Supporting Information

Wild birds of declining European species are dying from a thiamine deficiency syndrome

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Contents

Materials and Methods.

Bird material. The three main species investigated in this study were the herring gull (*Larus argentatus*), the common starling (*Sturnus vulgaris*), and the common eider (*Somateria mollissima*). They represent the following three major bird orders respectively: *Charadriiformes*, *Passeriformes*, and *Anseriformes*. The herring gull is a fish or omnivorous feeder, whereas the common starling is to a large extent an invertebrate feeder and the common eider is primarily a mussel feeder. On the European mainland, the herring gull is a non-migrant, whereas the common starling is a middle-distance to long-distance migrant and the common eider is a short-distance migrant. In Iceland all three species are non-migrants, although some juvenile herring gulls may migrate to the coast of western Europe during the winter. Eggs, pulli, and full-grown individuals were collected from 10 regions in the Baltic Sea area and Iceland during 2004–2007. The specific locations are shown in Fig. S1 a–j. The locations constitute a relatively random sample of areas, traditionally noted for their rich bird life, very often bird preservation areas. (They were never selected just because of reports about outbreaks of the paralytic disease.) Additional species, investigated less extensively, were the Canada goose (*Branta canadensis*), the common black-headed gull (*Chroicocephalus ridibundus*), the mew gull (*Larus canus*), the great black-backed gull (*Larus marinus*), and the hooded crow (*Corvus cornix*). Moreover, farmed domestic chicken (*Gallus gallus*) was used as an additional control. The domestic chicken pulli were obtained from SweHatch, Väderstad, Sweden.

Thiamine treatment control common starling eggs were produced in the County of Södermanland (G) during the spring 2006. Adult females were temporarily trapped in nest boxes and injected intramuscularly in the breast muscle with a thiamine (T) solution, at a dose of 50 mg T per kg body weight and with pH = 6.9, 19–24 days before egg laying. They were also given a ring, which could be spotted at a distance with a telescope to secure that the thiamine treatment control eggs were really produced by injected females. Thiamine treatment control common starling pulli were produced in the County of Blekinge (I) during the spring 2006. The pulli were taken out of the nest box and force-fed ~0.3 mL of a T solution at a dose of 50 mg T per kg body weight. They were

then immediately put back into the nest box and left to continued parental care for another 11 days. The survival rate of the thiamine treated specimens was 97%, whereas the survival rate of the untreated specimens was lower, though not exactly quantified.

For all species, great care was taken to obtain the first and/or second egg in each female's first clutch of the breeding season. Only newly laid eggs were used, and the criterion for this was absence of blood vessels. For herring gull eggs and pulli we found confounding heterogeneities within Iceland. Therefore, we were forced to use a sub-sample of Icelandic specimens to ensure that the control values were representative for relatively healthy individuals, even though we knew that these values were not representative for Iceland as a whole. The documented fact that three eggs per nest is the natural clutch size for the herring gull (1), combined with our knowledge about the positive relationship between egg laying ability and yolk T concentration in this species, led us to maximise the probability to obtain proper Icelandic control eggs, *i.e*. normal eggs from healthy females, by using only the first or second egg from complete three egg clutches. For Icelandic herring gull control pulli, areas with considerable human disturbance were excluded. Pulli of any species were always collected from other locations than the eggs in order to maximise the inclusion of first clutches of the breeding season. Herring gull pulli and common eider pulli were collected at different development stages. The investigation of herring gull pulli was confined to individuals with a body weight of >75 g, *i.e*. individuals that were at least 50% larger than at hatch and with only traces (<0.6% of the body weight) of the yolk sac left. This was done in order to minimize the influence of the previous conditions in the egg and to increase the influence of the colony environment and the food provided by the parents. The investigation of common eider pulli, on the other hand, was confined to hatchlings (at most 2–3 days old). Hence, there was an important difference in the influence of the colony environment and the food between the herring gull pulli and the common eider pulli. In the County of Södermanland (G) we had severe difficulties to find any common eider pulli at all for our investigations. We visited eleven islands, with known previous breeding of the common eider, without finding a single living pullus. Finally, on the 12th island, we found seven specimens from two clutches. This, of course, agrees with our previous observations that the common eider pulli have disappeared from this region, but

it may be questionable whether these two clutches really constituted a random sample, representative for the whole region. Hence, the County of Södermanland (G) was excluded from the statistical evaluation of the Baltic Sea area as a whole. Full-grown individuals were caught with a cannon net in Iceland, and with walk-in traps or a hand net in the Baltic Sea area. Individual nests were identified with a Garmin GPSMAP 60C® navigator purchased from Garmin International Inc. (Olathe, KA, USA).

Chemicals. Bovine serum albumin (A4378), coenzyme A (C3144), α-glycerophosphate dehydrogenase (G6751), HEPES (H4030), α-ketoglutaric acid (K1875), $MgCl_2$ (Ultra M2670), NaCl (S7653), NAD^+ (N1511), NADH (N8129), D-ribose 5-phosphate (R7750), sucrose (Ultra S7903), thiamine (T; T4625), thiamine diphosphate (TDP; C8754), thiamine monophosphate (TMP; T8637), triosephosphate isomerase (T2391), Tris-Cl (T3253), Triton X-100 (T9284), and D-xylulose 5-phosphate (15807) were purchased from Sigma (St. Louis, MO, USA). DL-dithiothreitol (43819) was purchased from Fluka (Riedel-deHaën, Germany). Acetonitrile LiChrosolv® (1.14291.2500), hydrochloric acid 30% Suprapur (1.00318.1000), potassium dihydrogen phosphate *p.a*. (1.04873.1000), potassium hexacyanoferrate (III) *p.a*. (1.04973.0250), and trichloroacetic acid *p.a*. (1.00807.1000) were purchased from Merck (Darmstadt, Germany). Ethyl acetate, Baker-analyzed (8037), hexane, Baker-analyzed (8044), and potassium hydrogen phosphate, anhydrous (0241) were purchased from J. T. Baker (Deventer, Holland). Sodium hydroxide, EKA pellets (1.3303-1) was purchased from AkzoNobel (Bohus, Sweden).

Thiamine treatment of paralysed full-grown birds. Twenty paralysed full-grown herring gulls from regions along the Swedish Baltic Sea coast were subjected to a thiamine treatment experiment. These specimens were unable to fly and, in most cases (95%), also unable to walk. Within a few hours after capture, they were injected in the breast muscle with either a T solution, at a dose of 50 mg T per kg body weight, in a volume of 1 mL per kg body weight, and with pH = 6.9, or a 0.9% saline. The specimens were placed in individual cages, and during the first 48 hours they had access to water *ad lib* but not food. Four specimens (20%) died during this time. This mortality rate was, in fact, the expected mortality rate of a random sample of paralysed herring gulls uniformly distributed over a range of symptom phases occurring over a period of approximately 10–20 days, from paralysis of wings and legs until death, where recovery is impossible

during the last 2–4 days. It is well known that thiamine deficiency is irreversible at late stages (2, 3), so the four specimens that died within 48 hours were considered to be beyond possible recovery at the time of the injection, and only the 16 specimens that survived the first 48 hours were evaluated for any effect of the thiamine treatment (Fig. 2b; Table S2). The specimens were observed regularly for up to 14 days, and they had access to water and food *ad lib* during this time. For the gulls, the food consisted of fish from the area where they were caught, and the fish was frozen directly when caught and thawed before it was delivered as food. Three to five days after injection, the thiamine-treated specimens regained their appetite, whereas the control specimens did not. In addition to the herring gulls, one Canada goose, one common eider, two great black-backed gulls, one mew gull and one hooded crow, all of them paralysed, were subjected to similar thiamine or saline treatment.

Sampling. The field material was transported by land, sea, or air to the laboratory, or to temporary laboratory facilities built up in the field, and sampled within hours after collection. The material was sampled rapidly and efficiently by a team of experienced laboratory workers. Egg sampling was performed by 1–3 persons, whereas pulli and full-grown specimens were sampled by 3–5 persons working together. For some full-grown specimens, though, the liver was dissected directly in the field, when no other samples were taken.

Egg length and width were measured with a slide caliper to the nearest 0.1 mm. Eggshells were dried at room temperature, and later, eggshell thickness was measured with a micrometer to the nearest 0.01 mm at four points around the equator and expressed as the arithmetic mean of the four measurements. Egg and eggshell weight was measured to the nearest 0.01 g. Yolk colour was determined with the NCS Natural Color System©® (Scandinavian Colour Institute AB, Stockholm, Sweden). The redness of the yolk was then calculated from the colour code by multiplying the chromaticness with the hue percentage of red and dividing the product with 100. Egg yolk was sampled with a 2 mL syringe (without needle) to avoid contamination with albumen, and put into cryotubes, which were immediately submerged in liquid nitrogen. Egg weight loss due to water evaporation between laying and sampling was obtained as the difference between the initial weight and the measured weight. The initial weight was calculated from a species-

specific value (determined by us) of the fresh egg density and the volume, which in turn was calculated (4) from length, width, and a species-specific shape constant (5–7). The yolk T concentration was adjusted for the water loss, using a species-specific literature value (8–10) of the yolk water content. The egg water loss was generally 0–10%, resulting in a 0–5% adjustment of the yolk T concentration, since the white contained about twice as much water as the yolk.

Pulli were sacrificed by decapitation, and liver and brain were dissected. Liver body index (LBI) was defined as the liver weight expressed as percent of the body weight, which in turn was defined as the total weight subtracted by the weight of any remaining yolk sac. All weights were measured to the nearest 0.01 g. The brain was divided into two symmetric halves along the medial plane. Samples for thiamine analysis were, as quickly as possible, put into cryotubes, which were immediately submerged in liquid nitrogen. A central piece of the liver and one half of the brain were homogenised for enzyme analysis. The tissue was homogenised in an equal volume of 0.25 M sucrose using a 10 mL Potter-Elvehjem homogeniser (size 21) with five up and down strokes at 400 rpm and under constant cooling with ice. The homogenate was diluted to 20% with 0.25 M sucrose and centrifuged at $9000g_{av}$ and 4 ºC for 10 min. Aliquots of the $9000g_{av}$ supernatant and pellet were put into cryotubes, which were immediately submerged in liquid nitrogen. Samples collected on Iceland were transported to Sweden by land and sea in liquid nitrogen containers. All samples were later transferred to a –140 ºC freezer for storage until analysis.

Full-grown specimens were sacrificed by decapitation and a central piece of the liver was dissected, either in the field or in the laboratory. The samples were put into cryotubes, which were immediately submerged in liquid nitrogen, and later transferred to a –140 ºC freezer for storage until analysis. These liver pieces were used only for measurement of α-ketoglutarate dehydrogenase (KGDH) activity. Before analysis, the samples were homogenised in an equal volume of 0.25 M sucrose using a 10 mL Potter-Elvehjem homogeniser (size 21) with five up and down strokes at 400 rpm and under constant cooling with ice. The homogenate was diluted to 20% with 0.25 M sucrose and then used directly (*i.e.* without preparation of a $9000g_{av}$ pellet) for the KGDH activity measurement.

Enzyme measurements. In a biomarker context, a measured enzyme activity is generally assumed to reflect the amount of active enzyme. If the TDP cofactor is missing in a thiamine-dependent enzyme, this enzyme will be inactive. Hence, in a situation of thiamine deficiency, it will be important to measure both the amount of enzyme with the TDP cofactor, as well as the amount of enzyme without the TDP cofactor. This is achieved by measuring the enzyme activity both before and after addition of excess TDP to the sample. The obtained respective activities are referred to as endogenous activity (obtained before addition of TDP) and maximum activity (obtained after addition of TDP). The difference between the maximum activity and the endogenous activity will then reflect the original amount of enzyme without the TDP cofactor, commonly referred to as the apoenzyme. Two common ways to express the relative amount of apoenzyme are latency and TPP-effect (thiamine pyrophosphate effect). The latency is defined as the difference between maximum and endogenous activity expressed as percent of the maximum activity (*i.e*. the proportion of apoenzyme), whereas the TPP-effect is defined as the difference between maximum and endogenous activity expressed as percent of the endogenous activity (*i.e*. the relative increase in activity after TDP addition). In this study we chose to express the relative amount of apoenzyme as latency for the following reasons: Firstly, the latency is always a number between 0 and 100 percent, whereas the TPP-effect becomes infinitely large as the endogenous activity approaches zero. This makes the latency easier to work with when there is much variation in the endogenous activity. Secondly, it can be shown mathematically that the latency always has a smaller coefficient of variation than the TPP-effect, and hence is a more sensitive measure than the TPP-effect. It should be pointed out that all measured activities presented in this study are endogenous activities.

Transketolase (TK) activity. Liver and brain TK activity was measured in the $9000g_{av}$ supernatant prepared during the sampling. The measurement was performed in 25 mM Tris-Cl buffer, pH = 7.0, at 40 ºC and otherwise according to Tate and Nixon (11). For measurement of maximum activity, TDP was added to the cuvette to a final concentration of 100 µM. Protein was quantitated according to Lowry *et al*. (12) with bovine serum albumin as the standard.

α-Ketoglutarate dehydrogenase (KGDH) activity. Liver and brain KGDH activity was measured in the resuspended $9000g_{av}$ pellet prepared during the sampling, except when the sample consisted of a piece of tissue (and no pellet was prepared during the sampling). In the latter case, the KGDH activity was measured directly in the homogenate. A control experiment with domestic chicken confirmed that the latency was the same irrespective of whether a resuspended $9000g_{av}$ pellet or a homogenate was used, although the specific activity differed between the two methods. The measurement was performed according to Lai and Cooper (13) and Lai *et al*. (14). For measurement of maximum activity, TDP was added to the cuvette to a final concentration of 200 μM. Protein was quantitated according to Lowry *et al*. (12) with bovine serum albumin as the standard.

Thiamine quantitation. T, TMP, and TDP concentrations were determined in liver, brain, and egg yolk. Samples were extracted and prepared according to Brown *et al*. (15) with modifications suggested by Kankaanpää *et al*. (16), with the exception of liver samples, which were prepared with 0.2 g sample and 0.4% $K_3[Fe(CN)_6]$ reagent on account of matrix effects. The analysis was performed on a LaChrom HPLC system (Merck Hitachi, Tokyo, Japan) with fluorescence detection (excitation $\lambda = 375$ nm, emission $\lambda = 433$ nm) and chromatographic conditions according to Mancinelli *et al*. (17). The chromatographic column was a Luna® 5μ NH_2 column 250x4.6 mm (00G-4378E0) with an appropriate guard column (SecurityGuard™ NH_2 4x3.0 mm, AJ0-4302), both from Phenomenex (Torrance, CA, USA). A homogeneous reference material consisting of six domestic chicken livers that were pooled, submerged in liquid nitrogen, and ground to a powder was used to check the method reproducibility. Coefficients of variation for measurements in this material were 25% for free thiamine, 8% for thiamine monophosphate and 11% for thiamine diphosphate. A similarly prepared egg yolk reference material gave a coefficient of variation for free thiamine of 13%.

Data analysis. Statistical analyses were made with the software Intercooled Stata 9.2 (StataCorp LP, College Station, TX, USA) and included regression models, analysis of variance (ANOVA), the Wald test, the F-test, the 1-tailed *t*-test, the Z-test, the Wilcoxon-Mann-Whitney test (with exact *P*-values), the Pearson correlation, the Spearman rank correlation, and Fisher's exact test. Since a small sample was analysed with the Spearman

rank correlation, an exact *P*-value was obtained with a permutation test. The Shapiro-Wilk normality test and Bartlett's test for equal variances, as well as diagnostic plots, were used to determine if assumptions of normality and homoscedasticity were met. Except for the 1-tailed *t*-test, only 2-tailed tests were used. Unless otherwise stated, the basic units were the clutch means, which were calculated before the statistical analysis. Since the number of individuals from each clutch varied, this procedure made the material more balanced. Moreover, data were pooled between years and locations unless otherwise stated. Dispersion was measured as standard deviation (SD). The term location×year denotes a combination of a location and a year. All tests were performed at the 95% significance level ($\alpha = 0.05$). Where appropriate, significance was determined after *P*-value adjustment for multiple comparisons (not shown) according to Holm (18).

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Text S1. Additional bird species affected by the paralytic disease.

Symptoms, similar to those observed in relatively large birds, were occasionally observed also in small birds: one common starling (*Sturnus vulgaris*) crawling on the ground in the County of Blekinge (I); one white wagtail (*Motacilla alba*) and one Eurasian pied flycatcher (*Ficedula hypoleuca*) with hanging wings, one chaffinch (*Fringilla coelebs*) flitting in circles and unable to fly properly, one barn swallow (*Hirundo rustica*), four European greenfinches (*Carduelis chloris*), and one yellowhammer (*Emberiza citrinella*) unable to fly in the County of Södermanland (G).

One Canada goose (*Branta canadensis*), one common eider (*Somateria mollissima*), one great black-backed gull (*Larus marinus*), one mew gull (*Larus canus*), and one hooded crow (*Corvus cornix*), all of them paralysed, were cured by a thiamine injection in the breast muscle, similar to the herring gulls described earlier (Table S2).

One paralysed great black-backed gull and one paralysed hooded crow in the County of Blekinge (I) had liver α-ketoglutarate dehydrogenase (KGDH) latencies of 88% and 34%, respectively. A negative linear relationship between liver KGDH activity and latency was also found in paralysed full-grown individuals and pulli of common black-headed gull (*Chroicocephalus ridibundus*) in the County of Blekinge (I; Fig. S3; $P = 0.0021$, $P = 0.020$). The range of latencies in the common black-headed gull was 6.5–40%.

Text S2. Additional results for eggs.

In the herring gull, the redness of the yolk, mainly reflecting the amount of carotenoid pigments, was significantly lower in the Baltic Sea area as a whole compared with the Icelandic control (A, B; Table S4; $P = 0.011$). In 156 eggs, there was only a weak positive correlation between the yolk redness and yolk thiamine (T) concentration ($r = 0.26$, $P = 0.0011$). A weak positive correlation between these variables was observed also in eggs of Baltic Sea salmon (*Salmo salar*), suffering from varying degree of thiamine deficiency (1). There was no significant difference in eggshell thickness or eggshell density between any of the investigated regions, or the Baltic Sea area as a whole, and the Icelandic control (A, B; Table S4).

In the common starling, the mean yolk redness did not differ significantly between any of the investigated regions and controls (Table S6), and there was no correlation between the yolk redness and yolk T concentration ($r = 0.056$, $P = 0.59$). Eggshell thickness and eggshell density did not differ significantly between any of the two regions in southern Sweden (G, I) and the two controls (A, T treatment; Table S6), whereas the eggshells in the County of Västerbotten (C) seemed to be somewhat more porous than in Iceland (A), indicated by a few percent higher thickness and a few percent lower density (Table S6; $P < 0.01$). This difference in porosity has no immediate interpretation.

In the common eider, the mean yolk redness and eggshell thickness did not differ significantly between any of the investigated regions in the Baltic Sea area (D, F–I) and Iceland (A; Table S8), and there was no significant correlation between the yolk redness and yolk T concentration ($r = 0.13$, $P = 0.083$). The eggshell density was 2% higher both in the County of Stockholm (F) and the County of Kalmar (H) compared with Iceland (A; Table S8), but not significantly different in the Baltic Sea area as a whole (D, F–I).

There were no signs of eggshell thinning in any of the investigated species. This was taken as an important indication that none of the classical persistent pollutants, known to cause eggshell thinning in birds (2–5), were responsible for the observed effects, especially on the yolk T concentration, in the Baltic Sea area.

In 19 herring gull colonies in Sweden (E, G) 2004–2008, the number of eggs per nest was 1.6 ± 0.23 (mean ± 95% CI), and the proportion of three egg clutches was

42 ± 8.8% (mean ± 95% CI). These findings form a sharp contrast to the reported normal number of eggs per nest of ~3 and the normal proportion of three egg clutches of at least 95% in non-thiamine deficient herring gulls (6). Conspicuously low proportions of three egg clutches were reported also in the lesser black-backed gull (*Larus fuscus*, a close relative to the herring gull) in Lancashire, UK, 1981–1994, although thiamine deficiency was not considered as a possible cause (7). In the common eider, the number of eggs per clutch decreased by ~25% in Northumberland, UK, 1958–1998 (8). The larger decrease in clutch size in the herring gull (~50%) during approximately the same period (our results compared with the situation around 1950 (6)) may be related to the fact that the common eider is able to produce eggs practically devoid of thiamine, whereas the herring gull seems to be unable to produce eggs with a yolk T concentration lower than ~10 nmol/g.

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Text S3. Results for liver body index (LBI) in pulli.

In the herring gull, there was no significant difference in mean LBI (the liver weight expressed as percent of the body weight) between any of the investigated regions, or the Baltic Sea area as a whole, and the Icelandic control (B), whereas the dispersion was significantly lower in the Baltic Sea area as a whole (E–G, I; Table S5; $P = 0.0033$). In a previous study, 7 days old (corresponding to a body weight of ~153 g in our study) herring gull pulli had a median LBI of 5.6% (1), whereas the mean LBI in our study, including Iceland (B), was 4.5–5.0%. This may be a result of thiamine deficiency, since previous studies have shown that lack of thiamine may cause a long-lasting decrease in LBI (2, 3), eventually followed by a dramatic, short-lasting increase in LBI during the final stages of the disease (4, 5). The higher dispersion of LBI in the Icelandic control (B) was explained by a main cluster of clutches with LBI values around 4.9% and a smaller cluster of clutches with LBI values around 3.5%. This may be a result of incipient thiamine deficiency also in Iceland.

In the common starling, mean LBI did not differ between any of the investigated regions and the two controls (Table S7). LBI was, however, significantly less dispersed in the County of Blekinge (I) than in Iceland (A; Table S7; $P = 0.0031$). This observation may indicate incipient thiamine deficiency in Iceland, similar to the herring gulls. Since thiamine deficiency may initially reduce LBI (2, 3), the difference in dispersion between Iceland (A) and the County of Blekinge (I), combined with a 22% (though not significantly) lower mean value in the latter region, may be explained by varying degree of thiamine deficiency in Iceland, whereas the majority of the clutches in the County of Blekinge had LBI values close to a lower limit for this variable, due to more advanced thiamine deficiency. Also, the observation that LBI did not respond to thiamine treatment in the County of Blekinge (I; Table S7; $P = 0.47$) would be compatible with an irreversible reduction in LBI owing to previous thiamine deficiency in the egg.

In the common eider, LBI did not differ significantly between any of the investigated regions in the Baltic Sea area (F, I) and Iceland (A; Table S9). This may be a result of thiamine deficiency in both Iceland and the Baltic Sea area.

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Text S4. Breeding output and population estimates.

For the herring gull, the number of fledglings per breeding female was recorded in a total of ten colonies in the County of Södermanland (G) during 2004–2007 and in a total of six colonies in the County of Värmland (E) during 2004–2005. The average number of fledglings per breeding female was 0.21 ± 0.087 (mean ± 95% CI) in the County of Södermanland and 0.78 ± 0.25 (mean ± 95% CI) in the County of Värmland. For the common eider, the number of pulli per female during the first three weeks after hatch was recorded at a total of five occasions in the County of Södermanland during 2004–2007 and at three occasions in southwestern Iceland (A) 2006. The average number of pulli per female was 0.012 ± 0.034 (mean ± 95% CI) in the County of Södermanland and 0.24 ± 0.27 (mean ± 95% CI) in southwestern Iceland. We used a simple population model (1) to investigate whether breeding failure may have contributed to the observed population declines for the herring gull and the common eider in Sweden (2–4):

$$N_t = s_A N_{t-1} + s_{F4} N_{t-4} b/2 \qquad \text{(herring gull)}$$

$$N_t = s_A N_{t-1} + s_{P3} N_{t-3} b/2 \qquad \text{(common eider)}$$

where N_t is the number of breeding females in year t, s_A is the annual survival rate for adults, s_{F4} is the survival rate for fledged juveniles until first breeding, s_{P3} is the survival rate for pulli until first breeding, and b is the average number of fledglings (herring gull) or pulli (common eider) per breeding female. The model assumes a closed population with a sex ratio of 1:1 and that all surviving juveniles become adult (start to breed) in their fourth (common eider) or fifth (herring gull) calendar year (when they are three respectively four years old). The model does not account for density dependence so infinite growth is possible. The seed was three (common eider) or four (herring gull) years with a stable population, and the tested s_{F4} or s_{P3} was introduced in the first seed year, whereas the tested s_A was introduced in the last seed year.

For the herring gull, the s_{F4} has been estimated to 0.63 (5), whereas the s_A has been estimated to 0.88 (6). For the common eider, the s_A has been estimated to 0.87 (7), and if the same annual survival rate is assumed for fledged juveniles, this yields an s_{P3} of 0.66. The sex ratio of the common eider is sometimes male biased (8), so modelled population sizes may be over-estimated. Also the use of the number of pulli per female during the

first three weeks after hatch, instead of the number of fledglings per female, will result in an over-estimation of any modelled population size for the common eider.

The herring gull population in Lake Vänern (the largest lake in Sweden) was stable 1994–2006 (9, 10). A curve was fitted to these observations and the observed b of 0.78 in the County of Värmland by reduction of the annual survival rate by 7% (Fig. S5). The literature value for s_{F4} of 0.63 was reduced to 0.47 and the literature value for s_A of 0.88 was reduced to 0.82. The herring gull population in Sweden decreased 1980–2007 (2). A curve was fitted to these observations and an s_{F4} of 0.47 and an s_A of 0.82 by adjustment of b to 0.46 (Fig. S5), which is close to the average b of the County of Södermanland and the County of Värmland (0.50). A population curve for the County of Södermanland was estimated by combining an s_{F4} of 0.47 and an s_A of 0.82 with the observed b of 0.21 (Fig. S5). The resulting curve indicated a stronger population decrease in the County of Södermanland than in Sweden as a whole, which agrees with our observations that the herring gull population in the County of Södermanland is nearly extinct. The observed breeding output for the herring gull in the County of Södermanland of 0.21 fledglings per breeding female was insufficient to maintain a stable population even at the survival rates reported in the literature (5, 6) (not shown).

For the common eider an average of 0.4 pulli per female was sufficient to maintain a stable population at the survival rates reported in the literature (not shown). The common eider population in Sweden decreased 1980–2007 (2, 4). A curve was fitted to these observations and the hypothetical b for a stable population of 0.4 by reduction of the annual survival rate by 5% (Fig. S6). The literature value for s_A of 0.87 was reduced to 0.83, yielding a corresponding s_{P3} of 0.57. The observed breeding output for the common eider in the County of Södermanland of 0.012 pulli per female and in southwestern Iceland of 0.24 pulli per female was in both cases insufficient to maintain a stable population even at the survival rates reported in the literature (not shown).

These calculations show that breeding failure most probably has contributed to the observed population declines, and that reduced survival of juveniles and adults may have added to these declines. In this work we demonstrate relationships between thiamine deficiency and breeding failure and excess mortality. Hence, thiamine deficiency is

strongly suspected to be an important cause of the observed population declines in northern Europe.

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Text S5. Elaborated discussion of important aspects.

Specific biomarkers of thiamine deficiency. In this work we primarily measured biomarkers that directly demonstrate thiamine deficiency, such as thiamine concentrations and thiamine diphosphate (TDP) dependent enzyme activities. Other biomarkers, like pyruvate and lactate concentrations as well as glucose, amino acid, and neurotransmitter metabolism, may be more directly related to the observed symptoms, but were not measured here, since they are not exclusively dependent on thiamine status. The general pattern was poorer thiamine status in the Baltic Sea area than in Iceland, but there were several indications of incipient thiamine deficiency also in Iceland. The latter observation was made possible by the use of additional controls with more general validity. Firstly, for the herring gull, historical data asserted that the normal number of eggs per nest is three and that at least 95% of the nests in a colony should have three eggs (1). Knowing the positive relationship between thiamine status and egg laying ability, the observed lower proportions of nests with three eggs in the Baltic Sea area were interpreted as manifestations of thiamine deficiency. Secondly, previous investigations asserted that the thiamine concentration ratio between the liver and brain in healthy vertebrate individuals should be 2–3 (2-5), and that thiamine deficiency decreases this ratio (4, 5). Hence, the observed ratios of 0.71–1.4 in common starling and common eider were interpreted as strong manifestations of thiamine deficiency. Thirdly, the liver and brain α-ketoglutarate dehydrogenase (KGDH) latency in healthy domestic chicken pulli was ~4%, and similar values were obtained in studies of *e.g.* rat (6–8). Assuming that a latency of ~4% is normal for healthy individuals also in other species, any higher value is *per se* a manifestation of thiamine deficiency.

Our observations of substantially higher latencies for KGDH than for transketolase (TK) in the liver agree well with a previous investigation of rats, where a thiamine deficient diet gave rise to liver KGDH latencies up to ~43% and liver TK latencies up to ~8% (6). In the brain, however, we found no such difference between KGDH and TK, which also agrees with previous investigations of rats (9). Another investigation, where the supply of thiamine was restored after one period of thiamine deficiency, demonstrated a permanent reduction in brain TK activity, although the latency was restored to zero

(10). A similar permanent loss of activity may explain the relatively moderate increase in TK activity in the brain of thiamine-treated common starling pulli. In this context it should be pointed out that there is no reason to believe that the activity was higher than normal in the thiamine-treated common starling pulli owing to induction by the presence of excess thiamine. Studies of TK activity in the brain and liver in non-thiamine deficient rats have shown that excess thiamine in the food, up to 872 times more than necessary, did not increase the activity, *i.e.* there was a natural maximum activity in non-thiamine deficient individuals (11). This maximum activity was reached at a certain critical low level of thiamine in the food.

Species differences in minimum yolk thiamine (T) concentration. For the herring gull and common starling eggs there was a minimum for the yolk T concentration of ~10 nmol/g, whereas there was no such lower limit for the common eider eggs. This phenomenon has at least two important implications. Firstly, as the relative differences in yolk T concentration between the Baltic Sea area and Iceland were smaller for the herring gull and common starling eggs than for the common eider eggs, the question may be raised whether these differences would be larger if there were no such minimum for the yolk T concentration in the herring gull and common starling eggs. Secondly, for species with a yolk T concentration minimum (other than zero) any attempt to estimate the thiamine status of the adult individuals in the population by measurement of the yolk T concentration in their eggs will generate an overestimation, when a significant proportion of the individuals produces fewer eggs than naturally owing to thiamine deficiency.

Non-invasive biomarkers of thiamine deficiency. Difficulty in keeping the wings folded along the side of the body (hanging wings) when resting, and pigmentation changes in the iris, were two characteristics of the paralytic disease that deserve special attention, since their possible relationship with thiamine deficiency has not been pointed out in previous studies. Hanging wings in this context implies that both wings are equally affected. This phenomenon was frequently observed during our field investigations as one of the first paralytic symptoms. Also, when the symptoms of the paralytic disease were reversed by thiamine treatment, and the symptoms disappeared in the reverse order as they appeared, hanging wings was one of the last symptoms to disappear. Pigmentation changes in the iris occurred frequently, but were not systematically

documented. They resemble, however, the pigmentation changes occurring in the eye disorder *retinitis pigmentosa*, which is caused by reduced α-oxidation of 3-methyl-branched and 2-hydroxy straight chain fatty acids in the peroxisomes owing to inhibited uptake of thiamine (12–14). Hence, inhibition of this enzyme due to thiamine deficiency in birds may result in similar pigmentation changes. Both the hanging wings and the pigmentation changes in the iris are non-invasive characteristics with potential to be used in the field. The pigmentation changes can, however, be seen with the unaided eye only when the iris is pale.

Sub-lethal thiamine deficiency causes secondary effects. Our observations of advanced thiamine deficiency strongly suggest that sub-lethal thiamine deficiency also occurs among the affected species, and that there is a broad range of thiamine deficiency. Previous laboratory studies have revealed a number of effects of sub-lethal thiamine deficiency, like reduced feeding (15, 16), memory and learning disturbances (17–20), immune suppression (21–27), and damage to the blood-brain barrier (28, 29). Relationships have also been demonstrated between sub-lethal thiamine deficiency and neurodegenerative diseases, cardiovascular diseases, diabetes, cancer, and cataract (30).

Reduced feeding owing to sub-lethal thiamine deficiency may result in weight loss (31, 32) that is indistinguishable from starvation owing to lack of food. This means that the occurrence of starved or emaciated individuals in the field does not necessarily imply lack of food. Weight loss due to loss of appetite, as a secondary effect of thiamine deficiency, has been demonstrated in mice (31) and rats (33). Loss of appetite was also observed in all specimens in our thiamine treatment experiments before administration of the thiamine, whereas appetite was always regained in the thiamine-treated specimens soon after administration of the thiamine. Moreover, experimental starvation did not induce thiamine deficiency in rats (8), neither did it cause mortality at hatch in domestic chicken (34), nor affect the yolk thiamine concentration (34). In order to test the generality of these observations, we performed a starvation experiment with domestic chicken pulli, in which we measured TK and KGDH activity and latency in the liver. The treatment group was starved day 14–18 (after hatch) and received only water *ad libitum* during this time, whereas the control group received both food and water *ad libitum*. On day 18 the starved specimens weighed 36% less than the control group. TK and KGDH

activities were not lower, and latencies were not higher, in the starved group (1-tailed *t*-test, $P > 0.05$). Moreover, there was no relationship between liver KGDH activity and latency in the starved group (Fig. S4 d; $P = 0.98$). In summary, there is plenty of evidence that thiamine deficiency causes starvation owing to loss of appetite, but not that starvation induces thiamine deficiency.

Behavioural changes, observed by us and suspected to be caused by sub-lethal thiamine deficiency, include reduced aggression rates (attack diving) and low noise level in herring gull colonies (compared with historical data (35)), as well as reduced (and often only rudimentary) nest building in the common starling and the common eider. A possible example of immune suppression induced by sub-lethal thiamine deficiency is herring gulls that were infected by the opportunistic pathogen *Aspergillus fumigatus* when fed a thiamine deficient diet (36). Further suspected effects of sub-lethal thiamine deficiency related immune suppression include the recent observations of viral (37, 38) and bacterial (39) infections in connection with high mortality of the common eider (38). We are also open to the possibility of increased risk of botulism as a secondary effect of thiamine deficiency. A study of thiamine deficiency in domestic chicken demonstrated *i.a*. anorexia and degeneration of the intestinal mucosa (40). Anorexia may, owing to prolonged duration of the passage through the gastrointestinal tract, favour the growth of the *Clostridium botulinum* bacterium and its production of the botulinum toxin, and degeneration of the intestinal mucosa may favour the uptake of the botulinum toxin in the blood (41–44). Factors like these may explain the occurrence of botulism in some Swedish herring gulls with symptoms fitting the paralytic disease studied in this work (45). Support for botulism as only a secondary effect in these herring gulls came from the fact that a large fraction of the paralysed specimens tested negative for the botulinum toxin, and that many non-paralysed specimens tested positive. It is a very important fact that general immune suppression, induced by thiamine deficiency, will facilitate the proliferation of practically any pathogen. A topical example, the avian flu virus (46), is just one pathogen that may be favoured by thiamine deficiency among wild birds. Sub-lethal thiamine deficiency may also lead to altered habitat preferences and migration routes. For example, we have observed that herring gulls that fail to breed often move from the archipelago to urban areas when they do not have any young to attend, which in

turn may give the general public an erroneous impression of a population increase. As another example, we have observed how an increased number of common eider stay and attempt to breed in the County of Blekinge (I) instead of migrating further north to their normal breeding areas. The reason for this is fatigue, which may be due to sub-lethal thiamine deficiency. This may also give an erroneous impression of a population increase for the common eider in southern Sweden.

Causative agent(s). The primary aim of this work was to demonstrate the relationship between the paralytic disease and thiamine deficiency. Also important was to form an idea about the temporal and geographical distribution of the proposed thiamine deficiency syndrome and its distribution among species. Further investigations focusing on causation are urgently needed. Examination of a large number of paralysed specimens of several species indicates that the disease is not caused by any known pathogen (47). Classical persistent organic pollutants known to cause eggshell thinning among birds are not suspected, since eggshell thickness was unaffected. The classical transport and fate model, with enrichment of lipophilic pollutants in the food web and the strongest effects among the top predators, does not necessarily apply here. Thiamine deficiency may be induced either by a causative agent(s) acting directly on the affected individual, and/or by insufficient transfer of thiamine between the trophic levels in the food web. This opens the possibility for the causative agent(s) to act also on any trophic level below that of the affected individual. The strategy used here was to study the thiamine deficiency syndrome in a wide range of species, differing in parameters like physiology, food preferences, habitat preferences, breeding biology, and migration patterns. This strategy will be important also in further work to find the causative agent(s). Our results obtained so far point towards naturally occurring sulphur and/or nitrogen containing toxic substances present in unnatural concentrations owing to altered biogeochemical cycles (48).

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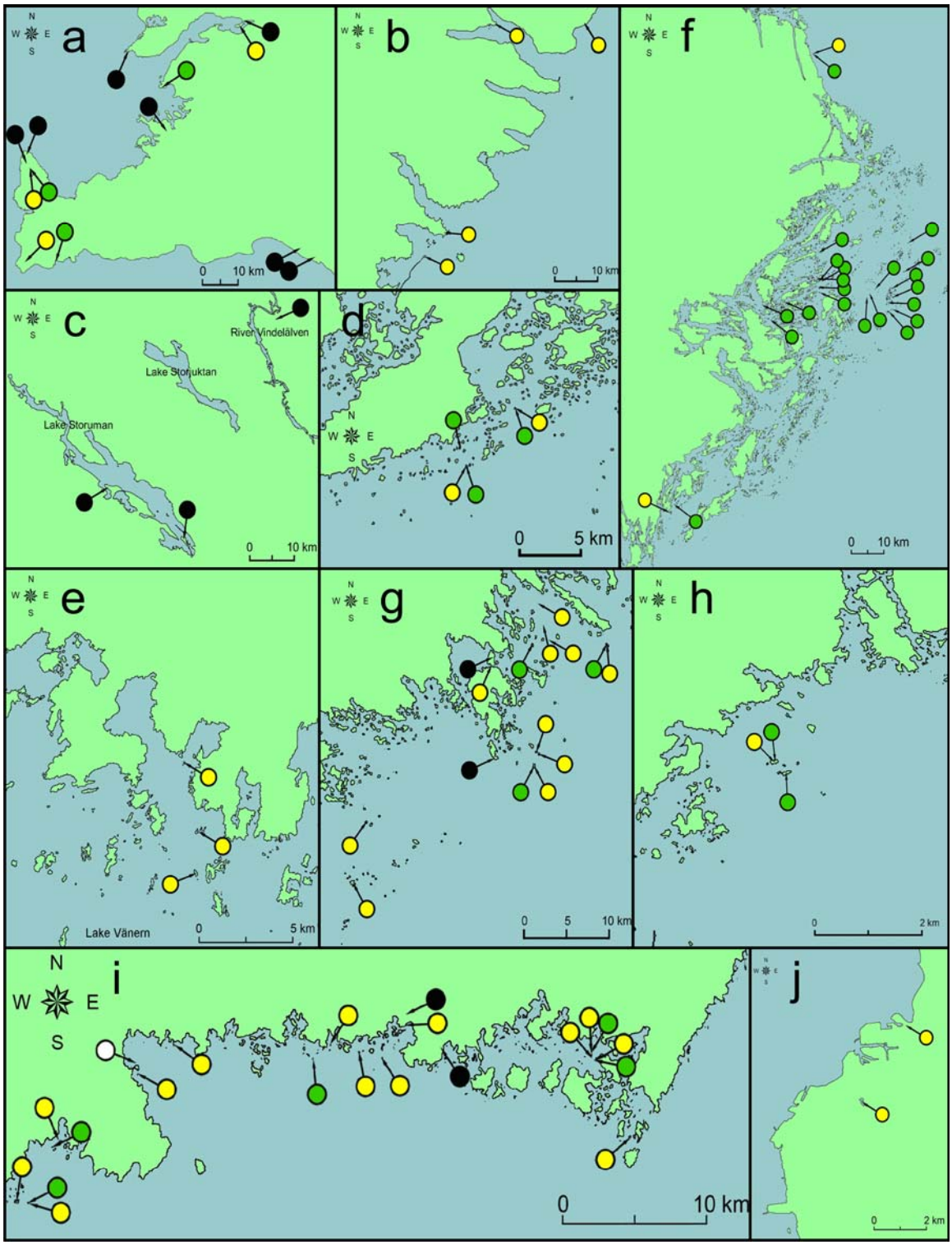

Fig. S1 a–j. The 83 locations where samples were collected. a, Southwestern Iceland (A). **b,** Eastern Iceland (B). **c,** County of Västerbotten (C). **d,** County of Södra Finland (D). **e,** County of Värmland (E). **f,** County of Stockholm (F). **g,** County of

Södermanland (G). **h,** County of Kalmar (H). **i,** County of Blekinge (I). **j,** County of Skåne (J). Yellow: herring gull (*Larus argentatus*). Black: common starling (*Sturnus vulgaris*). Green: common eider (*Somateria mollissima*). White: common black-headed gull (*Chroicocephalus ridibundus*). [In figure **i** a few locations in the southwest, formally belonging to the County of Skåne, were assigned to the County of Blekinge, since they are situated very close to the border between the two regions and may be regarded as an extension of the Blekinge archipelago.]

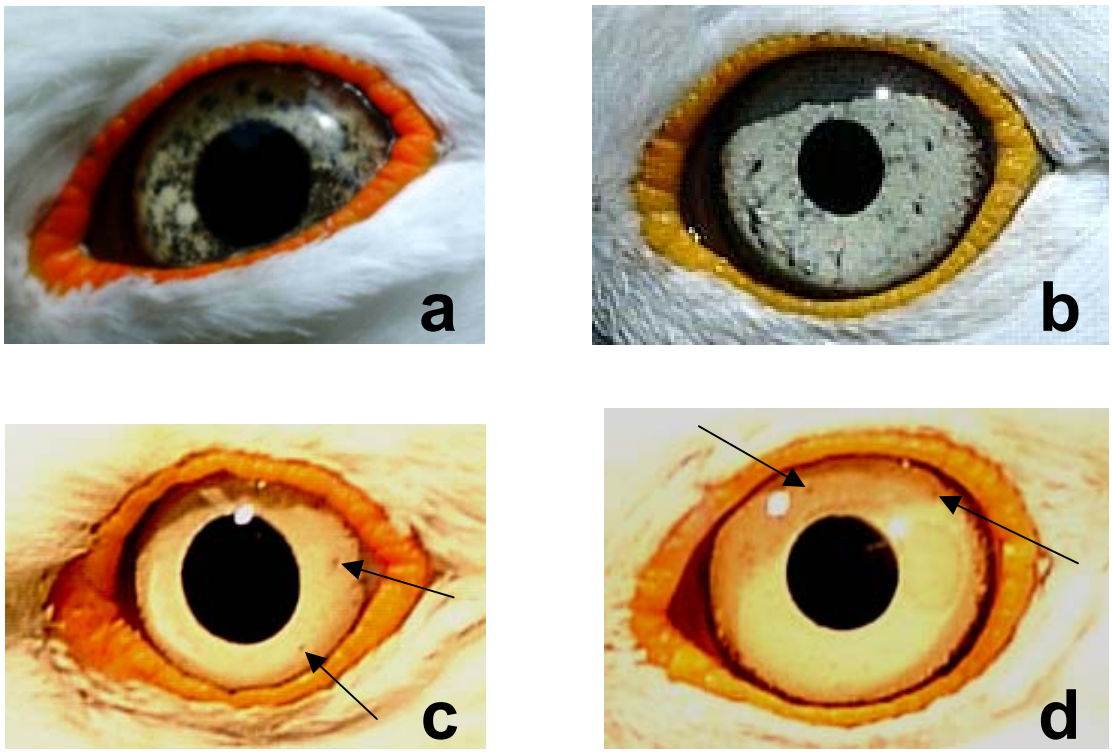

Fig. S2 a–d. Pigmentation changes in the iris of the herring gull (*Larus argentatus*). The pigmentation changes are often reminiscent of ash flakes or black spots. Photographs **a** and **b** show two specimens from the Baltic Sea area with conspicuous pigmentation changes, whereas **c** and **d** show two less affected specimens from Iceland, with only incipient pigmentation changes (arrows). The pigmentation changes in the iris were not systematically quantified, but our impression is that this phenomenon was much more abundant in the Baltic Sea area than in Iceland. [The central black, round area is the pupil, and the surrounding white area is the iris. The eye is surrounded by a yellow to orange orbital ring, whose colour may depend on subspecies (*i.a*. *Larus argentatus omissus* [**a**]) as well as age, season, and perhaps also carotenoids in the diet (1). Photographs **a** and **b** were taken in daylight, whereas photographs **c** and **d** were taken in strip light.]

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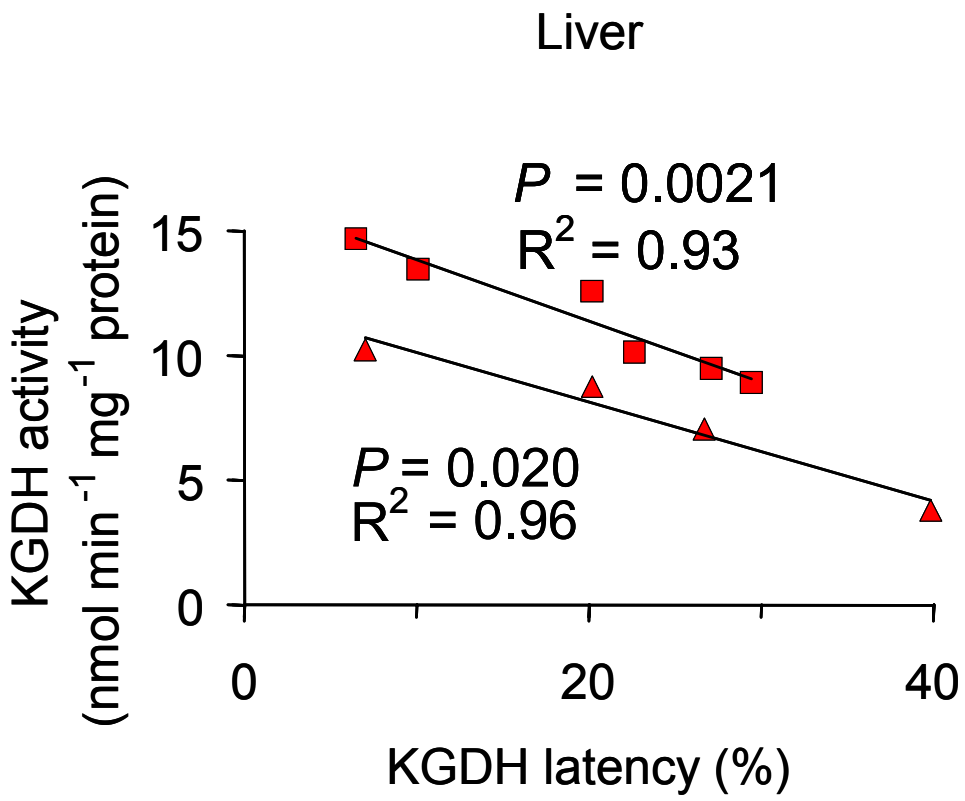

Fig. S3. Liver α-ketoglutarate dehydrogenase (KGDH) in common black-headed gull (*Chroicocephalus ridibundus*). There was a negative linear relationship between activity and latency in both full-grown individuals and pulli from the County of Blekinge (I). The occurrence of high latency values indicated thiamine deficiency in this species. [These KGDH activities were measured in the homogenate, whereas most other KGDH activities were measured in a mitochondrial suspension. Owing to a difference in protein content, the activities are not directly comparable between the two methods, whereas the latencies are independent of protein content and are thus directly comparable with all other latencies.] Red squares: Full-grown individuals. Red triangles: Pulli.

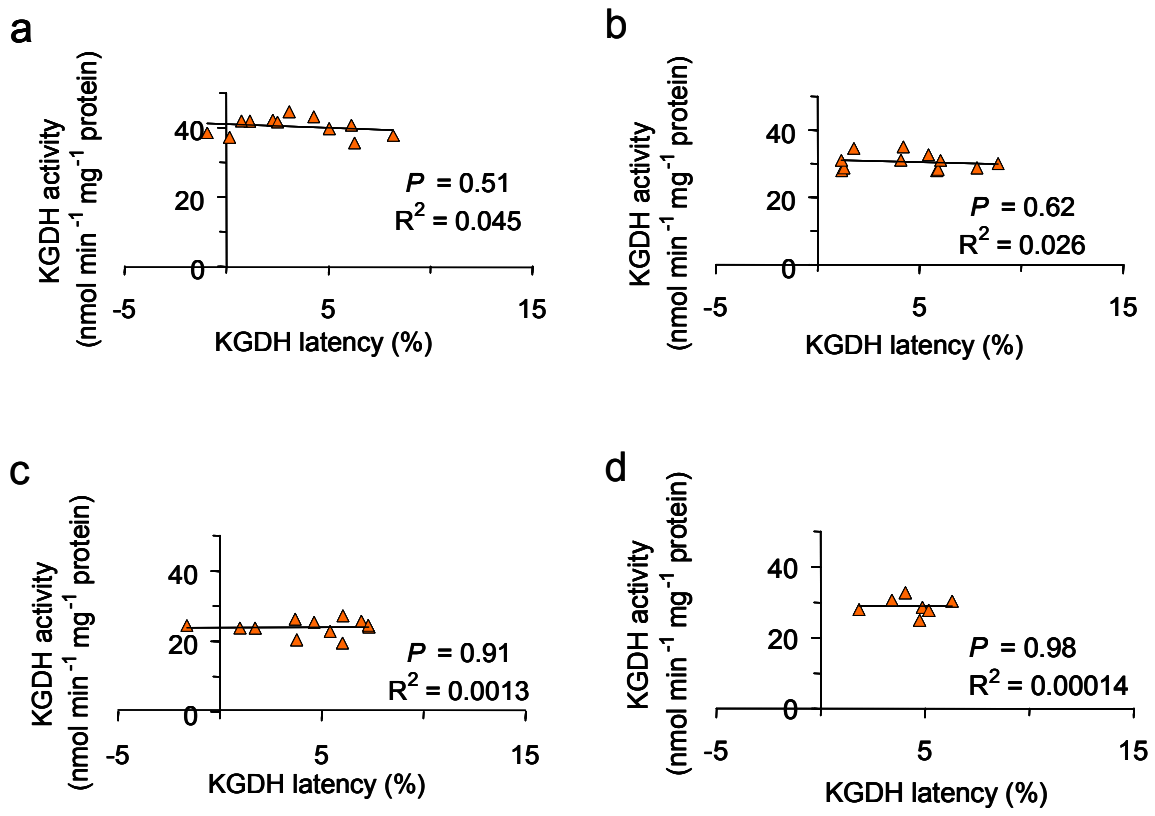

Fig. S4 a–d. Liver α-ketoglutarate dehydrogenase (KGDH) in control (a–c) and severely starved (d) domestic chicken (*Gallus gallus*) pulli. a, Non-thiamine deficient specimens ~10 hours after hatch. **b,** Non-thiamine deficient specimens 5 days after hatch. **c,** Non-thiamine deficient specimens 18 days after hatch. **d,** Specimens severely starved day 14–18 and sampled on day 18. There was no relationship between activity and latency in any of the four investigated groups. Moreover, there was no difference in activity or latency between the non-starved group (**c**) and the starved group (**d**) (1-tailed *t*-test, $P > 0.05$).

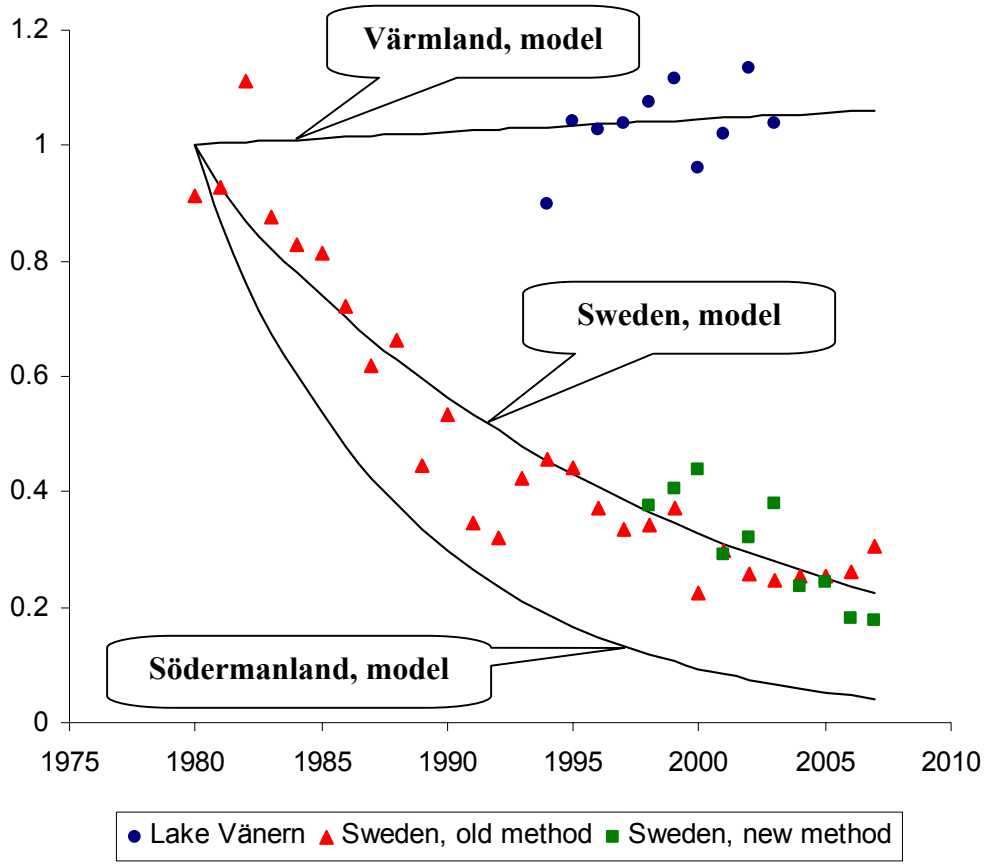

Fig. S5. Population trends for the herring gull (*Larus argentatus*) in Sweden. Blue circles: Lake Vänern (the largest lake in Sweden). Observed population development according to Vänerns Vattenvårdsförbund (1, 2). Red triangles: Sweden (old method). Observed population development according to the Swedish Bird Survey (3). Green squares: Sweden, (new method). Observed population development according to the Swedish Bird Survey (4).

Värmland, model: Population curve for the County of Värmland (E) fitted to the observations in Lake Vänern, $s_{F4} = 0.47$, $s_A = 0.82$, $b = 0.78$. Sweden, model: Population curve for Sweden fitted to the observations in Sweden, $s_{F4} = 0.47$, $s_A = 0.82$, $b = 0.46$. Södermanland, model: Estimated population curve for the County of Södermanland (G), $s_{F4} = 0.47$, $s_A = 0.82$, $b = 0.21$. Y-axis: Population index. X-axis: Year.

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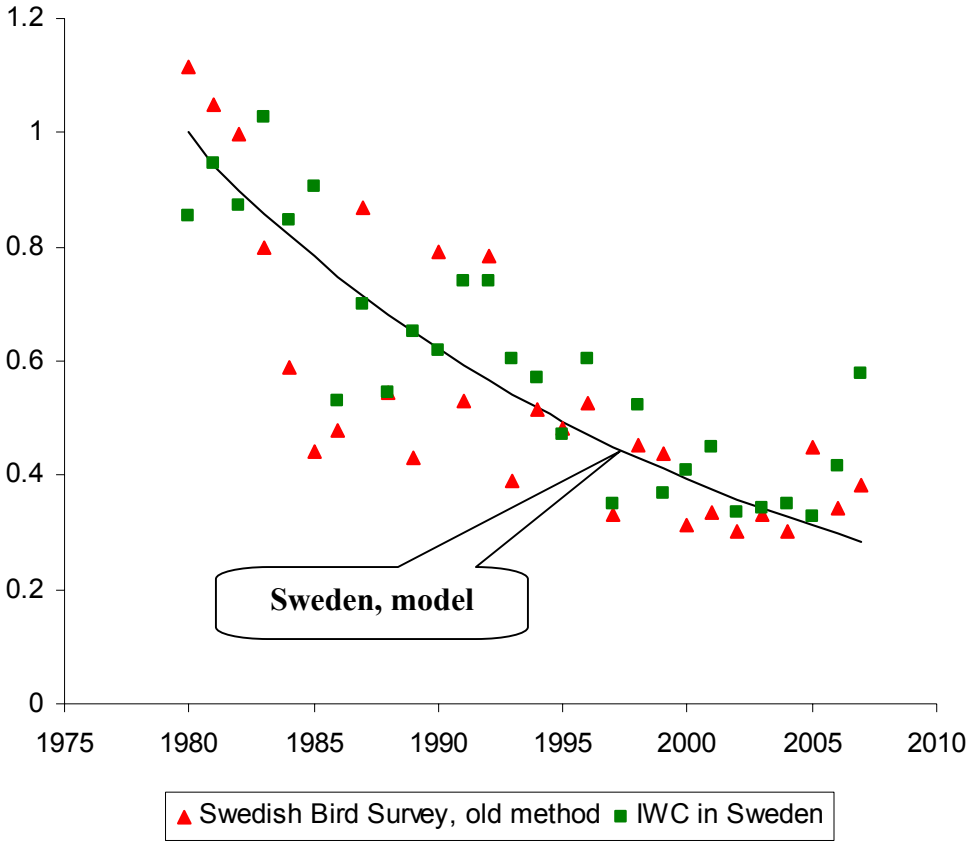

Fig. S6. Population trends for the common eider (*Somateria mollissima*) in Sweden. Red triangles: Swedish Bird Survey (old method). Observed population development in Sweden according to the Swedish Bird Survey (1). Green squares: IWC in Sweden. Observed population development in Sweden according to the International Waterfowl Census (IWC) in Sweden (2).

Sweden, model: Population curve for Sweden fitted to the observations, $s_{P3} = 0.57$, $s_A = 0.83$, b = 0.40. Y-axis: Population index. X-axis: Year.

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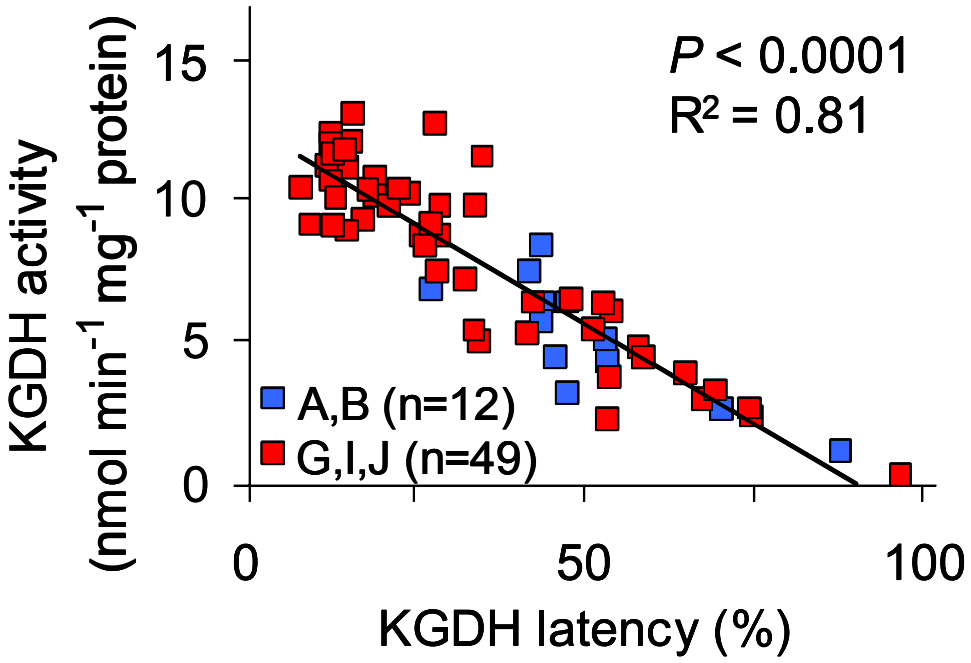

Fig. S7. Liver α-ketoglutarate dehydrogenase (KGDH) in full-grown herring gulls (*Larus argentatus*). There was a negative linear relationship between activity and latency both in the Baltic Sea area (G, I, J) and in Iceland (A, B), and the range of latency values was extremely large (8–96%). The many high latency values indicated advanced thiamine deficiency in the investigated regions. [These KGDH activities were measured in the homogenate, whereas most other KGDH activities were measured in a mitochondrial suspension. Owing to a difference in protein content, the activities are not directly comparable between the two methods, whereas the latencies are independent of protein content and are thus directly comparable with all other latencies.] Blue squares: Iceland. Red squares: Baltic Sea area.

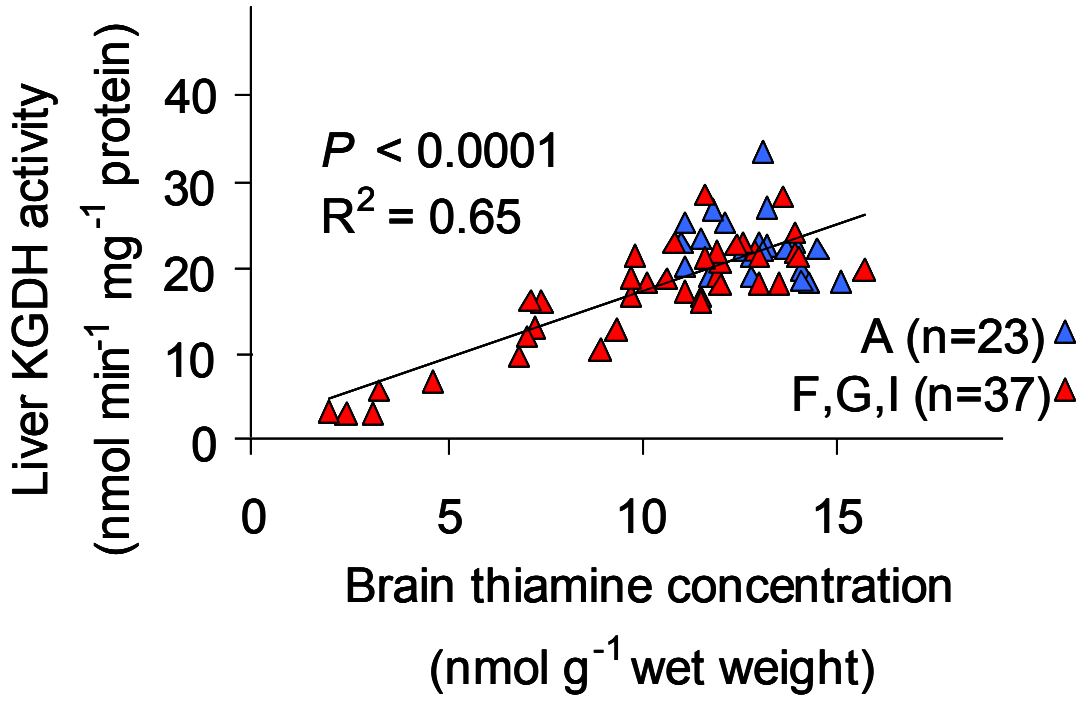

Fig. S8. Liver α-ketoglutarate dehydrogenase (KGDH) activity and brain thiamine concentration in common eider (*Somateria mollissima*) pulli. There was a positive linear relationship between liver KGDH activity and brain T+TMP+TDP concentration, which illustrates the systemic nature of the thiamine deficiency. Blue triangles: Iceland. Red triangles: Baltic Sea area.

Movie S1.

A paralysed herring gull (*Larus argentatus*). This movie shows a specimen from the County of Södermanland (G). Both wings are equally paralysed and the beak has no strength, whereas mobility and control of the head still remain. In this work we demonstrate that the probability to remedy an individual in this condition by thiamine treatment is very high. The movie is taken in the field, but the specimen is placed on a black tablecloth in order to remove disturbing background and enhance contrast.

Table S1. Occurrence of the paralytic disease studied in this work and other diseases, injuries, and causes of death in the County of Skåne (J)[a].

Species	Total n	The paralytic disease studied in this work[b]		Other disease or injury, or diagnosis not possible[c]	
		n	%[d]	n	%
Anas clypeata (Northern shoveler)	2	1	50	1	50
Anas crecca (Eurasian teal)	58	53	91	5	9
Anas penelope (Eurasian wigeon)	2	0	0	2	100
Anas platyrhynchos (Mallard)	58	47	81	11	19
Anser anser (Greylag goose)	21	1	5	20	95
Anser fabalis (Bean goose)	1	0	0	1	100
Aythya fuligula (Tufted duck)	2	2	100	0	0
Branta canadensis (Canada goose)	5	0[e]	0	5	100
Branta leucopsis (Barnacle goose)	8	2	25	6	75
Bucephala clangula (Common goldeneye)	1	0	0	1	100
Buteo buteo (Common buzzard)	1	0	0	1	100
Chroicocephalus ridibundus (Com. black-headed gull)	48	6	13	42	87
Corvus cornix (Hooded crow)	5	1	20	4	80
Corvus frugilegus (Rook)	2	0	0	2	100
Cygnus olor (Mute swan)	15	3	20	12	80
Fulica atra (Common coot)	21	12	57	9	43
Haematopus ostralegus (Eurasian Oystercatcher)	2	0[e]	0	2	100
Larus argentatus (Herring gull)	361	210	58	151	42
Larus canus (Mew gull)	21	8	58	13	62
Larus fuscus (Lesser black-backed gull)	2	1	50	1	50
Larus marinus (Great black-backed gull)	82	37	45	45	55
Mergus serrator (Red-breasted merganser)	1	0	0	1	100
Motacilla alba (White wagtail)	2	0[e]	0	2	100
Phalacrocorax carbo (Great cormorant)	13	6	46	7	54
Philomachus pugnax (Ruff)	7	7	100	0	0
Phoenicurus phoenicurus (Common redstart)	2	0	0	2	100
Pica pica (Eurasian magpie)	2	0	0	2	100
Recurvirostra avosetta (Pied avocet)	1	1	100	0	0
Somateria mollissima (Common eider)	13	0[e]	0	13	100
Sterna hirundo (Common tern)	1	0[e]	0	1	100
Tadorna tadorna (Common shelduck)	57	38	67	19	33
Tringa erythropus (Spotted redshank)	1	1	100	0	0
Tringa glareola (Wood sandpiper)	3	3	100	0	0
Tringa nebularia (Common greenshank)	2	2	100	0	0
Tringa totanus (Common redshank)	7	6	86	1	14
Vanellus vanellus (Northern lapwing)	7	3	43	4	57
Sum of all specimens	837	451	54	386	46

[a] Sick, dying, or dead full-grown specimens diagnosed by members of Bird-Protection Spillepeng (Malmö, Sweden) during 07/01/2002 to 02/01/2008 (month/day/year).

[b] The specimens diagnosed as suffering from the paralytic disease were unable to fly and walk, owing to paralysis of wings and legs, but were still able to move the head and drink water without any problem. Also recently dead specimens with typical characteristics of the paralytic disease were included. Several of these specimens were observed at a distance with a telescope and displayed typical symptoms of the paralytic disease before death. Specimens with the paralytic disease were observed all the year round (not shown).

[c] This category includes all specimens that could not be diagnosed as certainly suffering from the paralytic disease. Examples of other diagnoses were traffic injury, window collision, fish net injury, and gunshot injury. This category also includes decaying specimens where diagnosis was not possible.

[d] These percentages are probably underestimations, since only specimens that could be diagnosed as certainly suffering from the paralytic disease were included. Specimens with other diseases or injuries, or specimens that were not possible to diagnose, may have suffered from the paralytic disease as well. Moreover, frequent observations of the paralytic disease in various bird species were made by the ornithologist Le Carlsson, Ronneby, Sweden. In 2000–2007 he invented a ~50–75 km long stretch of coast in the County of Blekinge (I) and recorded a total of >18.000 dying or dead specimens, many of which displayed the typical symptoms of the paralytic disease.

[e] Full-grown specimens of these species, suffering from the paralytic disease, were, however, observed by us in the County of Södermanland (G) and/or the County of Blekinge (I). Hence, the paralytic disease was observed in 28 of 36 species (78%).

Table S2. Remediation of paralysis in birds from the Swedish Baltic Sea coast: Thiamine-treated and control (saline) specimens.

Species	Maturation[a]	Symptoms at capture[b]	Treatment[c]	Treatment date[d] [month/day/year]	Observation period[e] [days]	Final status[b]	Recovery[f]
Larus argentatus	Adult	IF, IW	Saline	5/2/2005	14	IF, IW	No
Larus argentatus	Adult	IF, IW	Saline	5/2/2005	14	IF, IW	No
Larus argentatus	Adult	IF, IW	Saline	5/2/2005	14	IF, IW	No
Larus argentatus	Adult	IF, IW	Saline	7/28/2005	14	Dead	No
Larus argentatus	Adult	IF, IW	Saline	5/20/2007	14	IF, IW	No
Larus argentatus	Adult	IF, IW	Saline	5/30/2007	14	IF, IW	No
Larus marinus	Adult	IF, IW	Saline	7/29/2007	2	Dead	No
Larus argentatus	Adult	IF, IW	Thiamine	7/17/2004	2	Dead	No
Larus argentatus	Adult	IF, IW	Thiamine	7/17/2004	2	Dead	No
Larus argentatus	Adult	IF, IW	Thiamine	8/16/2004	1	Dead	No
Larus argentatus	Adult	IF, IW	Thiamine	8/16/2004	1	Dead	No
Larus argentatus	Adult	IF, IW	Thiamine	6/16/2004	10	Dead	No
Larus argentatus	Adult	IF	Thiamine	6/4/2004	7	AF	Yes
Larus argentatus	Adult	IF, IW	Thiamine	7/17/2004	11	AF, AW	Yes
Larus argentatus	Adult	IF, IW	Thiamine	7/17/2004	11	AF, AW	Yes
Larus argentatus	Adult	IF, IW	Thiamine	4/10/2005	14	AF, AW	Yes
Larus argentatus	Adult	IF, IW	Thiamine	5/2/2005	14	AF, AW	Yes
Larus argentatus	Adult	IF, IW	Thiamine	5/25/2005	7	AF, AW	Yes
Larus argentatus	Adult	IF, IW	Thiamine	7/28/2005	10	AF, AW	Yes
Larus argentatus	Juvenile	IF, IW	Thiamine	9/17/2005	13	AF, AW	Yes
Larus argentatus	Adult	IF, IW	Thiamine	5/20/2007	9	AF, AW	Yes
Larus marinus	Juvenile	IF, IW	Thiamine	5/13/2004	9	AF, AW	Yes
Larus canus	Juvenile	IF, IW	Thiamine	12/2/2004	10	AF, AW	Yes
Som. mollissima	Adult	IF, IW	Thiamine	5/19/2005	1	AF, AW	Yes
Branta candensis	Juvenile	IF	Thiamine	8/5/2005	2	AF	Yes
Corvus corone[g]	Juvenile	IF	Thiamine	7/4/2004	7	AF	Yes

[a] A juvenile is fledged, and hence full-grown, but sexually immature.

[b] IF=Inability to fly; IW=Inability to walk; AW=ability to walk; AF= ability to fly

[c] Injection in the breast muscle with a thiamine (50 mg/kg) solution (1 mL/kg) or a saline (9.0 g/L; 1 mL/kg). The injection was always performed within a few hours after capture, and all specimens had permanent access to water, whereas food was withheld during the first 48 hours after the injection, unless otherwise stated.

[d] The paralytic disease was observed in various bird species throughout the year. The numerous observations of affected water birds in breeding colonies do not mean that the paralytic disease is confined to water birds during the breeding season, but rather is likely an effect of a high discovery rate. The herring gull, for example, is a large white bird, which is easily discovered in colonies. It should be stressed, that in the field it is necessary to approach the birds to discover whether they are affected by the paralytic disease because they may appear normal from a distance, *e.g.*, from a boat.

[e] During this period each specimen was regularly observed, and at the end of the period the final status was determined.

[f] An analysis of the entire material (n = 26), irrespective of species and time of recovery, showed that thiamine treatment had a highly significant effect on recovery (Fisher's exact test, $P = 0.0012$).

[g] This specimen received the thiamine injection two days after capture. It was offered food for 48 hours without eating, but 25 minutes after the thiamine injection it started to feed.

Table S3. Comparison of the paralytic disease studied in this work with experimental thiamine deficiency and experimental botulism in large (~0.5–1 kg) birds.

Characteristic	The paralytic disease studied in this work	Experimental thiamine deficiency[a]	Experimental botulism[b]
Time from the first symptoms until death	> 1 week	> 1 week[1,2]	< 4 days[c,3–9]
Paralysis of the neck (limberneck)	No	No[1]	Yes[4–8,10,11]
Paralysis of the nictitating membrane[d]	No	No[1]	Yes[4,5,10,11]
Raising of hackles	No	No[e,1]	Yes[5–7]
Spontaneous recovery from paralysis of the legs	No	No[1]	Yes[4,5,10,11]
Complete loss of appetite	Yes[f]	Yes[1,12,13]	No[5]
Opisthotonus (star-gazing)	Yes	Yes[1,2,12–14]	No[4–6,8]
Paralysis of the wings before paralysis of the legs	Yes	Yes[1]	Yes[6]/No[4,5,7,10,11]
Squinting eyes[d]	Yes	Yes[1]	Not reported
Loss of voice	Yes	Not reported	No[6,7]
Laboured breathing[g]	Yes	Yes[1]	No[h,5]
Positive response to thiamine treatment[i]	Yes	Yes[1,2,12–15]	Not investigated[j]

[a] Induced by insufficient supply of thiamine in the diet.

[b] Induced by *Clostridium botulinum* spores and/or toxin.

[c] In the seven cited investigations the typical time from the first symptoms until death was a few hours up to two days. In one dose group, where some specimens died and others survived, the course of the disease was longer than usual, and one specimen died 17 days after the first symptoms (5). This specimen was, however, the only exception, where the time from the first symptoms until death was > 4 days.

[d] Here we discriminate between "paralysis of the nictitating membrane" and "squinting eyes". Paralysis of the nictitating membrane may result in clogging and/or gradual closure of the eye owing to lack of moisture. The partial closure of the eye then resembles the reaction to sleepiness, whereas the squinting resembles the reaction to strong light. An example of squinting is shown in Fig. S2 a.

[e] The cited investigation describes in detail the main characteristics of experimental thiamine deficiency. Our interpretation is that raising of hackles would be mentioned if it occurred.

[f] The paralysed specimens studied in this work refused to eat but not to drink. Even in relatively late stages of paralysis of wings and legs, they drank water voluntarily and without any problem, whereas specimens in late stages of botulism were reported to be unable to swallow (16).

[g] Here we discriminate between "laboured breathing", which has the sense of difficulty to breathe, and "gasping for air", which has the sense of panting.

[h] The cited investigation describes in detail the main characteristics of experimental botulism. Our interpretation is that laboured breathing would be mentioned if it occurred.

[i] A positive response includes *i.a.* reversal of the paralysis of legs and wings as well as regained appetite and voice. The paralytic disease studied in this work was irreversible during the late stages, occurring during approximately 5–20% of the time between paralysis of wings and legs until death.

[j] There are no documented or suspected effects of thiamine on botulism, and there are large differences in the cellular biochemistry of thiamine deficiency and botulism.

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Table S4. Data from measurements in herring gull (*Larus argentatus*) eggs[a].

Variable [unit]	Statistic[b]	Iceland SW, E (A, B)	County of S. Finland (D)	County of Värmland (E)	County of Södermanland (G)	County of Kalmar (H)	County of Blekinge (I)	Baltic Sea area (D, E, G–I)
T in egg yolk[c] [nmol/g wet weight]	Mean	29	17*	19*	19*	18*	21*	19*
	95% CI	25–34	15–20	17–20	18–21	16–21	19–23	18–20
	P-value	Control	<0.0001	<0.0001	<0.0001	<0.0001	0.0014	<0.0001
	n_l, n_c, n_s	2, 7, 7	2, 8, 12	8, 48, 49	9, 41, 42	1, 5, 6	8, 51, 51	28, 153, 160
Redness of egg yolk [%]	Mean	38	23*	24*	26	25	27	25*
	95% CI	28–48	18–27	21–27	23–29	20–31	24–29	23–27
	P-value	Control	0.0054	0.0092	0.021	0.027	0.032	0.011
	SD	11	4.8	5.7	5.7	4.1	4.0*	5.1*
	P-value	Control	0.073	0.017	0.027	0.081	0.00010	0.0014
	n_l, n_c, n_s	2, 7, 7	2, 8, 12	8, 48, 49	6, 30, 31	1, 5, 6	8, 51, 51	25, 142, 149
Eggshell thickness [μm]	Mean	358	348	352	357	374	356	357
	95% CI	345–370	331–364	344–359	349–364	359–389	349–363	352–362
	P-value	Control	0.36	0.44	0.91	0.089	0.82	0.97
	n_l, n_c, n_s	2, 5, 5	2, 8, 12	8, 48, 49	9, 41, 42	1, 5, 6	8, 51, 51	28, 153, 160
Eggshell density [g/cm^3]	Mean	1.80	1.81	1.78	1.73	1.74	1.80	1.77
	95% CI	1.74–1.86	1.78–1.84	1.77–1.80	1.71–1.75	1.63–1.85	1.78–1.81	1.75–1.80
	P-value	Control	0.66	0.70	0.050	0.41	0.98	0.49
	n_l, n_c, n_s	2, 5, 5	2, 8, 12	8, 48, 49	9, 41, 42	1, 5, 6	8, 51, 51	28, 153, 160

[a] The basic units of the analysis were the clutch means, which were calculated from the specimen values. A regression model with the regions represented as dummy variables, and with robust variance estimates (1–3), was fitted to the data for each variable. A mixed model was used, with location×year as a random factor nested within region as a fixed factor. If necessary, the data were log-transformed. Each region of the Baltic Sea area, as well as the Baltic Sea area as a whole, was compared with control. Means of the model were compared with the Wald test. For comparison of residual standard deviations (SD), within location×year residuals were obtained for each group separately from the model. Residual standard deviations were compared with the F-test and are only presented when they contributed with information not already established by comparison of the means.

[b] *P*-value before adjustment for multiple comparisons with control, n_l = total number of location×year combinations, n_c = total number of clutches, n_s = total number of specimens.

[c] Regression model fitted to log-transformed data, and results back-transformed to the original quantity by simple inverse log-transformation.

* Significantly different from the Iceland control at $\alpha = 0.05$. For the five regions of the Baltic Sea area, significance was determined after *P*-value adjustment (not shown) for five comparisons with control according to Holm (4).

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Table S5. Data from measurements in herring gull (*Larus argentatus*) pulli with a body weight of >75g[a].

Variable [unit]	Statistic[b]	Iceland E (B)	County of Värmland (E)	County of Stockholm (F)	County of Södermanland (G)	County of Blekinge (I)	Baltic Sea area (E–G, I)
Endogenous TK in liver of pulli [nmol/min/mg protein]	Mean	32.0	33.4	29.3	25.2*	29.9	29.5
	95% CI	29.2–34.8	31.6–35.1	27.8–30.9	23.6–26.8	28.3–31.5	28.6–30.3
	P-value	Control	0.42	0.097	<0.0001	0.19	0.083
	SD	4.90	3.06	3.18	3.12	3.09	3.12*
	P-value	Control	0.13	0.11	0.11	0.11	0.027
	n_l, n_c, n_s	3, 12, 19	3, 12, 12	2, 17, 17	5, 15, 15	2, 15, 15	12, 59, 59
TK latency in liver of of pulli[c] [%]	Mean	0	0.054	1.1*	2.2	0.29	0.92*
	95% CI	0–0.14	0–0.87	0.38–1.8	0.080–4.4	0–1.2	0.28–1.6
	P-value	Control	0.45	0.0014	0.025	0.23	0.0026
	SD	0.77	1.4	1.5	4.2*	1.7*	2.5*
	P-value	Control	0.052	0.036	<0.0001	0.013	0.00018
	n_l, n_c, n_s	3, 12, 19	3, 12, 12	2, 17, 17	5, 15, 15	2, 15, 15	12, 59, 59
Endogenous KGDH in liver of pulli[d] [nmol/min/mg protein]	Mean	25.8	27.6	27.3	24.7	26.6	26.5
	95% CI	23.9–27.7	26.1–29.1	25.3–29.2	22.5–26.9	25.0–28.1	25.7–27.4
	P-value	Control	0.14	0.28	0.49	0.55	0.51
	n_l, n_c, n_s	3, 12, 19	3, 12, 12	2, 17, 17	5, 15, 15	2, 15, 15	12, 59, 59
KGDH latency in liver of pulli[c,d] [%]	Mean	25	25	27	32*	26	28
	95% CI	22–28	22–28	24–29	28–37	24–29	26–29
	P-value	Control	0.83	0.43	0.012	0.56	0.18
	n_l, n_c, n_s	3, 12, 19	3, 12, 12	2, 17, 17	5, 15, 15	2, 15, 15	12, 59, 59
T in liver of pulli [nmol/g wet weight]	Mean	1.5	1.2	0.92*	0.90*	1.2	1.1*
	95% CI	1.2–1.9	1.1–1.4	0.83–1.0	0.80–0.99	1.0–1.3	0.99–1.1
	P-value	Control	0.079	0.00033	0.00020	0.026	0.0031
	SD	0.54	0.27*	0.19*	0.17*	0.22*	0.21*
	P-value	Control	0.029	0.00031	0.00044	0.0023	<0.0001
	n_l, n_c, n_s	3, 12, 19	3, 12, 12	2, 16, 16	3, 13, 13	2, 15, 15	10, 56, 56
TMP in liver of pulli [nmol/g wet weight]	Mean	4.0	3.7	3.5	3.3	3.8	3.6*
	95% CI	3.6–4.5	3.5–3.9	3.1–3.8	3.0–3.7	3.3–4.3	3.4–3.7
	P-value	Control	0.14	0.026	0.014	0.44	0.040
	n_l, n_c, n_s	3, 12, 19	3, 12, 12	2, 16, 16	3, 13, 13	2, 15, 15	10, 56, 56
TDP in liver of pulli [nmol/g wet weight]	Mean	14.3	15.4	14.6	12.9*	14.3	14.3
	95% CI	13.7–14.8	14.3–16.5	13.8–15.4	12.1–13.7	13.3–15.2	13.8–14.8
	P-value	Control	0.072	0.48	0.0068	0.98	0.91
	SD	0.892	1.94	1.66	1.44	1.79	1.71*
	P-value	Control	0.016	0.044	0.12	0.025	0.023
	n_l, n_c, n_s	3, 12, 19	3, 12, 12	2, 16, 16	3, 13, 13	2, 15, 15	10, 56, 56
T+TMP+TDP in liver of pulli [nmol/g wet weight]	Mean	19.8	20.3	19.0	17.2*	19.2	18.9
	95% CI	18.9–20.7	19.0–21.6	17.9–20.0	16.1–18.2	17.9–20.5	18.3–19.5
	P-value	Control	0.55	0.21	0.00022	0.44	0.091
	n_l, n_c, n_s	3, 12, 19	3, 12, 12	2, 16, 16	3, 13, 13	2, 15, 15	10, 56, 56
Liver body index[e] (LBI) [%]	Mean	4.7	4.5	4.8	5.0	4.9	4.8
	95% CI	4.3–5.0	4.4–4.7	4.6–5.0	4.8–5.3	4.8–5.1	4.7–5.0
	P-value	Control	0.47	0.49	0.11	0.18	0.42
	SD	0.62	0.30	0.35	0.39	0.31	0.34*
	P-value	Control	0.023	0.039	0.11	0.017	0.0033
	n_l, n_c, n_s	3, 12, 19	3, 12, 12	2, 17, 17	5, 15, 15	2, 15, 15	12, 59, 59

[a] The basic units of the analysis were the clutch means, which were calculated from the specimen values. A regression model with the regions represented as dummy variables, and with robust variance estimates (1–3), was fitted to the data for

each variable. If necessary, any confounding variable was included as a covariate in the model. Each region of the Baltic Sea area, as well as the Baltic Sea area as a whole, was compared with control. Means of the model were compared with the Wald test. For comparison of residual standard deviations (SD), residuals were obtained for each group separately from the model, while still controlling for the effect of any covariate. Residual standard deviations were compared with the F-test and are only presented when they contributed with information not already established by comparison of the means.

[b] *P*-value before adjustment for multiple comparisons with control, n_l = total number of location×year combinations, n_c = total number of clutches, n_s = total number of specimens.

[c] Negative values truncated after the statistical analysis.

[d] Predicted values for a body weight of 153 g. The confounding variable body weight was included as a linear covariate in the model.

[e] Predicted values for a body weight of 153 g. The confounding variable body weight was included as a quadratic covariate in the model.

* Significantly different from the Iceland control at $\alpha = 0.05$. For the four regions of the Baltic Sea area, significance was determined after *P*-value adjustment (not shown) for four comparisons with control according to Holm (4).

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Table S6. Data from measurements in common starling (*Sturnus vulgaris*) eggs[a].

Variable [unit]	Statistic[b]	Iceland SW (A)	Thiamine Treatment[c] (G)	County of Västerbotten (C)	County of Södermanland (G)	County of Blekinge (I)	Baltic Sea area (C, G, I)
T in egg yolk [nmol/g wet weight]	Mean	22	23	23	20	18*†	20†
	95% CI	20–25	21–25	20–26	16–24	16–20	18–22
	P-value	Control	0.50	0.69	0.33	0.013	0.21
	P-value	0.50	Control	0.85	0.13	0.00031	0.021
	n_l, n_c, n_s	8, 20, 30	1, 4, 13	2, 5, 10	2, 6, 15	2, 18, 25	6, 29, 50
Redness of egg yolk[d] [%]	Mean	19	23	17	21	20	19
	95% CI	14–24	17–32	15–19	16–27	18–21	17–21
	P-value	Control	0.30	0.49	0.55	0.66	0.86
	P-value	0.30	Control	0.081	0.62	0.36	0.27
	CV[e] [%]	53	28	13	29	9.8*†	15*
	P-value	Control	0.36	0.047	0.30	<0.0001	<0.0001
	P-value	0.36	Control	0.24	0.98	0.0035	0.078
	n_l, n_c, n_s	8, 20, 30	1, 4, 13	2, 5, 10	2, 6, 15	2, 18, 25	6, 29, 50
Eggshell thickness [μm]	Mean	135	139	143*	134	141	139
	95% CI	132–139	137–141	139–146	128–140	137–145	136–142
	P-value	Control	0.10	0.0028	0.75	0.049	0.082
	P-value	0.10	Control	0.039	0.18	0.35	0.71
	SD	8.8†	2.3*	4.7	9.2	9.6	9.0†
	P-value	Control	0.046	0.34	0.80	0.75	0.94
	P-value	0.046	Control	0.25	0.044	0.035	0.041
	n_l, n_c, n_s	8, 20, 30	1, 4, 13	2, 5, 10	2, 6, 15	2, 18, 25	6, 29, 50
Eggshell density [g/cm^3]	Mean	1.85	1.83	1.76*	1.83	1.88	1.82
	95% CI	1.81–1.88	1.74–1.93	1.70–1.81	1.78–1.88	1.81–1.94	1.79–1.85
	P-value	Control	0.83	0.0067	0.58	0.39	0.30
	P-value	0.83	Control	0.18	0.91	0.47	0.80
	SD	0.0516	0.0991	0.0680	0.0435	0.111*	0.0977*
	P-value	Control	0.086	0.42	0.80	0.010	0.025
	P-value	0.086	Control	0.55	0.15	0.97	0.80
	n_l, n_c, n_s	8, 20, 30	1, 4, 13	2, 5, 10	2, 6, 15	2, 18, 25	6, 29, 50

[a] The basic units of the analysis were the clutch means, which were calculated from the specimen values. A regression model with the regions represented as dummy variables, and with robust variance estimates (1–3), was fitted to the data for each variable. A mixed model was used, with location×year as a random factor nested within region as a fixed factor. If necessary, the data were log-transformed. Each region of the Baltic Sea area, as well as the Baltic Sea area as a whole, were compared with each of the two controls, which also were compared with each other. Means of the model were compared with the Wald test. For comparison of residual standard deviations (SD), within location×year residuals were obtained for each group separately from the model. Residual standard deviations were compared with the F-test and are only presented when they contributed with information not already established by comparison of the means.

[b] *P*-value before adjustment for multiple comparisons with control, n_l = total number of location×year combinations, n_c = total number of clutches, n_s = total number of specimens.

[c] Injection of females with a thiamine (T) solution at a dose of 50 mg T per kg bodyweight as a single dose 19–24 days before egg laying.

* Significantly different from the Iceland control at $\alpha = 0.05$. For the three regions of the Baltic Sea area, significance was determined after *P*-value adjustment (not shown) for three comparisons with control according to Holm (4).

† Significantly different from the thiamine treatment control at $\alpha = 0.05$. For the three regions of the Baltic Sea area significance was determined after *P*-value adjustment (not shown) for three comparisons with control according to Holm (4).

[d] Regression model fitted to log-transformed data, and results back-transformed to the original quantity by simple inverse log-transformation. Back-transformation of the standard deviations yielded the coefficient of variation (CV) by the formula $CV = 100(\exp(SD^2) - 1)^{0.5}$, commonly used for log-normally distributed data.

[e] CV = coefficient of variation (standard deviation expressed as percent of the mean).

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Table S7. Data from measurements in common starling (*Sturnus vulgaris*) pulli[a].

Variable [unit]	Statistic[b]	Iceland SW (A)	Thiamine Treatment[c] (I)	County of Västerbotten (C)	County of Södermanland (G)	County of Blekinge (I)	Baltic Sea area (C, G, I)
Endogenous KGDH in liver of pulli[d,e] [nmol/min/mg protein]	Mean	28.1†	23.5*	29.3†	23.1	19.8*†	23.8*
	95% CI	24.6–32.1	22.2–25.0	24.8–34.6	19.9–26.8	17.3–22.6	22.0–25.7
	P-value	Control	0.019	0.70	0.047	0.00058	0.031
	P-value	0.019	Control	0.016	0.81	0.020	0.86
	CV[f] [%]	18.7†	7.61*	14.9	19.3	20.9	19.3†
	P-value	Control	0.047	0.69	0.96	0.83	0.97
	P-value	0.047	Control	0.14	0.039	0.024	0.030
	n_l, n_c, n_s	1, 7, 15	1, 7, 30	3, 5, 7	2, 8, 11	1, 11, 11	6, 24, 29
KGDH latency in liver of pulli[g] [%]	Mean	11†	14*	12	13	18*	14*
	95% CI	9.1–13	12–16	7.5–17	11–15	13–22	12–16
	P-value	Control	0.011	0.62	0.072	0.0079	0.019
	P-value	0.011	Control	0.37	0.47	0.17	0.96
	SD	2.2	2.4	5.1	2.7	7.1*†	5.6*†
	P-value	Control	0.86	0.073	0.67	0.010	0.030
	P-value	0.86	Control	0.10	0.80	0.015	0.044
	n_l, n_c, n_s	1, 7, 15	1, 7, 30	3, 5, 7	2, 8, 11	1, 11, 11	6, 24, 29
T in liver of pulli[d,e] [nmol/g wet weight]	Mean	2.6	—	2.5	3.0	2.6	2.7
	95% CI	2.0–3.5	—	1.8–3.6	2.2–4.2	2.1–3.2	2.3–3.2
	P-value	Control	—	0.87	0.45	0.96	0.81
	n_l, n_c, n_s	1, 7, 15	0, 0, 0	3, 5, 7	2, 8, 11	1, 12, 12	6, 25, 30
TMP in liver of pulli [nmol/g wet weight]	Mean	4.4	—	4.2	5.4	5.2	4.9
	95% CI	3.8–4.9	—	3.3–5.1	4.4–6.5	4.6–5.7	4.5–5.4
	P-value	Control	—	0.74	0.077	0.045	0.13
	n_l, n_c, n_s	1, 7, 15	0, 0, 0	3, 5, 7	2, 8, 11	1, 12, 12	6, 25, 30
TDP in liver of pulli[e] [nmol/g wet weight]	Mean	14.8	—	13.1	14.7	13.6*	13.8*
	95% CI	14.3–15.4	—	11.4–14.8	13.0–16.5	12.9–14.3	13.0–14.6
	P-value	Control	—	0.043	0.89	0.0092	0.032
	SD	0.566	—	1.74*	2.11*	1.13	1.61*
	P-value	Control	—	0.019	0.0051	0.10	0.015
	n_l, n_c, n_s	1, 7, 15	0, 0, 0	3, 5, 7	2, 8, 11	1, 12, 12	6, 25, 30
T+TMP+TDP in liver of pulli[e] [nmol/g wet weight]	Mean	21.9	—	19.9	23.6	21.6	21.7
	95% CI	20.6–23.2	—	18.0–21.8	21.7–25.5	20.3–22.8	20.8–22.6
	P-value	Control	—	0.067	0.16	0.72	0.78
	n_l, n_c, n_s	1, 7, 15	0, 0, 0	3, 5, 7	2, 8, 11	1, 12, 12	6, 25, 30
Endogenous TK in brain of pulli[d,h] [nmol/min/mg protein]	Mean	8.89†	10.5*	9.02†	8.71†	9.19†	8.97†
	95% CI	8.37–9.44	10.1–10.9	8.36–9.73	8.23–9.21	8.81–9.59	8.65–9.30
	P-value	Control	<0.0001	0.78	0.58	0.34	0.79
	P-value	<0.0001	Control	0.00062	<0.0001	<0.0001	<0.0001
	n_l, n_c, n_s	1, 7, 15	1, 7, 30	3, 5, 7	2, 8, 11	2, 20, 42	7, 33, 60
TK latency in brain of pulli[g] [%]	Mean	0.038	0	1.2	2.6*†	1.5*†	1.8*†
	95% CI	0–0.75	0–0.29	0–3.0	1.9–3.2	0.70–2.3	1.1–2.5
	P-value	Control	0.57	0.22	<0.0001	0.0084	0.0010
	P-value	0.57	Control	0.13	<0.0001	0.00070	<0.0001
	n_l, n_c, n_s	1, 7, 15	1, 7, 30	3, 5, 7	2, 8, 11	2, 20, 42	7, 33, 60

Table S7. Continued.

Variable [unit]	Statistic[b]	Iceland SW (A)	Thiamine Treatment[c] (I)	County of Västerbotten (C)	County of Södermanland (G)	County of Blekinge (I)	Baltic Sea area (C, G, I)
T in brain of pulli[h] [nmol/g wet weight]	Mean	1.2	—	1.2	1.2	0.97	1.1
	95% CI	0.82–1.6	—	0.75–1.7	0.97–1.3	0.85–1.1	0.93–1.3
	P-value	Control	—	0.97	0.75	0.23	0.56
	SD	0.43	—	0.37	0.15	0.20	0.23*
	P-value	Control	—	0.80	0.017	0.029	0.028
	n_l, n_c, n_s	1, 7, 15	0, 0, 0	3, 5, 7	2, 8, 11	1, 12, 12	6, 25, 30
TMP in brain of pulli[h] [nmol/g wet weight]	Mean	1.7	—	1.4	1.7	1.5	1.6
	95% CI	1.5–1.8	—	1.1–1.8	1.6–1.9	1.3–1.7	1.4–1.7
	P-value	Control	—	0.20	0.58	0.29	0.31
	n_l, n_c, n_s	1, 7, 15	0, 0, 0	3, 5, 7	2, 8, 11	1, 12, 12	6, 25, 30
TDP in brain of pulli[h] [nmol/g wet weight	Mean	13.8	—	13.5	13.8	13.2	13.5
	95% CI	12.0–15.5	—	10.7–16.2	12.2–15.4	12.2–14.3	12.2–14.8
	P-value	Control	—	0.84	0.97	0.61	0.79
	n_l, n_c, n_s	1, 7, 15	0, 0, 0	3, 5, 7	2, 8, 11	1, 12, 12	6, 25, 30
T+TMP+TDP in brain of pulli[h] [nmol/g wet weight]	Mean	16.6	—	16.1	16.7	15.7	16.2
	95% CI	14.6–18.6	—	13.4–18.9	15.0–18.3	14.5–17.0	14.9–17.4
	P-value	Control	—	0.72	0.97	0.44	0.66
	n_l, n_c, n_s	1, 7, 15	0, 0, 0	3, 5, 7	2, 8, 11	1, 12, 12	6, 25, 30
Liver body index[h,i] (LBI) [%]	Mean	4.5	3.6	3.9	3.5	3.5	3.6
	95% CI	3.3–5.7	2.7–4.4	2.8–5.1	2.4–4.5	2.6–4.4	3.0–4.2
	P-value	Control	0.23	0.54	0.19	0.18	0.21
	P-value	0.23	Control	0.60	0.87	0.47	0.88
	SD	0.81†	0.19*	0.70†	0.47	0.36*	0.42*
	P-value	Control	0.0024	1.00	0.20	0.0031	0.0085
	P-value	0.0024	Control	0.013	0.048	0.14	0.061
	n_l, n_c, n_s	5, 14, 34	1, 7, 30	3, 5, 7	2, 8, 11	2, 20, 42	7, 33, 60

[a] The basic units of the analysis were the clutch means, which were calculated from the specimen values. A regression model with the regions represented as dummy variables, and with robust variance estimates (1–3), was fitted to the data for each variable. If necessary, the data were log-transformed and any confounding variable was included as a covariate in the model. Each region of the Baltic Sea area, as well as the Baltic Sea area as a whole, were compared with each of the two controls, which also were compared with each other. Means of the model were compared with the Wald test. For comparison of residual standard deviations (SD), residuals were obtained for each group separately from the model, while still controlling for the effect of any covariate. Residual standard deviations were compared with the F-test and are only presented when they contributed with information not already established by comparison of the means.

[b] *P*-value before adjustment for multiple comparisons with control, n_l = total number of location×year combinations, n_c = total number of clutches, n_s = total number of specimens.

[c] Gavage of a thiamine (T) solution at a dose of 50 mg T per kg bodyweight as a single dose 11 days before sampling.

[d] Regression model fitted to log-transformed data, and results back-transformed to the original quantity by simple inverse log-transformation. Back-transformation of the standard deviations yielded the coefficient of variation (CV) by the formula $CV = 100(\exp(SD^2) - 1)^{0.5}$, commonly used for log-normally distributed data.

[e] Predicted values for a body weight of 57 g. The confounding variable body weight was included as a linear covariate in the model.

† Significantly different from the thiamine treatment control at $\alpha = 0.05$. For the three regions of the Baltic Sea area significance was determined after *P*-value adjustment (not shown) for three comparisons with control according to Holm (4).

* Significantly different from the Iceland control at $\alpha = 0.05$. For the three regions of the Baltic Sea area, significance was determined after *P*-value adjustment (not shown) for three comparisons with control according to Holm (4).

[f] CV = coefficient of variation (standard deviation expressed as percent of the mean).

[g] Negative values truncated after the statistical analysis.

[h] Predicted values for a body weight of 57 g. The confounding variable body weight was included as a quadratic covariate in the model.

[i] Regression model further elaborated to a mixed model with location×year as a random factor nested within region as a fixed factor. Within location×year residuals were used for comparison of residual standard deviations.

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Table S8. Data from measurements in common eider (*Somateria mollissima*) eggs[a].

Variable [unit]	Statistic[b]	Iceland SW (A)	County of S. Finland (D)	County of Stockholm (F)	County of Södermanland (G)	County of Kalmar (H)	County of Blekinge (I)	Baltic Sea area (D, F–I)
T in egg yolk [nmol/g wet weight]	Mean	17	5.9*	6.9*	4.7*	12	7.1*	7.2*
	95% CI	13–21	2.1–9.6	5.2–8.6	2.1–7.3	6.9–16	3.8–10	5.7–8.8
	P-value	Control	0.00014	<0.0001	<0.0001	0.12	0.00034	<0.0001
	n_l, n_c, n_s	3, 22, 22	3, 12, 17	17, 51, 84	3, 17, 23	2, 7, 11	4, 22, 22	29, 109, 157
Redness of egg yolk [%]	Mean	22	23	25	18	21	24	22
	95% CI	19–25	19–26	23–27	17–20	16–26	22–26	21–23
	P-value	Control	0.88	0.18	0.030	0.67	0.38	0.93
	SD	4.2	3.6	4.8	1.7*	5.0	2.6	3.8
	P-value	Control	0.68	0.57	0.0013	0.51	0.049	0.57
	n_l, n_c, n_s	3, 22, 22	3, 12, 17	17, 51, 84	3, 17, 23	2, 7, 11	4, 22, 22	29, 109, 157
Eggshell Thickness[c] [μm]	Mean	390	401	390	407	400	400	399
	95% CI	378–402	384–418	384–396	394–419	379–421	391–409	393–406
	P-value	Control	0.30	1.00	0.049	0.41	0.19	0.15
	n_l, n_c, n_s	3, 22, 22	3, 12, 17	17, 51, 84	3, 17, 23	2, 7, 11	4, 22, 22	29, 109, 157
Eggshell density [g/cm^3]	Mean	2.05	2.05	2.10*	2.04	2.10*	2.07	2.07
	95% CI	2.02–2.08	2.01–2.09	2.08–2.11	2.00–2.08	2.07–2.14	2.04–2.10	2.06–2.09
	P-value	Control	0.81	0.0063	0.85	0.011	0.33	0.14
	n_l, n_c, n_s	3, 21, 21	3, 12, 17	17, 51, 84	3, 17, 23	2, 7, 11	4, 21, 21	29, 108, 156

[a] The basic units of the analysis were the clutch means, which were calculated from the specimen values. A regression model with the regions represented as dummy variables, and with robust variance estimates (1–3), was fitted to the data for each variable. A mixed model was used, with location×year as a random factor nested within region as a fixed factor. Each region of the Baltic Sea area, as well as the Baltic Sea area as a whole, was compared with control. Means of the model were compared with the Wald test. For comparison of residual standard deviations (SD), within location×year residuals were obtained for each group separately from the model. Residual standard deviations were compared with the F-test and are only presented when they contributed with information not already established by comparison of the means.

[b] *P*-value before adjustment for multiple comparisons with control, n_l = total number of location×year combinations, n_c = total number of clutches, n_s = total number of specimens.

* Significantly different from the Iceland control at $\alpha = 0.05$. For the five regions of the Baltic Sea area, significance was determined after *P*-value adjustment (not shown) for five comparisons with control according to Holm (4).

[c] Icelandic eggshell thickness normalised by 4.2% (length scale), based on the relative difference in mean volumes between Icelandic and Baltic common eider eggs (13.2%, volume scale). Original eggshell thickness values: mean 374; CI: 363-385.

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Table S9. Data from measurements in common eider (*Somateria mollissima*) pulli[a].

Variable [unit]	Statistic[b]	Iceland SW (A)	County of Stockholm (F)	County of Södermanland (G)	County of Blekinge (I)	Baltic Sea area (F, G, I)
Endogenous TK in liver of pulli[c] [nmol/min/mg protein]	Median	25.1	17.1*	28.5	23.1	—
	95% CI	23.3–26.6	10.6–23.9	28.3–28.8	7.42–26.0	—
	P-value	Control	0.0019	0.030	0.073	—
	Minimum	21.5	3.89	28.3	6.31	—
	10 percentile	21.7	4.53	28.3	6.31	—
	25 percentile	23.4	10.4	28.3	14.6	—
	75 percentile	26.5	23.9	28.8	24.8	—
	90 percentile	27.0	25.3	28.8	26.2	—
	Maximum	27.0	25.5	28.8	26.2	—
	n_l, n_c, n_s	2, 10, 25	3, 14, 14	1, 2, 7	4, 6, 16	8, 22, 37
TK latency in liver of pulli[c,d] [%]	Median	0.040	10*	0	1.6	—
	95% CI	0–1.5	0.57–29	0	0–53	—
	P-value	Control	0.0073	0.12	0.31	—
	Minimum	0	0	0	0	—
	10 percentile	0	0	0	0	—
	25 percentile	0	0.55	0	0	—
	75 percentile	0.70	30	0	18	—
	90 percentile	2.2	68	0	59	—
	Maximum	2.3	69	0	59	—
	n_l, n_c, n_s	2, 10, 25	3, 14, 14	1, 2, 7	4, 6, 16	8, 22, 37
Endogenous KGDH in liver of pulli[c] [nmol/min/mg protein]	Median	22.8	18.3*	23.4	18.0*	—
	95% CI	19.1–24.2	16.1–20.8	23.4–23.5	4.24–20.1	—
	P-value	Control	0.0020	0.48	0.0075	—
	n_l, n_c, n_s	2, 10, 23	9, 28, 41	1, 2, 7	4, 6, 16	14, 36, 64
KGDH latency in liver of pulli[c,d] [%]	Median	36	41*	33	43*	—
	95% CI	28–39	35–50	32–35	36–83	—
	P-value	Control	0.012	0.76	0.0075	—
	n_l, n_c, n_s	2, 10, 23	9, 28, 41	1, 2, 7	4, 6, 16	14, 36, 64
TMP in liver of pulli [nmol/g wet weight]	Mean	0.85	0.44*	1.0*	0.69	0.72
	95% CI	0.77–0.94	0.19–0.69	0.95–1.1	0.44–0.94	0.60–0.84
	P-value	Control	0.0035	0.0048	0.21	0.069
	SD	0.13	0.44*	0.065	0.30	0.40*
	P-value	Control	0.0010	0.73	0.030	0.0019
	n_l, n_c, n_s	2, 10, 25	3, 14, 14	1, 2, 7	4, 6, 16	8, 22, 37
TDP in liver of pulli [nmol/g wet weight]	Mean	7.9	5.3*	8.9	7.2	7.1
	95% CI	7.3–8.5	3.5–7.0	7.8–10	4.2–10	5.9–8.3
	P-value	Control	0.0071	0.11	0.63	0.25
	SD	0.96	3.1*	1.0	3.7*	3.2*
	P-value	Control	0.0013	0.66	0.00090	0.00087
	n_l, n_c, n_s	2, 10, 25	3, 14, 14	1, 2, 7	4, 6, 16	8, 22, 37
T+TMP+TDP in liver of pulli[e] [nmol/g wet weight]	Mean	9.2	6.1*	10	8.3	8.2
	95% CI	8.5–9.8	4.1–8.1	9.2–12	5.0–11	6.9–9.6
	P-value	Control	0.0060	0.080	0.58	0.21
	SD	1.0	3.5*	1.1	4.0*	3.6*
	P-value	Control	0.00082	0.66	0.00083	0.00061
	n_l, n_c, n_s	2, 10, 25	3, 14, 14	1, 2, 7	4, 6, 16	8, 22, 37

Table S9. Continued.

Variable [unit]	Statistic[b]	Iceland SW (A)	County of Stockholm (F)	County of Södermanland (G)	County of Blekinge (I)	Baltic Sea area (F, G, I)
Endogenous TK in brain of pulli[c] [nmol/min/mg protein]	Median	12.5	11.6*	12.1	9.20*	—
	95% CI	12.1–13.5	10.0–12.1	11.8–12.5	4.47–10.0	—
	P-value	Control	0.016	0.48	0.00025	—
	n_l, n_c, n_s	2, 10, 24	3, 14, 14	1, 2, 7	4, 6, 16	8, 22, 37
TK latency in brain of pulli[c,d] [%]	Median	0.55	1.8	1.3	3.3*	—
	95% CI	0–1.1	0.085–9.1	1.1–1.5	2.4–33	—
	P-value	Control	0.14	0.18	0.00025	—
	Minimum	0	0	1.1	2.4	—
	10 percentile	0	0	1.1	2.4	—
	25 percentile	0	0.038	1.1	2.6	—
	75 percentile	1.0	11	1.5	12	—
	90 percentile	1.9	37	1.5	36	—
	Maximum	1.9	46	1.5	36	—
	n_l, n_c, n_s	2, 10, 24	3, 14, 14	1, 2, 7	4, 6, 16	8, 22, 37
Endogenous KGDH in brain of pulli[c] [nmol/min/mg protein]	Median	10.7	10.6	11.3	11.9	—
	95% CI	10.2–12.0	9.59–11.2	11.0–11.6	6.25–14.2	—
	P-value	Control	0.34	0.61	0.22	—
	Minimum	9.98	3.34	11.0	5.69	—
	10 percentile	10.0	6.13	11.0	5.69	—
	25 percentile	10.2	9.58	11.0	9.92	—
	75 percentile	12.0	11.2	11.6	12.8	—
	90 percentile	12.1	11.5	11.6	14.4	—
	Maximum	12.1	11.5	11.6	14.4	—
	n_l, n_c, n_s	2, 10, 25	3, 14, 14	1, 2, 5	4, 6, 16	8, 22, 37
KGDH latency in brain of pulli[c,d] [%]	Median	11	10	9.3	13	—
	95% CI	8.8–12	8.3–14	7.6–11	9.4–55	—
	P-value	Control	1.0	0.61	0.093	—
	Minimum	7.6	3.8	7.6	9.2	—
	10 percentile	7.7	5.1	7.6	9.2	—
	25 percentile	8.9	8.2	7.6	11	—
	75 percentile	12	14	11	27	—
	90 percentile	14	52	11	59	—
	Maximum	14	69	11	59	—
	n_l, n_c, n_s	2, 10, 25	3, 14, 14	1, 2, 5	4, 6, 16	8, 22, 37
T in brain of pulli [nmol/g wet weight]	Mean	0.30	0.21*	0.32	0.34	0.29
	95% CI	0.28–0.31	0.18–0.25	0.29–0.34	0.27–0.41	0.26–0.32
	P-value	Control	0.00020	0.22	0.23	0.60
	SD	0.023	0.066*	0.024	0.084*	0.070*
	P-value	Control	0.0031	0.66	0.0012	0.0017
	n_l, n_c, n_s	2, 10, 25	3, 14, 14	1, 2, 7	4, 6, 16	8, 22, 37
TMP in brain of pulli [nmol/g wet weight]	Mean	1.7	0.94*	1.6	1.1*	1.2*
	95% CI	1.6–1.8	0.65–1.2	1.5–1.7	0.71–1.5	1.0–1.4
	P-value	Control	<0.0001	0.16	0.0062	<0.0001
	SD	0.17	0.53*	0.10	0.49*	0.50*
	P-value	Control	0.0016	0.87	0.0062	0.0019
	n_l, n_c, n_s	2, 10, 25	3, 14, 14	1, 2, 7	4, 6, 16	8, 22, 37

Table S9. Continued.

Variable [unit]	Statistic[b]	Iceland SW (A)	County of Stockholm (F)	County of Södermanland (G)	County of Blekinge (I)	Baltic Sea area (F, G, I)
TDP in brain of pulli [nmol/g wet weight]	Mean	10.9	7.21*	10.4	8.68	8.78*
	95% CI	10.4–11.4	5.41–9.02	8.56–12.3	5.92–11.4	7.51–10.0
	P-value	Control	0.00036	0.63	0.11	0.0031
	SD	0.713	3.21*	1.73	3.39*	3.20*
	P-value	Control	<0.0001	0.077	0.00015	<0.0001
	n_l, n_c, n_s	2, 10, 25	3, 14, 14	1, 2, 7	4, 6, 16	8, 22, 37
T+TMP+TDP in brain of pulli [nmol/g wet weight]	Mean	12.9	8.37*	12.4	10.1	10.3*
	95% CI	12.4–13.5	6.24–10.5	10.3–14.4	6.94–13.3	8.84–11.7
	P-value	Control	0.00022	0.59	0.089	0.0016
	SD	0.818	3.78*	1.85	3.91*	3.74*
	P-value	Control	<0.0001	0.10	0.00014	<0.0001
	n_l, n_c, n_s	2, 10, 25	3, 14, 14	1, 2, 7	4, 6, 16	8, 22, 37
Liver body index[f] (LBI) [%]	Mean	4.1	4.1	4.4	4.4	4.3
	95% CI	3.6–4.6	3.8–4.4	4.2–4.5	4.2–4.6	4.2–4.4
	P-value	Control	0.98	0.30	0.32	0.49
	n_l, n_c, n_s	2, 10, 25	9, 28, 41	1, 2, 7	4, 6, 16	14, 36, 64

[a] The basic units of the analysis were the clutch means, which were calculated from the specimen values. A regression model with the regions represented as dummy variables, and with robust variance estimates (1–3), was fitted to the data for each variable, when the residuals met the assumptions of normality and homoscedasticity. Each region of the Baltic Sea area, as well as the Baltic Sea area as a whole, was compared with control. Means of the model were compared with the Wald test. For comparison of residual standard deviations (SD), residuals were obtained for each group separately from the model. Residual standard deviations were compared with the F-test and are only presented when they contributed with information not already established by comparison of the means. When the assumptions of normality and homoscedasticity were not met, the Wilcoxon-Mann-Whitney test was used instead. Confidence intervals (CI) of the median were calculated according to Mood and Graybill (4). Percentiles are given to illustrate the non-normal distributions when they contributed with information not already established by comparison of the medians.

[b] *P*-value before adjustment for multiple comparisons with control, n_l = total number of location×year combinations, n_c = total number of clutches, n_s = total number of specimens.

[c] No regression model. The groups were compared with control with the Wilcoxon-Mann-Whitney test instead.

* Significantly different from the Iceland control at $\alpha = 0.05$. For the three regions of the Baltic Sea area, significance was determined after *P*-value adjustment (not shown) for three comparisons with control according to Holm (5).

[d] Negative values truncated after the statistical analysis.

[e] Owing to a chromatographic disturbance, T in liver was estimated to 0.4 nmol/g wet weight (half limit of quantification) for all clutches.

[f] Regression model further elaborated to a mixed model with location×year as a random factor nested within region as a fixed factor. Within location×year residuals were used for comparison of residual standard deviations.

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