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Are yolk thiamine levels associated with stress hormone levels in seabirds?

Master's thesis in Biology

Supervisor: Børge Moe

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Abstract

Several Norwegian seabird populations are declining. The reason may be complex, and proposed explanatory factors include climate change, reduced food availability, pollution, alien species, and diseases. These factors are stress-inducing and may ultimately lead to reduced reproductive investment and survival. In recent years there has been an increased focus on diseases as a possible cause, especially diseases and symptoms associated with thiamine deficiency. Thiamine is an essential nutrient for seabirds that needs to be acquired through their diet. Low levels of thiamine could lead to e.g. neurological disorders and immunosuppression. Thiamine deficiency has been suggested as a contributing factor for reproductive failure and population decline in seabirds breeding in the Baltic Sea and the North-Atlantic Ocean. The hypothesis being that thiamine deficiency in seabirds is caused by episodes of deficiency of this specific vitamin in the marine food web. In this study we examined an alternative hypothesis that poor food availability could cause generally low levels of thiamine as well as other vitamins and nutrients. Corticosterone is the avian stress hormone, known to be sensitive to body condition and nutritional status. Under the assumption that corticosterone levels reflect nutritional status and food supply, it was tested if corticosterone and thiamine levels correlated. Egg yolk was collected from three common eider populations and one black-legged kittiwake population. No significant correlation between corticosterone and thiamine levels was found. Thiamine and corticosterone levels differed among the common eider populations. This study was correlational and cannot reveal any causal relationships, but the results indicate that thiamine levels are independent of corticosterone levels. The results therefore support the hypothesis that thiamine deficiency in seabirds comes from a specific deficiency of thiamine in the marine food web rather than poor food availability.

Sammendrag

Flere norske sjøfuglbestander minsker. Årsaken til populasjonsnedgangen kan være sammensatt, og ulike forklarende faktorer som klimaendringer, redusert mattilgang, forurensning, fremmede arter og sykdom har blitt foreslått. Dette er stress-induserende faktorer som til slutt kan føre til redusert reproduksjon og overlevelse. De siste årene har det vært økt oppmerksomhet på sykdom som en mulig forklarende årsak, og da spesielt sykdom og symptomer assosiert med tiaminmangel. Tiamin er et essensielt næringsstoff for sjøfugl som de må få gjennom dietten. For lave nivåer med tiamin kan blant annet føre til nevrologiske lidelser og nedsatt immunforsvar. Tiaminmangel har blitt foreslått som en medvirkende årsak til reproduksjonsvikt og populasjonsnedganger i Østersjøen og i Nord-Atlanteren. Hypotesen er at tiaminmangelen i sjøfugl skyldes mangel av dette spesifikke vitaminet i det marine næringsnett. I dette studiet undersøkte vi en alternativ hypotese, at dårlig mattilgang forårsaker generelt lave nivåer av tiamin, andre vitaminer og næringsstoffer. Kortikosteron er fuglenes stresshormon, kjent for å være følsomt for fugler sin ernæringsstatus. Under antagelsen at kortikosteron reflekterer ernæringsstatus og mattilgang, ble det testet om kortikosteron-nivåer og tiamin-nivåer korrelerer. Eggeplomme ble samlet inn fra tre ærfugl- og en krykkje-populasjon. Det ble ikke funnet noen signifikant korrelasjon mellom kortikosteron- og tiamin-nivå. Kortikosteron- og tiamin-nivåene i ærfugl-populasjonene var forskjellige fra hverandre. Dette studiet så på korrelasjon og kan derfor ikke si noe om kausale forhold, men resultatet indikerer likevel at tiamin nivåer er uavhengige av kortikosteron nivåer. Disse funnene støtter hypotesen om at tiaminmangel i sjøfugl kommer av et underskudd på dette vitaminet i det marine næringsnett fremfor dårlig mattilgang generelt.

Preface

This master's thesis was written at the Department of Biology, Norwegian University of Science and Technology (NTNU) in collaboration with the Norwegian Institute of Nature Research (NINA). My main supervisor has been Børge Moe (NTNU, NINA) and my co-supervisor has been Sveinn Are Hanssen (NINA). The field work in Svalbard was supported by an Arctic field grant from Svalbard Science Forum.

I want to thank my supervisors Børge Moe and Sveinn Are Hanssen for having me as their master student and for a fantastic field season at Svalbard. I appreciate the good atmosphere and the opportunity to learn techniques for field work. Thanks to Olivier Chastel, William Jouanneau, Don Jean Léandri-Breton, Céline Albert, Sveinn Are Hanssen and Børge Moe for collecting egg yolk samples. Thanks to Tom Zahl Pedersen and Nesodden kommune for logistical support at Nesodden as well as Haakon Braathu Haaverstad and Norwegian Nature Surveillance (SNO) for logistical support at Hvaler. Thanks to Charline Parenteau and Olivier Chastel at CEBC-CNRS for analysing corticosterone. Thanks to Lennart Balk and Hanna Gustavsson at the Department of Environmental Science and Analytical Chemistry (ACES) and Annbjørg Bøkevoll at the Institute for Marine Research (IMR) for analysing thiamine. Finally, I am grateful to those who have given me comments and feedback on my thesis, with a huge thank you to my friends and family for the support.

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Anne Wangen

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List of abbreviations

NINA	Norwegian Institute for Nature Research
ACES	Department of Environmental Science and Analytical Chemistry
IMR	The Institute for Marine Research
CEBC-CNRS	Centre for Biological Studies of Chizé in France
LOD	Limit of detection
LOQ	Limits for quantifications
ANOVA	Analysis of variance
SE	Standard error
CI	Confidence interval
ww	Wet-weight
T	Non-phosphorylated thiamine
TDP	Di-phosphorylated thiamine
TMP	Mono-phosphorylated thiamine

Introduction:

Population numbers for several seabird species in Norway are declining. This is observed both in the northern (Barents Sea and Norwegian sea) and southern (Skagerrak and North Sea) part of Norway (Fauchald et al., 2015a). The reason for the decline can be complex, and different explanations have been suggested. Some of the explanatory factors for this decline could be climate change, reduced food availability, alien species, ocean warming, pollution, over-fishing or harvesting, by-catch, hunting, disturbance, increased predator pressure and diseases (Fauchald et al., 2015b; Hanssen et al., 2013). None of these factors can explain the general problem alone, but complicated effects on the food web seem to play an important role. An example of this is food sources moving north because of increasing ocean temperatures, combined with increased predation in the seabird colonies. These factors are stress-inducing, which can lead to increased sensitivity for other factors and ultimately reduce reproductive investment and survival (Fauchald et al., 2015b).

Reduced food supply is assumed to be one of the most important explanatory factors for the population declines of seabirds in Norway (Fauchald et al., 2015b). Corticosterone is the avian stress hormone and it is known to be sensitive to the animal's body condition and nutritional status. Specifically, elevated corticosterone levels are expected with food shortages or poor body condition (Crisuolo et al., 2005, 2006; Kitaysky et al., 1999a, 2001; Lendvai et al., 2014). Corticosterone is involved in regulation of energy, stress responses and immune reactions. It regulates body maintenance processes mainly through modifying the behaviour of the bird e.g. facilitate foraging behaviour and/or interrupt reproduction. The response from this hormone can improve survival, but also lead to reproductive failure (Cherel et al., 1988; Criscuolo et al., 2005; Kitaysky et al., 1999b).

In recent years, there has been an increased focus on diseases as explanatory factors for the population declines, and especially diseases and symptoms associated with thiamine (vitamin B1) deficiency (Balk et al., 2009, 2016; Mörner et al., 2017). Thiamine is a water-soluble, essential nutrient for seabirds that needs to be acquired through their diet. In animal cells, non-phosphorylated thiamine becomes phosphorylated to thiamine diphosphate. Thiamine diphosphate functions as a cofactor for enzymes required for several biochemical processes that are crucial for the bird's life. It is known to be a co-factor in at least five vital enzymes in the birds' cellular metabolism. With further phosphorylation of the thiamine diphosphate to

thiamine triphosphate, it is essential for proper function of the neuronal membranes (Bettendorff et al., 1993; Cooper & Pincus, 1979; Manzetti et al., 2014). Low levels of thiamine can lead to memory and learning disorders, immunosuppression, and neurological disorders, and are ultimately lethal (Langlais & Savage, 1995; Mair et al., 1985; Manzetti et al., 2014; Prasad et al., 1980).

In Sweden, studies have documented thiamine deficiency in several fish species, shellfish, herring gulls (*Larus argentatus*) and common eiders (*Somateria mollissima*). Birds with clinical symptoms associated with thiamine deficiency have also been observed (Balk et al., 2009, 2016; Mörner et al., 2017). The hypothesis by Balk et al. (2016) is that the thiamine deficiency is a result of a deficiency of this specific vitamin in the marine food web and not starvation or poor food availability for seabirds.

As it is documented that thiamine deficiency has negative effect on both survival and reproduction, it is suggested to be a significant contributor to population declines in the Baltic Sea and the North-Atlantic Ocean (Balk et al., 2009, 2016; Mörner et al., 2017). A recent study investigated the thiamine levels in seabirds in Norway and Svalbard (Moe et al., 2020). The study revealed lower thiamine levels in egg yolk from common eiders in southern Norway than in common eiders in Svalbard. Thiamine levels in egg yolk from black-legged kittiwakes (*Rissa tridactyla*) in both northern Norway and Svalbard were much higher compared to the levels in common eiders. The common eiders are benthic feeding, and the black-legged kittiwakes are pelagic feeding. Hence, there seems to be variation among populations (areas), species and food webs.

There is a need for a better understanding of the possible causes of thiamine deficiency, but to our knowledge, no studies have investigated whether thiamine levels are associated with corticosterone levels. If the reason for low thiamine levels come from food shortages rather than a specific deficiency of thiamine in the marine food webs, we expect to see an association between corticosterone and thiamine. This is under the assumption that corticosterone levels reflect food supply, and that the food supply affects thiamine levels. Maternal corticosterone has shown to transfer to yolk during egg formation (Almasi et al., 2012; Cook et al., 2009; Hayward & Wingfield, 2004) and maternal thiamine nutrition has shown to affect the offspring's thiamine levels and metabolism in hens (Olkowski & Classen,

1999). We therefore assume that yolk corticosterone levels reflect food availability and that yolk thiamine levels reflect thiamine levels for the mother during egg formation.

The aim of this thesis is to measure whether thiamine levels are associated with corticosterone levels in seabirds. Our hypothesis is that thiamine deficiency in seabirds comes from food shortages, rather than thiamine deficiency in the marine food web. We predict a negative correlation between corticosterone and thiamine levels, where high corticosterone levels are related to low thiamine levels. The hypothesis of Balk et al. (2016), stating that thiamine deficiency in seabirds is expected to be a result of a specific deficiency of thiamine in the marine food web, predicts no connection between corticosterone and thiamine, and therefore no correlation between yolk levels of corticosterone and thiamine.

To test for a potential relationship between corticosterone and thiamine, eggs from benthic feeding common eider in Oslofjorden (Norway) and Kongsfjorden (Svalbard) and eggs from pelagic feeding black-legged kittiwake in Kongsfjorden were sampled and analysed for thiamine and corticosterone concentrations. Common eider was chosen as a study species because previous research showed thiamine deficiency in this species in the Baltic Sea (Balk et al., 2009, 2016). Black-legged kittiwakes was chosen as a study species for comparative reasons: 1) They have shown to have higher thiamine levels than common eiders in general (Moe et al., 2020), and 2) they feed on another level in the food web. This study design made it possible to investigate if the potential relationship between yolk corticosterone and thiamine levels differs among populations and between two species feeding in different parts of the marine food web.

Methods

Study species and locations

Common eiders are benthic feeding, and eat mainly molluscs, echinoderms, crustaceans, fish eggs and marine worms. These prey items can normally be found in depths of less than 10 meters below the water surface, even though they can dive much deeper. Common eider is the heaviest duck species in the northern hemisphere, and it is sexually dimorphic. The female usually lays 4-6 eggs on islands and islets and incubate and care for the chicks alone. By fasting during the incubation period, the female maximises nest attendance and protect the nest from predation (Bustnes & Lønne, 1997; Fauchald et al., 2015b; Waltho & Coulson, 2015).

Black-legged kittiwakes are pelagic feeding and eat mostly small fish. They can also eat zooplankton, small squids, jellyfish, and marine worms. Black-legged kittiwakes breed in the arctic and sub-arctic areas of the northern hemisphere. They build nests on cliffs in colonies along the shoreline. They exhibit bi-parental care during incubation and raise one to three chicks together (Suryan et al., 2000; Vihtakari et al., 2018).

The field work for this thesis was conducted by Norwegian Institute for Nature Research (NINA) (common eider) and Centre for Biological Studies of Chizé in France (CEBC-CNRS) (black-legged kittiwake). The eggs were collected from breeding colonies in Kongsfjorden, Svalbard in 2019 and Oslofjorden, Norway in 2019 and 2020 (Figure 1, Table 1).

In Oslofjorden, common eider eggs were collected at two different locations, Hvaler (59°11' N, 10°76' E) and Nesodden (59°82' N, 10°60' E). In Kongsfjorden, Svalbard, common eider eggs were collected at Storholmen (78°95' N, 12°18' E). Black-legged kittiwake eggs were collected at Krykkjefjellet in Kongsfjorden (78°89' N, 12°33' E).

The breeding season starts earlier in Oslofjorden than in Kongsfjorden (hereafter Svalbard). In the years where eggs were sampled in both places, the fieldwork therefore started in Oslofjorden. Only freshly laid eggs were analysed. Eggs that were incubated and under development were not included.

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

Table 1: Overview of study locations, years, and sample sizes for the study species.

Species	Region	Location	Year	Sample size (n)
Common eider	Oslofjorden, Norway	Hvaler	2020	16
	Oslofjorden, Norway	Nesodden	2020 + 2019	12 + 2
	Svalbard	Kongsfjorden	2019	14
Black-legged kittiwake	Svalbard	Kongsfjorden	2019	22



Figure 1: Overview of the study locations. Black-legged kittiwake and common eider eggs were collected in Kongsfjorden, Svalbard. Common eider eggs were also collected at Nesodden and Hvaler in the inner and outer part of Oslofjorden, Norway. Top picture: Common eider (Own photo). Bottom picture: Black-legged kittiwake (Own photo).

Data sampling

Freshly laid eggs from the different breeding colonies were located. Black-legged kittiwake nests were checked regularly until the eggs were laid and collected. Common eider eggs were assumed to be freshly laid based on the assumption that nests with few eggs (1-3) and no down were not incubated yet. Only one egg from each nest was collected and stored in a protective case. The eggs were then brought back to the field station and processed the same day. Egg mass was measured using a digital scale (precision of 0.01 g) and length and width

were measured using a calliper (precision of 0.1 mm). The eggs were then carefully cracked in a petri dish to preserve the egg yolk membrane. The eggs were confirmed to be freshly laid based on the absence of development in the egg. A picture was taken of each egg for documentation. By using a syringe without a needle, homogenous yolk samples were extracted from the eggs and then preserved in a 2 mL cryotube in a dry-shipper (-150 °C) until the end of the field season. This period varied in length dependent on the field location, from 1 to 14 days. They were then brought back to NINA and stored in a freezer (-80 °C) until they were sent to further analysis (thiamine and corticosterone). Samples sent to labs in other countries were sent with international carrier on dry ice and were delivered within 4-5 days. The samples were still frozen at delivery. Samples sent to the Norwegian lab were sent with national post service on freezer elements and delivered frozen within 19 hours.

Corticosterone analysis

Corticosterone analysis of the yolk samples were done at CEBC-CNRS. Yolk (100 mg) were homogenised in 1.0 mL of distilled water with three glass beads, using a vortex.

Corticosterone was extracted by adding 3 mL of diethyl-ether to 300 µL of the yolk mixture, vortexing and centrifuging (5 minutes at 2000 rpm, 4 °C). The diethyl-ether phase containing corticosterone was decanted and poured off after snap freezing the tube. This was done twice. The solvent was then evaporated (37 °C). Dried extracts were re-dissolved in phosphate buffer (0.01 M, pH 7.4). Corticosterone was then assayed using Radio-immunoassay (RIA). The extract (100 µL) was incubated overnight (4 °C) with 4000 cpm of H³-Corticosterone (Perkin Elmer, US) and polyclonal antiserum (Ab). Anti-corticosterone Ab was supplied by Merck, Sigma Aldrich, France. The bound fraction was then separated from the free fraction by addition of dextran-coated charcoal. The activity was counted on a Tri-Carb 2810TR scintillation counter (Perkin Elmer, US). Limit of detection (LOD) for corticosterone was 1.03 pg/mg. All samples were above LOD.

Thiamine analysis

Thiamine analyses of the yolk samples used in this project were done by two different laboratories. These two laboratories use their own separate method of analysing the thiamine content in the samples, but to be able to include more data to the analysis, results from both laboratories were included.

Samples from 2019 (Svalbard common eider and black-legged kittiwake, Nesodden common eider) were all analysed by the Department of Environmental Science and Analytical Chemistry (ACES, Sweden). ACES quantified the three forms of thiamine in the samples, (non-phosphorylated thiamine [T], di-phosphorylated thiamine [TDP], and mono-phosphorylated thiamine [TMP] which sums up to a total concentration of thiamine in the sample (hereafter thiamine). Their method consists of homogenization of the sample, followed by extraction, centrifugation, clean-up, derivatisation and then separation and analysis on high performance liquid chromatography (HPLC, NH₂ column) with fluorescence detection. Research done by Balk et al. (2009, 2016) and Mörner et al. (2017) on thiamine levels in different species were also analysed by this lab. For a more thorough description of the lab method, see Balk et al. (2016).

All concentrations from the ACES lab are quantified in nmol/g on a wet-weight basis (ww). LOD were 0.2 T, 0.05 TMP and 0.02 nmol/g TDP. Limits for quantifications (LOQ) were 0.2 T, 0.4 TMP and 0.1 nmol/g TDP. Concentrations lower than LOQ or LOD were assigned with 0.5 x LOQ and 0.5 x LOD.

Samples from 2020 (Nesodden and Hvaler, common eider) were analysed by The Institute for Marine Research (IMR) in Bergen (Norway). IMR is certified by the Norwegian Accreditation (reg no. TEST 050). They only quantified the total concentration of thiamine in the yolk sample. Their method (239- Thiamine -HC1) consist of extraction, hydrolysis, enzyme treatment, derivation of thiamine to thiochrome and quantification with reverse phase HPLC and fluorescence detection. All concentrations from the IMR lab were quantified in mg/kg ww. LOQ and LOD for total thiamine concentration in the samples were 0.1 and 0.03 mg/kg ww, respectively. Concentrations lower than LOQ or LOD were assigned with 0.5 x LOQ and 0.5 x LOD. The chromatograms for the samples had clear peaks and no interference.

In order to compare the result from both labs a predictive equation (Equation 1) was used to adjust values from IMR to values from ACER (Moe et al., 2020). This equation is based on a study where duplicated samples from common eider eggs were sent to both labs. The study found that total thiamine levels in the duplicated egg samples analysed by IMR and ACES

correlated significantly ($r=0.52$, $df=13$, $t=2.2$, $p=0.04$). The relationship was predicted by the following equation, in which Y is ACES thiamine concentration and X is IMR thiamine concentration (both in mg/kg).

Equation 1 $y= 0.90x+2.36$

Finally, the adjusted thiamine levels from the IMR lab were converted from mg/kg ww to nmol/g ww. These values were converted using the molecular weight of T since it is found that virtually 100% of the total thiamine content in the egg yolk is in the T form (Moe et al., 2020).

Statistical analysis

All statistical analyses and plotting of results were performed in RStudio version 4.0.2 (RStudio Team, 2020). Tests were run separately on the two species. Linear models were performed using the lm function in the stats package. Samples were independent (one egg yolk sample per nest), and the other assumptions underlying a linear model (linearity, normal distribution of residuals, constant variance of residuals along the values of x (homoscedasticity)) were checked using diagnostic plots (QQ, residuals vs fitted and Cook's distance).

Differences among means were tested with the anova function and parameter estimates were obtained with the summary function. The best model for explaining thiamine concentration in egg yolk for each species were selected based on rejection of non-significant parameters from a "full model". All tests were two-tailed, and null-hypothesis were rejected at an alfa-level of 0.05.

The full model for common eiders included the following explanatory terms: population (factor), corticosterone, corticosterone² (quadratic term) and the interactions population x corticosterone and population x corticosterone². The population term tests whether the thiamine levels differ among populations. Corticosterone tests for a linear relationship between corticosterone and thiamine. The interaction term "corticosterone x population" is

testing if the slopes of this relationship differ among populations. Corticosterone², which is the quadratic term of corticosterone, tests whether there is a curved (quadratic) relationship between corticosterone and thiamine. The interaction term “population x corticosterone²” is testing whether the curvature differs among populations. The full model for black-legged kittiwakes included corticosterone and corticosterone² (quadratic term). All models included intercept.

Estimates are provided with ± 1 standard error (SE) and 95% confidence intervals.

Histograms were made with hist function. Scatterplots and boxplots were made with the “ggplot2” package (Wickham, H., 2016). The upper and lower boundaries of the box in the boxplots represent the 25th and 75th percentile. The horizontal line in the middle represents the median. Whiskers extended from the box represent the smallest and highest value, at most 1.5 x interquartile range (IQR). Data beyond the whiskers are plotted as outliers (large points).

Results

Egg yolk corticosterone did not significantly explain variation in thiamine levels in egg yolk of common eiders ($F= 1.26$, $P=0.27$, Figure 2, Table 2), neither did the quadratic term of corticosterone (corticosterone², $F= 0.92$, $P=0.41$, Figure 2, Table 2) nor the interaction terms ‘corticosterone x population’ or ‘corticosterone² x population’ ($F< 0.53$, $P>0.72$, Figure 2, Table 2). Population, however, was a significant factor explaining egg yolk thiamine levels in common eiders ($F= 4.09$, $P= 0.024$, Figure 2, Table 2), and the final model only included this predictor variable (Table 2, Table 3). This means that thiamine levels differed significantly among common eider populations. There was no relationship between corticosterone and thiamine, linear or curved. The non-significant interaction terms showed that these potential relationships did not differ among populations, and the slopes are therefore not significantly different from zero (Figure 2).

Egg yolk corticosterone did not significantly explain variation in thiamine levels in egg yolk of black-legged kittiwake ($F= 1.99$, $P=0.17$, Figure 2, Table 4), nor did the quadratic term of corticosterone (corticosterone², $F= 0.98$, $P=0.39$, Figure 2, Table 4). The final model only included intercept, meaning that no tested variables significantly explained the variation in thiamine, and the mean of thiamine levels were significantly different from zero. There was therefore no curved or linear relationship between yolk corticosterone and thiamine levels in the tested black-legged kittiwake population.

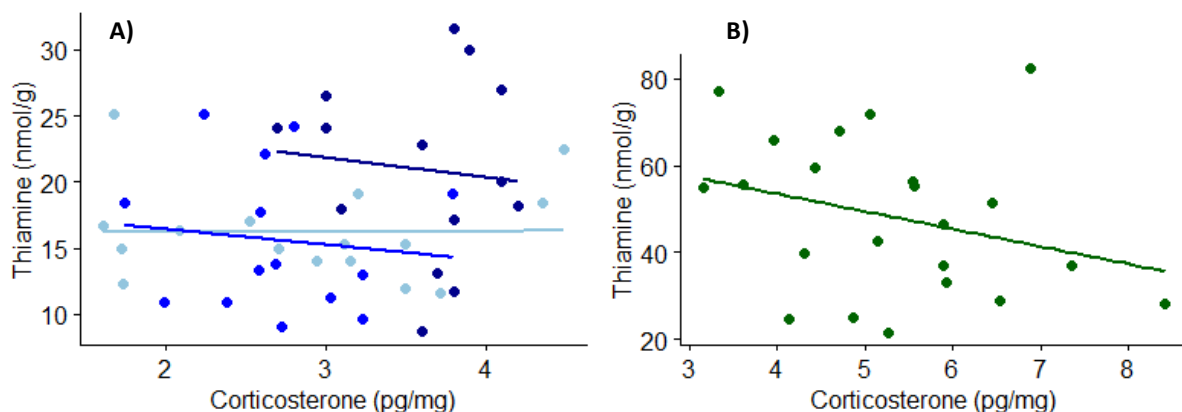


Figure 2: Thiamine and corticosterone levels in egg yolk from A) common eider breeding in Hvaler (light blue), Nesodden (blue) and Svalbard (dark blue) and B) black-legged kittiwake (green) breeding in Svalbard. Regression lines are shown for easier interpretation. The slopes did not differ among the common eider population and there was no significant relationship between corticosterone and thiamine in common eider or black-legged kittiwake. See Table 2 and Table 4 for test-statistics.

Table 2: Results from linear models analysing thiamine levels in common eider egg yolk. The final model included only population as explanatory variable, while the full model also included the rejected terms (corticosterone, corticosterone² and the interactions population x corticosterone and population x corticosterone²).

	d.f	F	p
Final model			
Population	2, 41	4.09	0.024
Rejected terms			
Corticosterone	1, 40	1.26	0.27
Corticosterone x Population	2, 38	0.13	0.88
Corticosterone ²	2, 39	0.92	0.41
Corticosterone ² x Population	4, 35	0.53	0.72

Table 3: Estimates and test-statistic for the final model analysing thiamine levels in common eider egg yolk. The final model for common eider included only population as predictor variable. The intercept represents the mean of the Hvaler population, while the estimates and test statistics of Nesodden and Svalbard represent the difference to Hvaler.

	Estimate (SE)	t	p
Intercept(Hvaler)	16.2 (1.36)	11.98	<0.0005
Nesodden	-0.63 (1.98)	-0.32	0.75
Svalbard	4.70 (1.98)	2.37	0.02

Table 4: Results from linear models analysing thiamine levels in black-legged kittiwake egg yolk. The final model included only intercept, while the full model also included the rejected terms corticosterone and corticosterone².

	d.f	F	p
Final model			
Intercept	-	-	-
Rejected terms			
Corticosterone	1, 20	1.99	0.17
Corticosterone ²	2, 19	0.98	0.39

Thiamine levels in common eiders differed among the populations. The Svalbard population had higher thiamine levels (20.9 ± 1.84 nmol/g) than the Hvaler (16.2 ± 0.92 nmol/g) and Nesodden (15.6 ± 1.46 nmol/g) populations (Figure 3, Figure 4, Table 5).

Corticosterone levels in common eiders also differed among the populations. The mean of the Svalbard population (3.60 ± 0.13 pg/mg) was higher than means of the populations from Nesodden (2.69 ± 0.14 pg/mg) and Hvaler (2.88 ± 0.23 pg/mg) (Figure A1, Figure A2, Table A2).

The black-legged kittiwake colony had the highest thiamine and corticosterone levels measured in this study at 48.3 ± 3.82 nmol/g and 5.29 ± 0.28 pg/mg respectively (Figure 3, Figure 4, Figure A1, Figure A2, Table 5, Table A2).

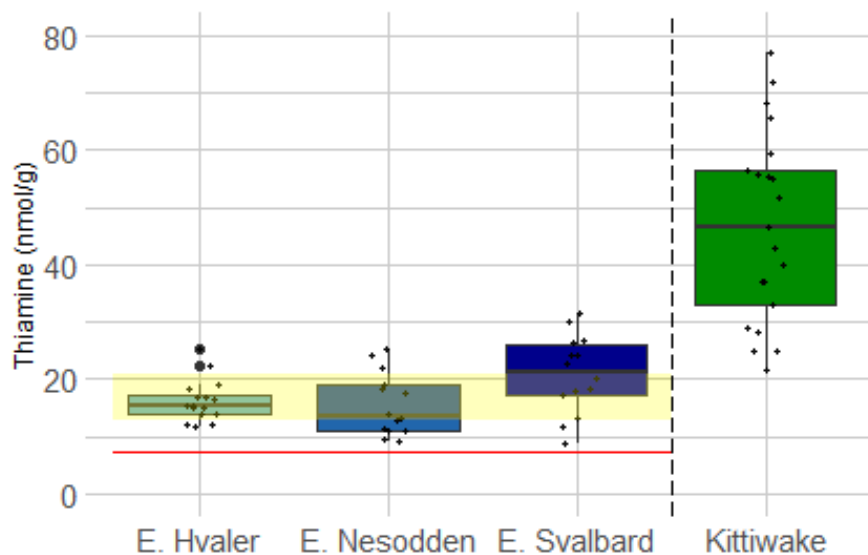


Figure 3: Boxplot with jitter of thiamine levels (nmol/g) in egg yolk for the different common eider populations, Hvaler (light blue), Nesodden (blue), Svalbard (dark blue), and for a black-legged kittiwake population in Svalbard (green). Red horizontal line shows for comparison the mean thiamine yolk levels of the populations in the Baltic Sea classified as thiamine deficient (7.2 nmol/g) (Balk et al. 2009). The yellow horizontal section shows suggested yolk thiamine levels for sub-lethal effects (13 - 21 nmol/g ww) by Balk et al. (2016).

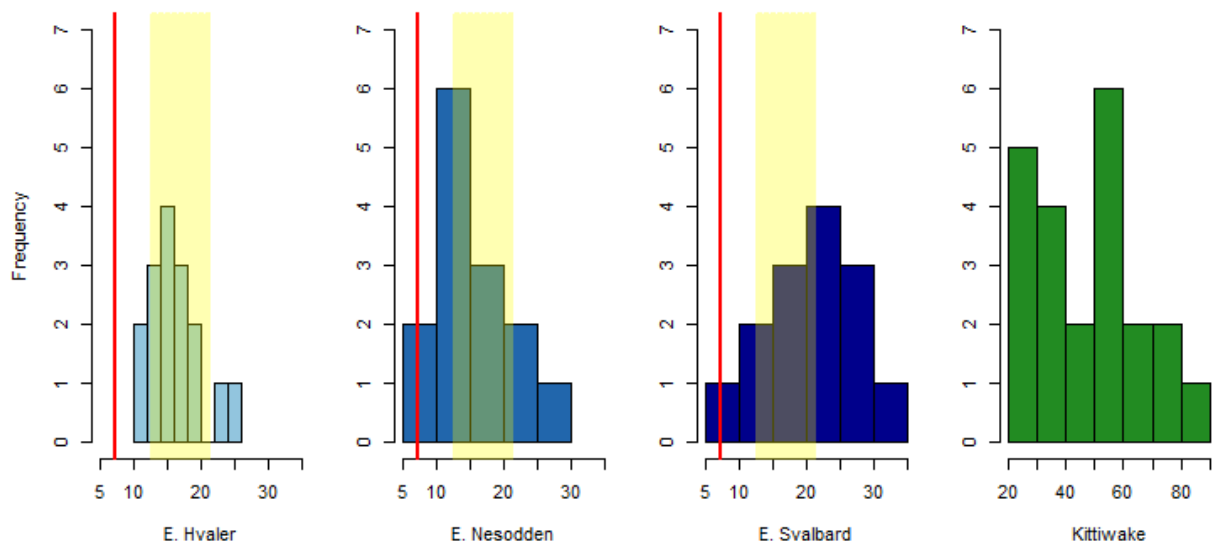


Figure 4: Distribution of thiamine levels (nmol/g) in egg yolk for the different common eider populations, Hvaler (light blue), Nesodden (blue), Svalbard (dark blue) and a black-legged kittiwake population in Svalbard (green). Red vertical line shows the mean thiamine yolk levels of the populations in the Baltic Sea classified as thiamine deficient (Balk et al. 2009). The yellow vertical section shows suggested yolk thiamine levels for sub-lethal effects (13 -21 nmol/g ww) by Balk et al. (2016).

Table 5: Descriptive statistics for thiamine levels in egg yolk (nmol/g ww) for common eider breeding in Oslofjorden (2019, 2020) and Svalbard (2019) and for black-legged kittiwake breeding in Svalbard (2019).

Population	mean	SE	min	max	2.5 % CI	97.5 % CI	n
Common eider, Hvaler	16.2	0.92	11.6	25.2	14.27	18.19	16
Common eider, Nesodden	15.6	1.46	9.06	25.2	12.45	18.76	14
Common eider, Svalbard	20.9	1.84	8.76	31.6	16.96	24.89	14
Black-legged kittiwake, Svalbard	48.3	3.82	21.6	82.4	40.35	56.25	22

Discussion

The main finding of this study was that egg yolk levels of corticosterone did not correlate with thiamine levels in any of the three common eider populations or the black-legged kittiwake population. The lack of relationship may support the hypothesis of Balk et al. (2016) that thiamine deficiency results from a deficiency of this specific vitamin in the marine food web and not starvation or poor food availability for seabirds.

Thiamine levels in common eider egg yolk differed among the populations. Egg yolk of common eiders from Svalbard had higher concentrations compared to those from Nesodden and Hvaler. The two populations from Oslofjorden had the lowest yolk thiamine levels in this study (Nesodden and Hvaler). Interestingly they still had approximately twice the levels of yolk thiamine compared to the Baltic populations (7.2 nmol/g ww) measured by Balk et al. (2009) which they classified as thiamine deficient. Balk et al. (2016) suggested sub-lethal effects for egg yolk concentrations of 13 -21 nmol/g ww and lethal effects for concentrations of 5.7-8.8 nmol/g ww based on different effect parameters. Although none of the populations in this study had several thiamine measurements within the suggested lethal effect concentrations, most measurements of the thiamine egg yolk concentrations were within the suggested levels for expecting sub-lethal effects (Balk et al., 2016). It is possible that the measured levels of thiamine and corticosterone in our study were not within the range for seeing a potential relationship. However, Balk et al.'s (2016) suggestions about sub-lethal thiamine levels imply that at least the egg yolk in the tested common eider populations comes from birds experiencing some degree of thiamine deficiency.

The black-legged kittiwake colony had the highest thiamine and corticosterone levels measured in this study. Much less is known about thiamine levels in black-legged kittiwakes than in the common eider. The thiamine data on black-legged kittiwakes in this study originates from Moe et al. (2020) which showed that eggs from black-legged kittiwakes breeding in Svalbard and Nordland had rather similar thiamine levels (48.3 ± 3.82 and 47.3 ± 3.8 nmol/g respectively). To be able to assess whether these birds are deficient in thiamine or not, more studies are needed, and different parameters need to be measured (Balk et al., 2009, 2016).

The Svalbard common eider population did also have higher corticosterone levels than the populations in Nesodden and Hvaler. Based on our hypothesis that predicted a negative relationship between corticosterone and thiamine, it was surprising to find that the population with the highest corticosterone level, was also the population with the highest thiamine level. Since no data on food availability of the three common eider populations was recorded, this result may also warrant some cautions when using corticosterone as an indicator of food supply/nutritional status. Corticosterone can also be affected by other environmental, seasonal, and physical factors. Some examples are pollution (Fowler et al., 1995), extreme weather conditions (Romero et al., 2000), changes during the breeding season (Fowler et al., 1994; Wingfield et al., 1997) or habitat quality (Marra & Holberton, 1998). These inflicting factors can be troublesome when comparing corticosterone levels across different populations. However, when analysing potential relationships between corticosterone and thiamine within each population, the other factors affecting corticosterone will be as standardized as possible.

Even though our results may give some support to the hypothesis by Balk et al. (2009, 2016), the reason for the thiamine deficiency is still far from understood. The low levels of thiamine measured in the common eiders' main prey, blue mussels (*Mytilus edulis*) (Balk et al., 2016; Moe et al., 2020), could be the cause of the thiamine deficiency in the common eider populations. The size of the blue mussel stocks around each of the sampling sites in this study were not quantified, nor in the populations tested by Balk et al. (2016) to our knowledge. This makes it difficult to assess if the amount of available blue mussels is limiting as well.

In other species, other factors have proven to cause thiamine deficiency. For example, salmon (*Salmonidae*) and lake trout (*Salvelinus namaycush*) eating fish that were rich in thiaminase, an enzyme breaking down thiamine, lead to thiamine deficiency, and reduced reproduction and population declines in the Great Lakes (Brown et al., 2005). Other possible explanations for the documented thiamine deficiency can be a pollutant that somehow affects thiamine uptake or metabolism, or imbalance in bacteria and phytoplankton. Both bacteria and phytoplankton are primary producers of thiamine in the marine food webs (Sañudo-Wilhelmy et al., 2012, 2014). Fridolfsson et al (2018) found that during large blooms of cyanobacteria, which are common in the Baltic Sea in the summer, zooplankton were starved of thiamine. It

is therefore possible that thiamine does not get passed up the food chain during these episodes. However, limited data on dissolved ocean vitamin concentrations during different seasons and geographic areas leads to lack of evidence. Thus, the reason for the thiamine deficiency may be complex and it may differ from one species to another.

To further investigate if thiamine shortages in seabirds could be connected to limited food supply by looking for a correlation between thiamine and corticosterone levels, a wider range of species, populations, and locations needs to be investigated. It would also be important to do measurements over several years in order to capture variation between years both in thiamine levels and corticosterone levels. In addition, quantification of available prey items would be important. By including more vitamins and nutrients in the analyses, it would be possible to see if the birds are only deficient in thiamine, or if they have generally poor nutritional status. If thiamine is the only scarce nutrient, it would also give some support to the hypothesis of Balk et al. (2016) of a specific deficiency of thiamine in the marine food web.

This study focused on thiamine and corticosterone levels in egg yolk based on the assumption that the egg yolk levels reflect the nutritional/thiamine status of the mother during egg production (Almasi et al., 2012; Cook et al., 2009; Hayward & Wingfield, 2004; Olkowski & Classen, 1999). The advantage of focusing on eggs, is that egg yolk thiamine levels are well studied in common eiders (Balk et al., 2009, 2016; Moe et al., 2020; Mörner et al., 2017), and that physiological status of eggs can be linked to reproductive success and subsequent population dynamics (Benton et al., 2005; Buck et al., 2007; Hayward & Wingfield, 2004; Murphy, 1996; Nager, 2006). Looking at eggs, however, limits the knowledge to the females of the species, and it would not be possible to study birds that are too starved or thiamine deficient to lay eggs. It could therefore be relevant to sample blood or organ tissues from a more representative sample of the population. Blood can be easily sampled without injury to the birds but looking at organs requires invasive sampling. It is not desirable to sacrifice many seabirds under the current population declines, especially when we are screening for potential correlations and are still far from understanding the potential links between food availability, stress hormones and thiamine deficiency.

In order to control for corticosterone's many influencing factors one would ultimately need experimental manipulations to say something about causation. Conducting field experiments with different feeding treatments could be challenging with seabirds but are already carried out in a black-legged kittiwake population which nested in an artificial tower with easy access (Lanctot et al., 2003). Ultimately a lab experiment with domestic birds could be necessary in order to limit the food and thiamine supply. Since advanced thiamine deficiency also can lead to a loss of appetite (Harata et al., 1993), one could possibly expect elevated corticosterone levels from lack of feeding and a poor body condition. In this case we could get a relationship between thiamine and corticosterone where thiamine could indirectly affect corticosterone levels. By quantifying the amount of available food the birds have eaten in a controlled lab experiment, it would be possible to separate elevated corticosterone levels from reduced appetite and reduced food supply.

Conclusion

Analyses of egg yolk from three common eider populations and one black-legged kittiwake population did not show a significant correlation between corticosterone and thiamine levels in any of the populations sampled. This study was correlational and cannot reveal any causal relationships, but the results indicate that thiamine levels are independent of corticosterone levels. Under the assumption that the stress hormone levels reflect nutritional status, the findings may give some support to the hypothesis that thiamine deficiency comes from a specific deficiency of thiamine in the marine food webs rather than poor food availability. Research finding low levels of thiamine in common eiders main prey, blue mussels, may also give some support to this hypothesis. However, since there is no known research on the availability of blue mussels, it is difficult to see if food availability can be limiting as well. Different factors have proven to cause thiamine deficiency in different species (Brown et al., 2005; Fridolfsson et al., 2018), and the reason for the thiamine deficiency in the marine food webs may therefore be complex. With so many factors potentially affecting thiamine and corticosterone levels in birds, one would ultimately need to conduct experimental manipulations to properly test different hypotheses regarding the thiamine deficiency in seabirds.

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Appendix

Table A1: Data from the yolk corticosterone (pg/mg) and thiamine (nmol/g) analysis.

Species	Population	Year	Thiamine (nmol/g)	Corticosterone (pg/mg)
Common eider	Hvaler	2020	12.29	1.7
Common eider	Hvaler	2020	19.07	3.2
Common eider	Hvaler	2020	13.98	3.2
Common eider	Hvaler	2020	16.36	2.1
Common eider	Hvaler	2020	16.69	1.6
Common eider	Hvaler	2020	18.39	4.4
Common eider	Hvaler	2020	17.03	2.5
Common eider	Hvaler	2020	13.98	2.9
Common eider	Hvaler	2020	15.00	1.7
Common eider	Hvaler	2020	15.34	3.5
Common eider	Hvaler	2020	15.00	2.7
Common eider	Hvaler	2020	15.34	3.1
Common eider	Hvaler	2020	11.95	3.5
Common eider	Hvaler	2020	22.46	4.5
Common eider	Hvaler	2020	25.17	1.7
Common eider	Hvaler	2020	11.61	3.7
Common eider	Nesodden	2020	9.06	2.7
Common eider	Nesodden	2020	11.27	3.0
Common eider	Nesodden	2020	25.17	2.2
Common eider	Nesodden	2020	24.16	2.8
Common eider	Nesodden	2020	18.39	1.8
Common eider	Nesodden	2020	10.93	2.4
Common eider	Nesodden	2020	22.12	2.6
Common eider	Nesodden	2020	12.96	3.2
Common eider	Nesodden	2020	13.30	2.6
Common eider	Nesodden	2020	19.07	3.8
Common eider	Nesodden	2020	10.93	2.0
Common eider	Nesodden	2020	9.57	3.2
Common eider	Nesodden	2019	17.73	2.6
Common eider	Nesodden	2019	13.77	2.7
Common eider	Svalbard	2019	11.65	3.8
Common eider	Svalbard	2019	24.13	2.7

Common eider	Svalbard	2019	31.57	3.8
Common eider	Svalbard	2019	26.56	3.0
Common eider	Svalbard	2019	8.76	3.6
Common eider	Svalbard	2019	18.19	4.2
Common eider	Svalbard	2019	17.14	3.8
Common eider	Svalbard	2019	13.08	3.7
Common eider	Svalbard	2019	24.10	3.0
Common eider	Svalbard	2019	29.97	3.9
Common eider	Svalbard	2019	22.81	3.6
Common eider	Svalbard	2019	26.93	4.1
Common eider	Svalbard	2019	20.08	4.1
Common eider	Svalbard	2019	17.96	3.1
Black-legged kittiwake	Svalbard	2019	28.13	8.4
Black-legged kittiwake	Svalbard	2019	82.40	6.9
Black-legged kittiwake	Svalbard	2019	67.93	4.7
Black-legged kittiwake	Svalbard	2019	24.77	4.1
Black-legged kittiwake	Svalbard	2019	42.74	5.2
Black-legged kittiwake	Svalbard	2019	59.38	4.4
Black-legged kittiwake	Svalbard	2019	65.72	4.0
Black-legged kittiwake	Svalbard	2019	37.09	5.9
Black-legged kittiwake	Svalbard	2019	28.92	6.5
Black-legged kittiwake	Svalbard	2019	55.55	3.6
Black-legged kittiwake	Svalbard	2019	55.48	5.6
Black-legged kittiwake	Svalbard	2019	25.07	4.9
Black-legged kittiwake	Svalbard	2019	33.11	5.9
Black-legged kittiwake	Svalbard	2019	71.75	5.1
Black-legged kittiwake	Svalbard	2019	54.78	3.2
Black-legged kittiwake	Svalbard	2019	46.53	5.9
Black-legged kittiwake	Svalbard	2019	21.58	5.3
Black-legged kittiwake	Svalbard	2019	77.04	3.3
Black-legged kittiwake	Svalbard	2019	36.84	7.4
Black-legged kittiwake	Svalbard	2019	51.58	6.5
Black-legged kittiwake	Svalbard	2019	39.92	4.3
Black-legged kittiwake	Svalbard	2019	56.32	5.6

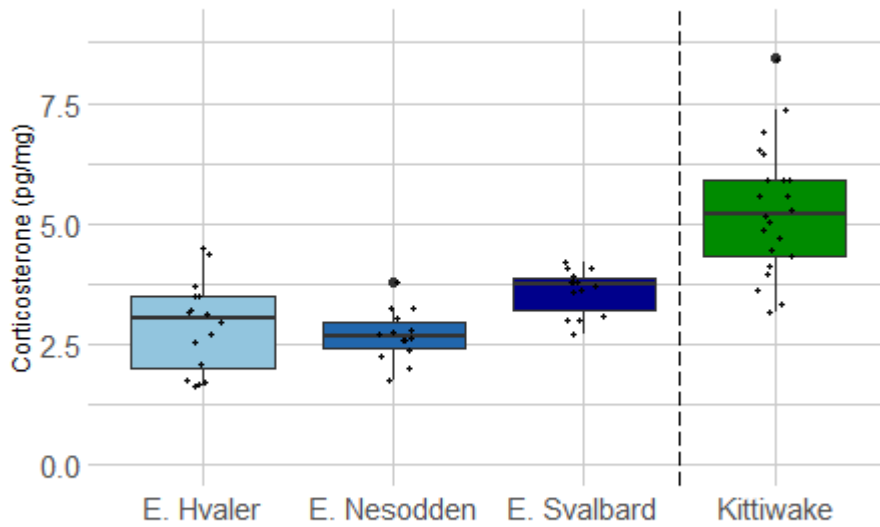


Figure A1: Boxplot with jitter of corticosterone levels (pg/mg) in egg yolk for the different common eider populations: Hvaler (light blue), Nesodden (blue), Svalbard (dark blue) and for a black-legged kittiwake population on Svalbard (green).

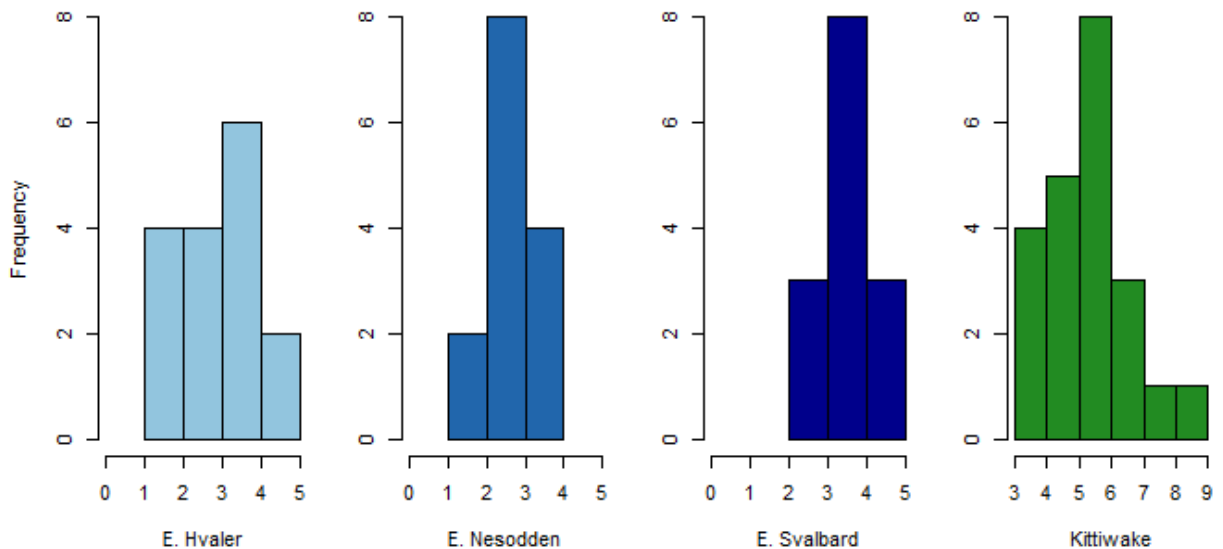


Figure A2: Distribution of corticosterone levels (pg/mg) in egg yolk for the different common eider populations, Hvaler (light blue), Nesodden (blue), Svalbard (dark blue) and a black-legged kittiwake population on Svalbard (green).

Table A2: Descriptive statistics for corticosterone levels in egg yolk (pg/mg ww) for common eider breeding in Oslofjorden (2019, 2020) and Svalbard (2019) and black-legged kittiwake breeding in Svalbard (2019).

Population	mean	SE	min	max	2.5 % CI	97.5 % CI	n
Common eider, Hvaler	2.88	0.23	1.61	4.49	2.38	3.37	16
Common eider, Nesodden	2.69	0.14	1.75	3.79	2.38	2.99	14
Common eider, Svalbard	3.60	0.13	2.70	4.20	3.33	3.87	14
Black-legged kittiwake, Svalbard	5.29	0.28	3.16	8.42	4.70	5.88	22

