laid and could be used in this analysis. There is therefore considerable uncertainty about the levels from this population, also reflected by the confidence intervals (2.5 and 97.5% confidence interval 0- 40.9 nmol/g, **Table 3.3**). The concentrations in Troms were lowest. The mean for the Troms population (9.9 ±1.7 nmol/g, **Table 3.3**) was close to the mean reported from common eider populations from the Baltic Sea (7.2 nmol/g, **Figure 3.6A**).

Hanssen et al. (2020b) analyzed a higher sample size of eggs from Nesodden (Oslofjord) and Hvaler (Oslofjord 2) collected in 2020, and we present the results here (**Figure 3.6B., Table 3.3**). After conversion from IMR to ACES levels using the predictive equation from the interlaboratory calibration, the mean concentrations (15.2 ± 1.3 and 16.2 ± 1.2 nmol/g) were intermediate of those from Troms and Svalbard (**Table 3.3**).

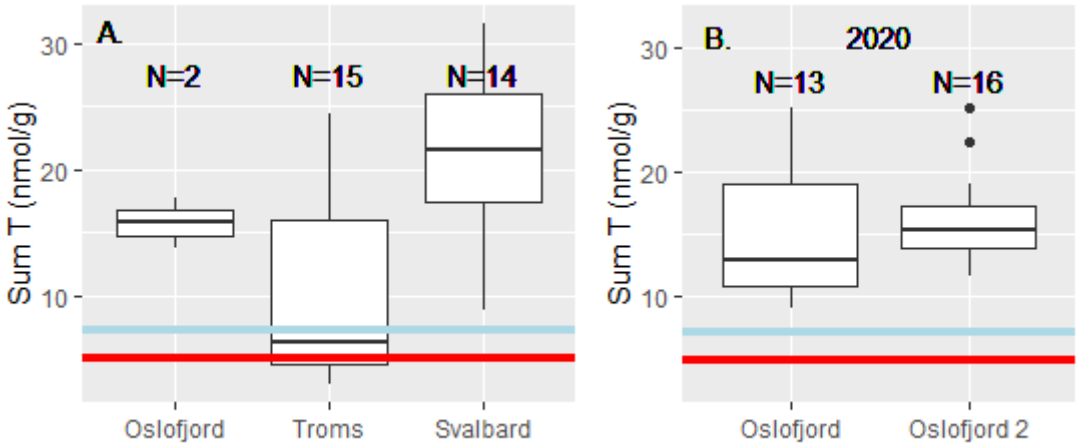

Figure 3.6. *Boxplot of sum T (nmol/g) in egg yolk from common eiders from Oslofjord, Troms and Svalbard in 2019 (A.) and two locations in Oslofjord in 2020 (B.). Results in B. are from Hanssen et al. (2020b) after conversion using the predictive equation obtained from interlaboratory calibration (see below). The blue horizontal line refers to average sum T in egg yolk from common eiders in the Baltic Sea and categorized as thiamine deficiency (Balk et al. 2009). The red line refers to a suggested threshold for viable offspring in common eider eggs (Balk et al. 2016).*

***Table 3.3**. Descriptive statistics for sum T (nmol/g ww) in egg yolk from common eiders breeding in Svalbard, Troms and Oslofjord in 2019 and two locations in Oslofjord in 2020. Note, only results for freshly laid eggs are included. Only two eggs from Oslofjord in 2019.*

Region	N	mean	se	max	min	2.5%ci	97.5%ci
2019							
Svalbard	14	20.9	1.8	31.6	8.8	17	24.9
Troms	15	9.9	1.7	24.3	2.9	6.2	13.6
Oslofjord	2	15.8	4.7	17.7	13.8	0	40.9
2020							
Oslofjord	13	15.2	1.3	25.2	9.1	11.6	18.7
Oslofjord 2	16	16.2	1.2	25.2	11.6	14.3	18.2

Oslofjord and Oslofjord 2 refers to Nesodden and Hvaler, which are located in the inner and

Virtually all thiamine was in the form av non-phosphorylated thiamine (T) in egg yolk samples from common eiders (**Figure 3.7**). The phosphorylated forms TMP and TDP were usually below detection limit (LOD) in most egg yolk samples and they constituted a negligible proportion of sum T. The bars for TMP and TDP in **Figure 3.7** are the footprints of non-detects being assigned with the value of 0.5 x LOD. The same pattern was apparent in egg samples from all the seabird species in this study.

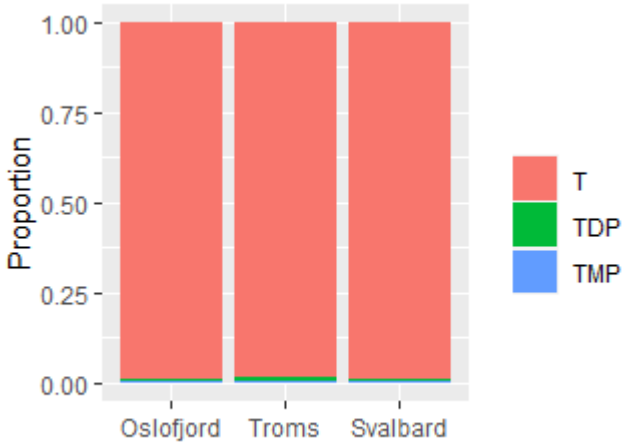

***Figure 3.7**. Proportion of the three forms of thiamine (T, TMP, TDP) in egg yolk from common eider.*

3.2.2 Herring gull

In egg yolk samples from herring gulls, sum T was highest in gulls breeding in Nordland and lowest in gulls in Oslofjord (**Figure 3.8, Table 3.4**). The sample size was very low for Oslofjord (n=3), causing wide confidence intervals (**Table 3.4**). Accordingly, the means are not significantly different among the two populations ($F_{1,16}$=1.7, p=0.21).

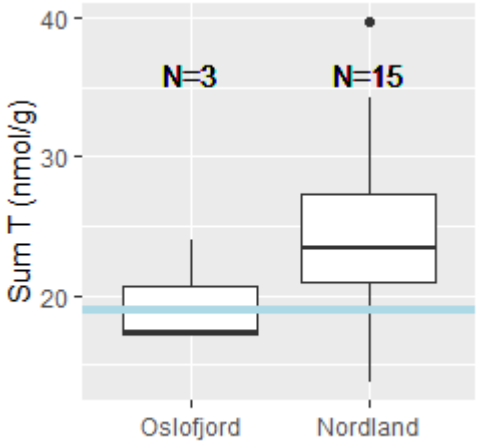

***Figure 3.8**. Boxplot of sum T (nmol/g) in egg yolk from herring gulls from Oslofjord and Nordland. Horizontal line (blue) refers to average sum T in egg yolk from herring gulls in the Baltic Sea and is categorized as thiamine deficiency (Balk et al. 2009).*

***Table 3.4**. Descriptive statistics for sum T (nmol/g ww) in egg yolk from herring gulls breeding in Nordland and Oslofjord. Note, only results for freshly laid eggs are included. Only three eggs from Oslofjord.*

Region	N	mean	se	max	min	2.5%ci	97.5%ci
Nordland	15	24.7	1.6	39.7	13.8	21.1	29.2
Oslofjord	3	19.5	3.6	24	17.1	9.8	29.2

The proportions of the three forms of thiamine in herring gull eggs were identical with that of common eider eggs. Virtually all thiamine was in the form av non-phosphorylated thiamine (T) in egg yolk samples. The phosphorylated forms TMP and TDP were usually below detection limit in most egg yolk samples and constituted a negligible proportion of sum T.

3.2.3 Kittiwake and Atlantic puffin

Sum T in egg yolk samples from kittiwakes were higher than in herring gulls and eiders. Kittiwakes from Svalbard had the highest mean level (47.3 nmol/g) but they were not significantly different from that of kittiwakes from Nordland (t=0.87, df= 28 and 1, p=0.39, **Figure 3.9A, Table 3.5**). Sum T in egg yolk samples from Atlantic puffins breeding in Nordland was slightly lower than that of kittiwakes (**Figure 3.9B),** with a mean of 33.9 nmol/g **(Table 3.6).**

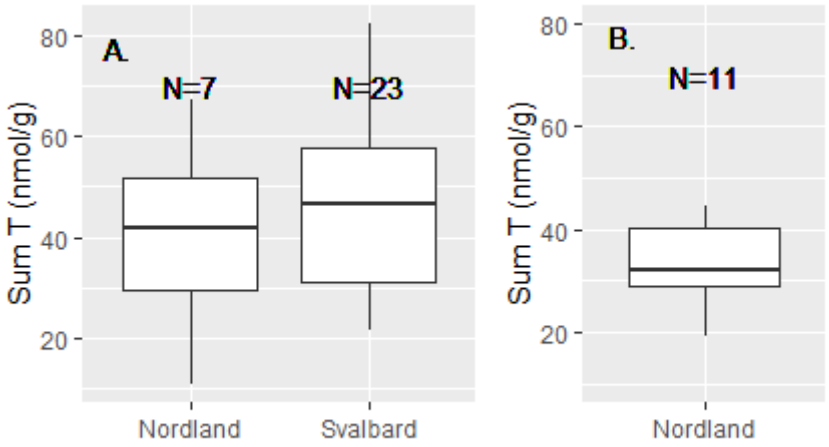

Figure 3.9. *Boxplot of sum T (nmol/g) in egg yolk from kittiwakes breeding in Nordland and Svalbard (A.) and Atlantic puffins breeding in Nordland (B.).*

Table 3.5*. Descriptive statistics for sum T (nmol/g ww) in egg yolk from kittiwakes breeding in Svalbard and Nordland.*

Region	N	mean	se	max	min	2.5%ci	97.5%ci
Svalbard	23	47.3	3.8	82.4	21.6	39.5	55.2
Nordland	7	40.4	6.9	67.1	10.9	22.8	58.1

Table 3.6*. Descriptive statistics for sum T (nmol/g ww) in egg yolk from Atlantic puffins breeding in Nordland.*

Region	N	mean	se	max	min	2.5%ci	97.5%ci
Nordland	11	33.9	2.5	44.6	19.1	28.4	39.3

3.3 Thiamine levels and composition in seabird prey

3.3.1 Blue mussel

Blue mussels are important prey for eiders in mainland Norway. Blue mussels are, however, rare in Svalbard and are not an important prey for Svalbard eiders. Average sum T was 0.98 ± 0.09 and 0.17 ± 0.0 nmol/g in blue mussel soft tissue from Oslofjord and Troms, respectively (**Figure 3.10A, Table 3.7**). The difference among the locations was statistically significant ($F_{1,28}$ =38.9, p<001). TDP was the most important part of sum T, constituting 75 and 92% in samples from Troms and Oslofjord, respectively (**Figure 3.10B**). T was below LOQ in all samples, while TMP was below LOQ in 15/15 and 3/15 samples from Troms and Oslofjord, respectively (**Table 3.8**).

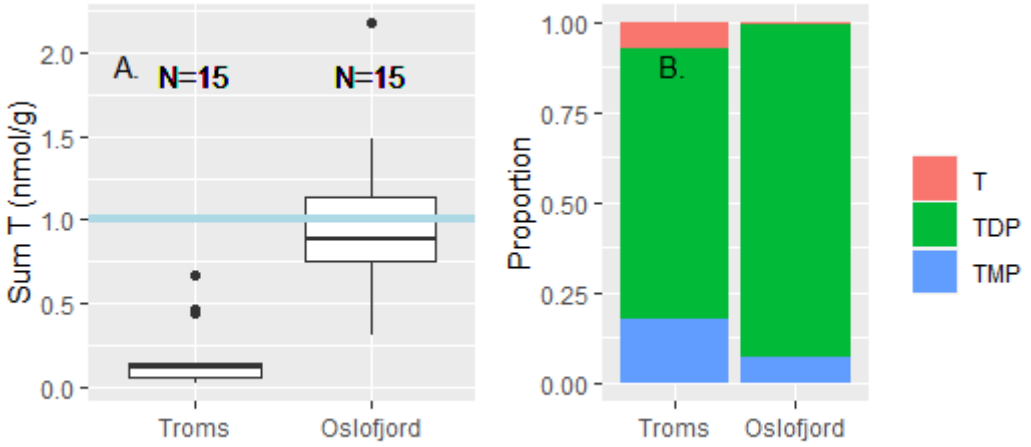

***Figure 3.10**. (A.) Boxplot of sum T (nmol/g) and (B.) proportion of the three forms of thiamine (T, TMP, TDP) in blue mussels from Troms and Oslofjord. The horizontal blue line in panel A refers to average sum T in blue mussels from the Baltic Sea and categorized as severe thiamine deficiency (Balk et al. 2016).*

***Table 3.7**. Descriptive statistics for sum T (nmol/g) in blue mussels collected in Troms and Oslofjord.*

Region	N	mean	se	max	min	2.5%ci	97.5%ci
Troms	15	0.17	0.09	0.67	0.02	0.07	0.28
Oslofjord	15	0.98	0.09	2.18	0.3	0.72	1.23

***Table 3.8.** Proportion (N / N_{total}) of samples >LOD and >LOQ for blue mussel samples from Troms and Oslofjord.*

Sample type	Region	T	TMP	TDP
Blue mussel	Troms	0/15	0/15	8/15
Blue mussel	Oslofjord	0/15	12/15	15/15

3.3.2 Diet samples collected from kittiwakes and Atlantic puffins

The diet samples reflect the prey items collected by the adults at sea and brought back to the nest, mainly to feed their chick(s) in the nest. All Atlantic puffin diet samples consisted of sandeel, whereas the kittiwake diet samples were more heterogenous (**Table 3.9)**. Kittiwakes in Nordland had mainly fish in their diet, and both herring and sandeel were identified in the diet samples. Some samples contained mashed fish as a result of digestive processing in the crop, and here identification of species was not possible. Kittiwakes in Svalbard did not catch sandeel, but herring and polar cod. In addition, krill and sea butterfly were present in the diet samples. Average sum T of diet samples from kittiwakes was 4.3 ± 1.24 and 1.8 ± 1.24 nmol/g, and did not differ significantly among kittiwakes from Svalbard and Nordland ($F_{1,18}$ =2.0, p=0.17, **Figure 3.11A**, **Table 3.10**). Average sum T of Atlantic puffin diet samples from Nordland was 6.1 ± 0.54 nmol/g (**Figure 3.11B. Table 3.11**). The variation in sum T was rather high within kittiwake samples (**Figure 3.11A, Table 3.10**), and several samples had T and TMP concentrations below LOQ (**Table 3.12**). The proportions of T, TMP and TDP were 29, 20 and 51 and 76, 13 and 11 % in kittiwakes from Svalbard and Nordland, respectively **(Figure 3.12**). T, TMP and TDP were above LOQ and LOD in all samples of Atlantic puffin diet (**Table 3.12**), and constituted 35, 50 and 15%, respectively.

Table 3.9*. Overview of prey groups and species identified in the collected diet samples of kittiwakes and Atlantic puffins from Svalbard and Nordland.*

Seabird	Region	Prey groups	Prey species identified
Kittiwake	Svalbard	Fish, krill, sea butterfly	Herring, polar cod, *Thysanoessa inermis*, *Thecosomata sp*
Kittiwake	Nordland	Fish, fish larvae	Herring, Sandeel
Atlantic puffin	Nordland	Fish	Sandeel

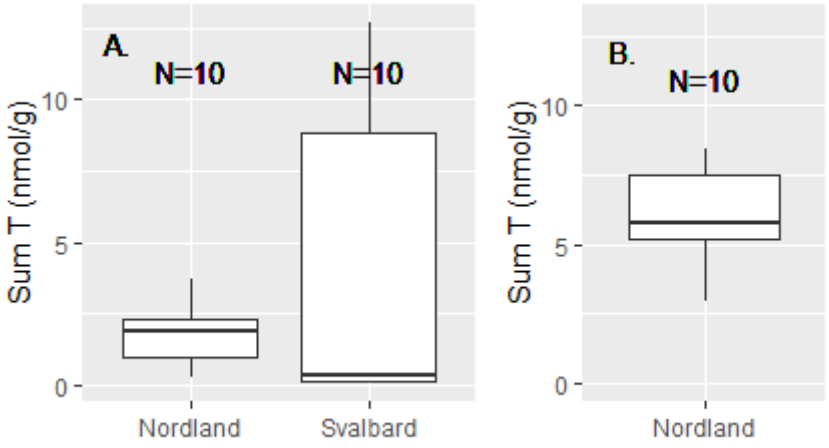

Figure 3.11. *Boxplot of sum T (nmol/g) in diet samples from kittiwakes breeding in Nordland and Svalbard (A.) and Atlantic puffins breeding in Nordland (B.).*

***Table 3.10**. Descriptive statistics for sum T (nmol/g ww) in diet samples from kittiwakes breeding in Svalbard and Nordland.*

Region	N	mean	se	max	min	2.5%ci	97.5%ci
Svalbard	10	4.3	1.24	12.7	0.05	0.4	8.2
Nordland	10	1.8	1.24	3.7	0.25	1	2.6

***Table 3.11**. Descriptive statistics for sum T (nmol/g ww) in diet samples from Atlantic puffins breeding in Nordland.*

Region	N	mean	se	max	min	2.5%ci	97.5%ci
Nordland	10	6.1	0.54	8.4	3	4.9	7.3

***Table 3.12.** Proportion (N / N_{total}) of samples >LOD and >LOQ for diet samples from kittiwakes and Atlantic puffins breeding in Svalbard and Nordland.*

Species	Region	T	TMP	TDP
Kittiwake	Svalbard	4/10	4/10	7/10
Kittiwake	Nordland	10/10	7/10	7/10
Atlantic puffin	Nordland	10/10	10/10	10/10

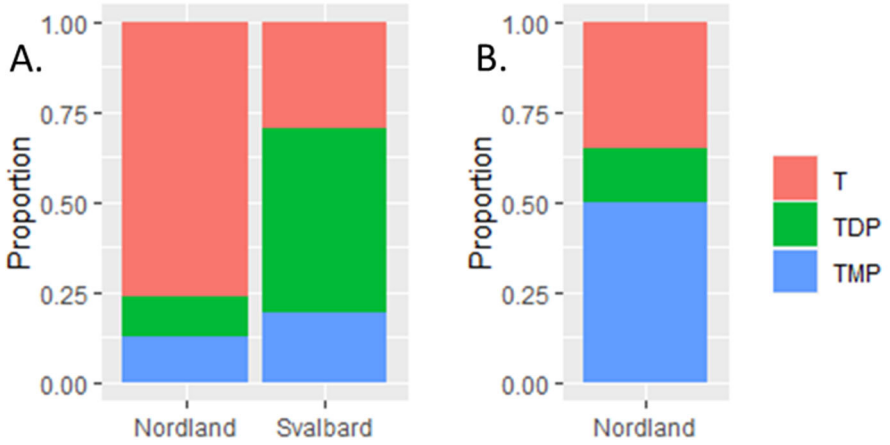

***Figure 3.12**. Proportion of the three forms of thiamine (T, TMP, TDP) in diet samples from kittiwakes (A.) breeding in Svalbard and Nordland, and Atlantic puffins (B.) breeding in Nordland.*

3.5 Clinical observations and pathological examinations:

During the field season in 2019, no cases of paralytic syndrome or mortality were reported in any of the focal seabird colonies (**Figure 2.1**). Hence, no birds or carcasses were subjected to such investigations. A single juvenile graylag goose (*Anser ansers*) found abandoned at Justøya in Lillesand with clinical signs of central nervous system disease (ataxia, confusion) was necropsied, but found to have a unilateral purulent meningoencephalitis and not subjected for further histopathological examination.

4 Discussion

4.1 Methodological tests

4.1.1 Storage freezing temperature

The results showed no statistical difference in thiamine levels among duplicate samples stored in -20 vs -150/-80°C. Average levels were similar among the two temperature treatments. Hence, it should be possible to use -20°C. However, -20°C would limit other potential biochemical analyses (e.g. enzyme activity), and dry-shipper should be preferred whenever practically possible. Nevertheless, we recommend consistency of procedures for the populations/type of samples that should be compared. Furthermore, this test was done with egg yolk, which only contains non-phosphorylated T. This is the most stable form of T. TMP and TDP are expected to be more unstable and more sensitive to higher temperature. Blue mussel and diet samples had substantial proportions of TMP and TDP, and this may question the use of -20C for blue mussels and diet samples.

4.1.2 Incubation time

An embryo will develop during incubation, and the growing embryo may metabolize thiamine. Our goal for testing the effect of incubation time on sum T was to establish a statistical relationship between the two which could be used for correcting sum T levels in eggs that have been incubated. However, our test cannot be used to correct statistically for incubation time in egg yolk thiamine levels. The reason is that egg yolk membrane brakes when opening the eggs that have been incubated. This happened in all incubated eggs in this study. Mixing of egg-yolk and albumen would cause dilution of thiamine levels. An alternative would be to homogenize eggs, but this has some drawbacks. The most important is that previous studies have analyzed thiamine in egg yolk and not in homogenate of yolk and albumen. Another is increased risk of obtaining concentrations below LOD. Another option would be to freeze eggs and sample yolk after subsequent thawing. Then yolk and albumen are better separated because albumen thaws before the yolk. However, additional freezing and thawing should generally be avoided. At should then be tested whether it increased degradation and affected thiamine levels.

4.1.3 Inter-laboratory calibration

ACES quantified the thiamine levels reported by Balk et al. (2009, 2016) and Mörner et al. (2017). These studies report levels from seabird populations in the Baltic Sea and in Iceland, which form an important basis for comparison of thiamine levels among species, populations and geographic regions. For practical reasons it is not realistic that ACES would do the quantification for all future studies, and it is also relevant to have independent results from other laboratories. In Norway, IMR is certified to analyze thiamine and we compared IMR and ACES using duplicate egg yolk samples in this study. The comparison demonstrated that the different methods provided different results. ACES concentrations were higher than IMR (2.0 mg/kg), and this underlines the need for inter-calibration of different methods and labs. The concentrations in duplicated samples correlated significantly between IMR and ACES, and our equation (1) can be used for correcting values from IMR and subsequent comparison with previous results from ACES. However, the inter-calibration was performed with egg yolk and concentrations ranging 1.7- 5.1 mg/kg (IMR). Applying this equation for other matrices should be avoided or done with great caution. Furthermore, the composition of the forms of thiamine (T, TMP and TDP) must be known for the focal matrix before converting IMR values (mg/kg) to ACES values (nmol/g). Finally, the results also stress caution about applying the predictive equation for concentrations outside the investigated range (extrapolation) due to higher uncertainty and wide confidence intervals.

4.2 Thiamine levels and composition in seabird prey

4.2.1 Common eider

Sum T concentrations varied significantly between the Norwegian common eider populations. The levels were lowest in the Troms population, intermediate in Oslofjord and highest in Svalbard.

We compare these results with those obtained from common eider populations in the Baltic Sea and Iceland by Balk et al. (2009, 2016) and Mörner et al. (2017). Balk et al. (2009) characterized the average thiamine level in eggs from the Baltic sea (7.2 nmol/g) as thiamine deficiency. Based on results for different effect parameters, Balk et al. (2016) suggested lethal effects for eider eggs at yolk concentrations ≤ 5.7-8.8 nmol/g and sub-lethal effects ≤ 13–21 nmol/g.

The average sum T in egg yolk samples from Troms (9.9 ±1.7 nmol/g) is very close to the average level for common eiders from the Baltic Sea (7.2 nmol/g, Balk et al. 2016). Balk et al. (2016) suggested a threshold at 5 nmol/g for viable offspring, and 33% of the samples (5/15) were below this threshold. This may warrant future attention and investigations whether low levels sustain in years to come and potentially impair reproduction. Interestingly, this population has shown very strong declines in population size over the last decades. Previous studies have shown that increased sea surface temperatures and predation are important causes for population decline (Bårdsen et al. 2018, Hanssen et al. 2013). Low thiamine levels may act as an additional stressor. In such a multi-stress perspective, low thiamine levels should be of concern.

Levels were higher in eider eggs from Oslofjord. However, only 2 eggs of those collected in 2019 were freshly laid and analysed for thiamine levels. The other eggs in 2019 were incubated and could thus not be reliable analyzed. Confidence intervals (N=2) shows that the result can not be used for inference on this population. However, we included results from Hanssen et al. (2020b) who collected eggs in Oslofjord in 2020. That project was launched after an incident of mass-mortality and collection of ~100 dead eiders in March 2020 (Hanssen et al. 2020a). Thiamine levels in eggs collected in Oslofjord in 2020 were higher than those from Troms, but lower than those from Svalbard, and can be characterized as intermediate. The average sum T in Nesodden and Hvaler (15.2 ± 1.3 and 16.2 ± 1.2 nmol/g) were close to that reported from Iceland (17 nmol/g) and higher than in eiders from the Baltic sea (7.2 nmol/g, Balk et al. 2009). The highest levels in eiders were in the Svalbard population, with an average of 20.9 ± 1.8 nmol/g.

4.2.2 Herring gull

The average thiamine level in herring gull eggs from Oslofjord was very low (19.5 ± 3.6 nmol/g), and at similar level reported for herring gulls from the Baltic Sea and categorized as thiamine deficiency (19 nmol/g, Balk et al. 2009). Our results for Oslofjord is, however, only based on 3 eggs analyzed and uncertainty and confidence intervals are large. The result still warrants some concern and need for additional samples from this population before stronger inference can be made. The average thiamine level in herring gulls from Nordland was higher (24.7 ± 1.6 nmol/g) but not statistically different from Oslofjord. Balk et al. (2009) found that average levels in the range of ~19-23 nmol/g correlated negatively to average clutch size, indicating an effect on fecundity and reproduction. Based on extrapolation of this relationship they suggested that average levels below 34 nmol/g could result in a reduced clutch size (Balk et al.2009). Balk et al. (2016) suggested lethal effects for herring eggs at yolk concentrations ≤ 18-20 nmol/g and sub-lethal effects ≤ 25–34 nmol/g. In this perspective, herring gull eggs from both Oslofjord and Nordland should be characterized as thiamine deficient. Eggs from both populations had lower levels than herring gulls from Iceland (29 nmol/g, Balk et al. 2009). The sum T levels associated with potential effects is consequently much higher in herring gulls compared to eiders differ. Such differences among species is apparently not understood.

4.2.3 Kittiwake and Atlantic puffin

Egg yolk samples from kittiwakes and Atlantic puffins had the highest levels among all species in this study. Average sum T was 40.4 ± 6.9 and 47.3 ± 3.8 nmol/g in kittiwake egg yolk samples from Svalbard and Nordland, respectively, and 33.9 ± 2.5 nmol/g in Atlantic puffin egg yolk samples from Nordland. To our knowledge, this is the first study to report thiamine levels in egg yolk samples from these species. As such we cannot yet compare with other populations. The results may form the basis of future comparison among populations but also comparisons within the same populations among years and changes over time.

Sum T consisted almost of 100% T in egg yolks from all species, and TMP or TDP was below LOQ or at very low levels. TMP and TDP are negatively charged, and this prevent TDP and TMP to be spontaneous transferred from the mother to the egg. The contribution, if any, of mediated or active transport of thiamine into the eggcell is still unknown.

4.2.4 Blue mussel

Average sum T in blue mussels from Oslofjord and Troms were 0.98 ± 0.09 and 0.17 ± 0.09 nmol/g, respectively. The levels differed significantly among the two locations. They were low compared to levels reported from Iceland, where the average sum T ranged from ~1 to 3.5 nmol/g (Balk et al. 2016). Average sum T from Oslofjord was similar to the average level reported for Baltic Sea (1.0 nmol/g) -which has been characterized as severe thiamine deficiency and suggested as threshold for survival of blue mussels (Balk et al. 2016). The average sum T from Troms were even much lower than that. This may challenge the suggested threshold for blue mussel survival. It also suggests that eiders in Troms feed on prey with extremely low thiamine levels, and further sampling and monitoring is needed.

4.2.5 Diet samples from kittiwakes and Atlantic puffins

The diet samples in this study reflect the prey items collected by the adults and brought back to feed their chicks. To our knowledge, this is the first study to report thiamine levels in diet samples from these species. The diet samples are partly composite samples, of different prey items, and partly uniform samples of single prey species. We are not aware of studies reporting thiamine levels for sandeel, polar cod, krill or sea butterfly. Balk et al. (2016) reported levels for specific tissues in females and males of Baltic sea herring (brain: 27.7 and 27.9 nmol/g, liver: 13.7 and 21.1 nmol/g, white muscle: 6.0 and 6.6 nmol/g). These adult herrings were categorized as clearly non-deficient of thiamine by Balk et al. (2016). The herring in our diet samples were young individuals only (0 and 1-group), and we analyzed whole fish or composite samples, and direct comparison cannot be made. As such we cannot yet compare with other populations. The results may form the basis of future comparison, though.

4.3 Clinical and pathological investigations

During this pilot study, we aimed to perform clinical, pathological investigations and necropsy of dying birds with strong symptoms associated with thiamine deficiency or dead birds with potential/suspected thiamine deficiency. However, no such cases were observed in any of the focal seabird colonies in the 2019 field season, and no birds or carcasses were subjected to such investigations.

Balk et al. (2009, 2016) and Mörner et al. (2017) have described clinical signs and pathological findings in birds proposed to suffer from thiamine deficiency. Specifically, they have linked thiamine deficiency to paralytic syndrome and suggested negative impact on population level.

The described clinical signs and pathological findings have been criticized as being imprecise and inconsistent from a veterinary medical perspective (Rocke & Barker 2010, Sonne et al. 2011, 2012), and not sufficiently validated with coherent histopathological findings. However these papers has been responded at, both from Balk et al. (2010) and other authors (Tillitt et al. 2012). There is also little knowledge about the reference values and threshold levels for thiamine for most wild bird species. From an ecological perspective, the description of reproductive failure and susceptibility of chicks to predation (e.g. Mörner et al. 2017), are also unspecific, and not convincingly justified as a clear effect of thiamine deficiency, as they can be easily caused by ecological factors. As such, it is not warranted to infer causality for all cases (Swaen & van Amelsvoort, 2009).

To elucidate if low levels of thiamine, clinical signs or mortality can be related to thiamine deficiency, necropsy should be performed on relevant cases. The carcass should be brought to a veterinary pathologists with experience in central nervous system histopathology to undertake such a necropsy. An extensive and high-standard necropsy will not only target findings relevant for thiamine deficiency but also the full range of wildlife disease. Most seabird field work are done in remote locations, and it is not always possible to transport a freshly dead bird to a veterinary pathologist. In such cases, it would be useful to undertake a field necropsy. To facilitate this, we have made a standard procedure for field necropsy (Ytrehus and Work, 2019). Proper and reliable necropsy results would though require training of key field personnel and cooperation with veterinary pathologist. To elucidate if, when and to what degree paralytic syndromes impact Norwegian bird populations, we need to survey their occurrence and nature. To facilitate standardized and reproducible registration of clinical signs we have included a draft for a report form for use in the field in Ytrehus and Work (2019).

4.4 Potential effects on Norwegian seabird population

Many Norwegian seabird populations have shown dramatic declines over the last decades (Fauchald et al. 2015a). There can be many and complex reasons for these decline, but food availability has been suggested as one of the most important factors (Fauchald et al. 2015b). Also, predation is important, and may act in concert with poor food conditions and cause additional stress on populations (Fauchald et al. 2015b). The potential role of diseases has not been investigated much, and this is the first time thiamine levels have been investigated in Norwegian seabird populations and food webs.

This pilot-study cannot answer whether Norwegian seabird populations sizes are affected by thiamine levels. The pilot study has, for the first time, investigated thiamine levels in seabird eggs from selected species and populations in Norway mainland and Svalbard, and in their food webs. The pilot study revealed variation among species, populations and prey types. Some of the results show low levels accordant with thiamine deficiency as defined by Balk et al. (2009, 2016). As such, we cannot rule out that thiamine can be a limiting factor in marine ecosystems in Norway for some seabird populations. Our results of low levels coincide partly with population trajectories. For example, the eider population in Troms had the lowest thiamine levels and has declined substantially over the last decades, while the Svalbard population had the highest levels and much better population trajectory (Hanssen et al. 2013). We also showed low levels in blue mussels, especially in Troms, the main prey of eiders form mainland Norway. Herring gull populations have also declined (Fauchald et al. 2015a) and their thiamine levels were rather low too. Thiamine levels were highest in kittiwakes, puffins and their prey. They are among the seabird species showing the strongest declines in populations sizes in Norway (Fauchald et al. 2015a) and are classified as endangered (EN) and vulnerable (VU), respectively, on the Norwegien IUCN red-list for mainland Norway (Henriksen & Hilmo 2015). However, the kittiwake and puffin populations we investigated, in Kongsfjorden (Svalbard) and Anda, have shown relatively better population trajectories.

4.5 Knowledge gaps and recommendations

There is a need to monitor and perform more sampling over several years to assess inter-annual variation for better understanding natural variation and reveal potential episodes of thiamine deficiency. Seabird species and populations may differ in their thiamine levels according to the thiamine content of their prey. Continued sampling in the same populations as the present study would fulfill this need. Also there is a knowledge gap for populations not investigated so far. For example, kittiwake and puffin populations showing strong declines in population sizes should be targeted. The populations from this report should also be included for comparative basis.

If thiamine affect population size, it must affect reproduction or survival negatively. For reproduction and reproductive success, there is a need to test whether thiamine levels are associated with clutch size, hatching success or fledging success. This can be achieved with observational studies. The potential links between stress hormones and thiamine, as well as contaminants and thiamine levels, are also interesting to investigate Species and populations with previous data on stress hormones and contaminants are good potential candidates for such studies, like kittiwakes and eiders. Comparative studies, using species with high and low thiamine levels, is also interesting for better understanding if and why the levels associated with potential effects (‘threshold levels/ranges’) vary considerable among species.

Experimental studies can be done using thiamine treatment. Injection of thiamine into eggs could be one alternative, but this will require further investigations before conducting such an experiment. It may be possible to only inject into the albumen, and this may not be available for the embryo. Experimental treatment on chicks is another alternative. Eider chicks are not very well suited for this in field studies, but it could work if eider chicks were kept in the lab or a captive situation. Kittiwake and herring gulls chicks would perhaps be better candidates for such an experimental approach under natural field conditions. Thiamine treatment has been debated (Rocke & Barker 2010, Balk et al. 2010), but from a study-design perspective, such experiments are generally very strong for investigating causal relationships.

There is also a need to investigate whether thiamine levels are linked to survival. This usually require substantial effort and so called capture-mark-recapture studies (CMR). This requires measurements of thiamine of individuals that have been banded/marked and subjected to demographic monitoring over several years. This will require large sample sizes. A study on avian cholera demonstrated a link between disease, survival and population dynamics (Descamps et al. 2011). In this study it was shown that the survival of the youngest age-classes of eiders and the population trajectory of eiders were negatively affected in years with avian cholera outbreak. Thiamine deficiency is probably not as potent as avian cholera, though.

Food shortage and predation are population limiting factors, and they are stress inducing. Increased stress may lead to increased sensitivity to other stress factors. In such a multiple-stress perspective (Sonne et al. 2012, Bårdsen et al. 2018, Sonne et al. 2020), thiamine deficiency would be an additional stress factor, and not only a single factor or the most important factor affecting population size. In such a perspective, we expect statistical interaction effects among stressors. In the case of food shortage, contaminants or predation, we expect thiamine levels to be a significant negative predictor only at relatively high levels of food shortage, predation or contaminant load.

It remains to be investigated whether disease occurs as a results of thiamine deficiency, such as paralytic disease and disorders. This require high standard necropsy and histopathological investigations. It remains to be revealed whether clear histopathological findings can be identified in Norwegian seabirds. Nevertheless, necropsy has the advantage that it can rule out or identify other underlying disease. Several diseases can cause abnormal behaviour in birds, and field workers may wrongly link such behaviour to paralytic disease and thiamine deficiency.

I remains to investigate if, when and where symptoms of paralytic disease occur in Norwegian seabird populations. It can be investigated from case to case, where symptoms are observed, and linked to proper diagnostics. If we are to provide an answer for how abundant this occurs and how prevalent this is, we need to use a large -scale balanced study design based on standardized observations in selected populations on a relative large geographic scale.

We have also identified some knowledge gaps on the methodological side. There is a need for more interlaboratory calibrations if we would like to use a Norwegian laboratory in future monitoring. In this study, we only calibrated with egg and at relatively high levels. Eggs with lower levels are needed to have less uncertainty in the predictive equation and avoid extrapolation. With the present equation (1) the LOQ at IMR (0.1 mg/kg) corresponds to 9.2 nmol/g at ACES. The uncertainty is high at these low levels, and predicting values at this range is extrapolation and should ideally be avoided. If the equation remain unchanged after additional calibration, using egg samples with lower levels, it can be a challenge that the LOQ is at this relatively high level. It is in line with the average for eiders form Troms, higher than eiders from the Baltic Sea (Balk et al. 2009, 2016) and much higher than the blue mussels (this study, Balk et al. 2009, 2016).

The test of storage temperature was promising for using -20°C. However, this test was done with egg yolk, which only contains non-phosphorylated T. this is the most stable form of T, and TMP and TDP are expected to be more unstable and more sensitive to higher temperature. As such, this may question the use of -20C for blue mussels and diet samples.

For the test of incubation time, we conclude that we should keep to collecting fresh eggs. We don’t have any good solutions for coping with eggs that have been incubated. We may cope with eggs that have been slightly warmed for a day or two, but it seems that proper incubation after clutch completion negatively affect the yolk membrane and cause mixing of yolk and albumen when opening the eggs. When eggs have been incubated for very long, however, the yolk becomes more compact. At that time it should be possible to take a clean yok sample, but that could maybe be used for other purposes, and not in this study context.

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